CENTER FOR DRUG EVALUATION AND RESEARCH

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PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
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CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

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Sponsor	TopoTarget A/S Copenhagen, Denmark
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Review Division	Division of Drug Oncology Products
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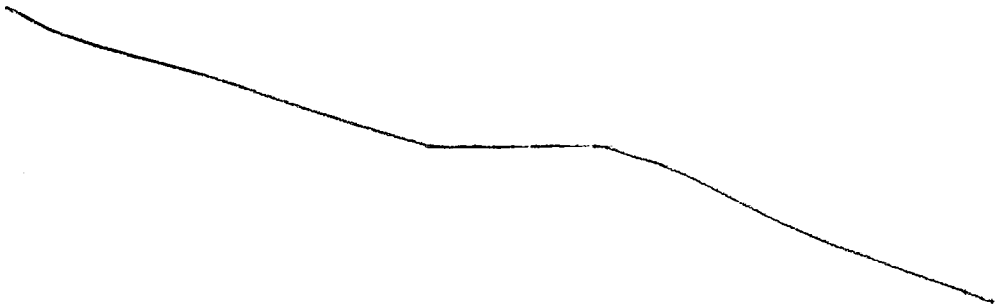

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Executive Summary

The US FDA has approved dexrazoxane for injection (ZINECARD®, NDA 20-212, May 26, 1995) for use in the prevention of the cardiomyopathy associated with doxorubicin cancer chemotherapy. The mechanism by which dexrazoxane exerts its cardio-protective activity is not well established. Dexrazoxane is a cyclic derivative of EDTA that, unlike EDTA, can cross cell membranes.

When a health care professional inadvertently extravasates an anthracycline drug, such as doxorubicin or daunorubicin, during an infusion into a peripheral vein, a serious painful open wound can form in the skin overlying the extravasation and in the underlying tissue. The most common treatment for such wounds is surgical repair. The results of such repair are frequently unsatisfactory. In the current application, the sponsor proposes that dexrazoxane is effective for the amelioration or prevention of subcutaneous damage associated with the accidental extravasation of anthracyclines during cancer chemotherapy. For ethical reasons, the sponsor claimed they could not conduct controlled clinical trials in cancer patients. They have submitted two uncontrolled clinical studies designed to demonstrate that dexrazoxane administration after anthracycline extravasation decreases the need for surgical repair at the injection site (see Dr. Robert Kane's medical review). Because of the paucity of clinical information I was ask to do an extensive analysis of the efficacy of this treatment in animal model studies. Specifically, we needed to determine if we might approve the application through the provisions of the animal rule (21 CFR 314.600).

The sponsor submitted numerous studies that clearly demonstrate that dexrazoxane administration soon after a subcutaneous injection of doxorubicin or daunorubicin prevents or diminishes the formation of cutaneous lesions in female mice. Nevertheless, the available studies do not provide sufficient evidence of efficacy to allow approval based on the requirements of the animal rule. The sponsor did not do the available studies under GLP conditions, the sample sizes were relatively small, the studies used only one species and one sex (female mice) and the studies do not establish a mechanism for this pharmacology. Indeed, the studies demonstrate that dexrazoxane probably does not mitigate damage by scavenging radicals at the damage site as the sponsor has proposed. Dexrazoxane probably works by binding to a site on DNA close to but distinct from the binding site of anthracyclines, thereby preventing the binding of the anthracycline and the resultant double-strand breaks associated with the inhibition of topoisomerase II, but the sponsor has not established this mechanism.

The sponsor proposes to give TOTECT™ clinically within six hours of anthracycline extravasation at a dose of 1000 mg/m^2 (not to exceed 2000 mg), with a second dose of 1000 mg/m^2 (not to exceed 2000 mg) on day 2 and a third dose of 500 mg/m^2 (not to exceed 1000 mg) on day three. Experimental results in mice demonstrate that dexrazoxane given immediately after anthracycline extravasation is more effective than delayed treatment. Efficacy in mice diminishes rapidly six hours after the anthracycline toxic insult. Thus, the available evidence in mice does

not support the efficacy of doses given on any day but the day of the initial insult. The most effective schedule in mice was IP injections of 62.5 mg/kg (187.5 mg/m^2) at time = 0, 3 and 6 hours (562.5 mg/m^2 total dose) after the subcutaneous injection of anthracycline. The next most effective schedule was a single IP injection of 250 mg/kg (750 mg/m^2) immediately after the anthracycline injection. In many cases, these two dosing regimens completely prevented the formation of an anthracycline-induced wound in mice, particularly with doxorubicin. Other experiments with daunorubicin show similar results. Single IP doses of dexrazoxane of 375 mg/kg in combination with daunorubicin caused unacceptable morbidity and mortality. IV dosing at equivalent doses provided no better protection than IP dosing. Neither does the animal data establish a clear dose effect, probably because most of the doses tested where above the range of the slope of the dose response curve and because of limitations of the efficacy assay (see below).

The body surface area of a mouse is about 1000 mm^2. A dose of 3 mg/kg (9 mg/m^2) of daunorubicin in the absence of treatment with dexrazoxane caused maximal wounds of about 110 mm^2. Larger doses caused significant mortality and morbidity. Thus, mice could sustain wounds over about 10% of their body surface area. In induction therapy for acute myelogenous leukemia, the usual dose of daunorubicin is 60 mg/m^2. In the treatment of breast cancer the dose of doxorubicin is also usually about 60 mg/m^2. So the doses used in the animal studies of this NDA to induce cutaneous wounds were about one sixth the total dose used clinically on a mg/m^2 basis. These studies in mice cannot predict the efficacy of dexrazoxane in the situation where a large fraction clinical dose is extravasated.

Recommendations

Recommendation on Approvability

The available Pharmacology and Toxicology information is adequate to support the approval of TOTECT™ for use in the proposed clinical indication.

Recommendation for Non-clinical studies

None

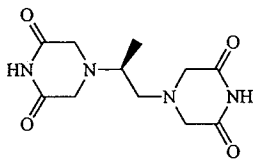
Recommendations on Labeling

The various sections of the product label should read as follows. The wording below represents a consensus among the members of the review team. I excerpted with modification some of the information for the following label changes from the ZINECARD™ product label to assure consistency between the two products.

DESCRIPTION

Totect™ (dexrazoxane) for injection is a sterile, pyrogen-free lyophilizate intended for intravenous (IV) administration.

Chemically, dexrazoxane is 2,6-piperazinedione,4,4'-(1-methyl-1,2-ethanediyl)bis-,(S)- or (S)-(+)-1,2-bis(3,5-dioxopiperazin-1-yl)propane. The following diagram shows the chemical structure:



CLINICAL PHARMACOLOGY

Mechanism of Action

The mechanism by which Totect™ ameliorates tissue damage resulting from the extravasation of anthracycline drugs is unknown. Some evidence suggests that dexrazoxane inhibits topoisomerase II reversibly.

WARNINGS

Pregnancy - *Pregnancy Category D* - Dexrazoxane was toxic to pregnant rats at doses of 2 mg/kg (1/80 the human dose on a mg/m^2 basis) and embryotoxic and teratogenic at 8 mg/kg (about 1/20 the human dose on a mg/m^2 basis) when given daily during the period of organogenesis. Teratogenic effects in the rat included imperforate anus, microphthalmia, and anophthalmia. In offspring allowed to develop to maturity, fertility was impaired in the male and female rats treated *in utero* during organogenesis at 8 mg/kg. In rabbits, doses of 5 mg/kg (about 1/16 the human dose on a mg/m^2 basis) daily during the period of organogenesis caused maternal toxicity and doses of 20 mg/kg (1/4 the human dose on a mg/m^2 basis) were embryotoxic and teratogenic. Teratogenic effects in the rabbit included several skeletal malformations such as short tail, rib and thoracic malformations, and soft tissue variations including subcutaneous, eye and cardiac hemorrhagic areas, as well as agenesis of the gallbladder and of the intermediate lobe of the lung.

There is no adequate information about the use of Totect ™ in pregnant women. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

Carcinogenesis/Mutagenesis/Impairment of Fertility

The carcinogenic potential of the optically pure S-enantiomer, dexrazoxane, is unknown. Nevertheless, a study by the National Cancer Institute has reported that 52 weeks of dosing with razoxane (the racemic mixture of dexrazoxane and its R-enantiomer) is associated with an increased incidence of malignancies. In this bioassay, rats were dosed with up to 96 mg/kg (576 mg/m^2) and mice with up to 80 mg/kg (240 mg/m^2) three times per week. The incidence of uterine adenocarcinomas increased with dose in rats. In female mice, the incidence of hematopoietic neoplasms also increased with increasing dose. Thus, razoxane was carcinogenic in female rats and mice.

Dexrazoxane was not mutagenic to bacteria *in vitro* (Ames test) but was found to be clastogenic to human lymphocytes *in vitro* and to mouse bone marrow erythrocytes *in vivo* (micronucleus test).

The possible adverse effects of TOTECT™ on the fertility of humans and experimental animals, male or female, have not been adequately studied. Testicular atrophy was seen with dexrazoxane administration at doses as low as 30 mg/kg weekly for 6 weeks in rats (about 1/5 the human dose on a mg/m^2 basis) and as low as 20 mg/kg weekly for 13 weeks in dogs (about half the human dose on a mg/m^2 basis).

Summary of Non-clinical findings

Overview of Non-clinical findings

Dexrazoxane is cytotoxic, genotoxic, fetotoxic, teratogenic and likely carcinogenic. Nevertheless, single doses in the range of the proposed clinical dose or somewhat higher on a mg/m^2 basis caused little acute toxicity in rodents. Longer term dosing (1200 mg/m^2/day for 28 days) is associated with profound myelotoxicity and anemia in rats. Gross and microscopic damage occurs in the spleen, thymus, heart, testes, lymph nodes, bone marrow, kidneys and liver. There is little evidence of any secondary pharmacology.

Dexrazoxane plasma concentration decreases in three distinct phases in rats, a rapid distribution phase that last for only a few minutes, an elimination phase lasting to about four hours, and a longer terminal elimination phase. Rats eliminated most of a dose of radioactivity associated with dexrazoxane in the urine within the first 8 hours after dosing (about 80%). Elimination is negligible after that. Rats excrete only 7 to 8% of a radiolabeled dose in the feces. Dexrazoxane hydrolyzes primarily to the open ring tetra-acetate.

Pharmacological activity

The mechanism of action of dexrazoxane in the prevention or amelioration of tissue damage after accidental anthracycline extravasation remains unknown. Dexrazoxane does prevent the formation of or lessen the severity of cutaneous lesions in mice after the subcutaneous injection of doxorubicin, daunorubicin and to a lesser extent epirubicin and idarubicin. This effect is time dependent. The sooner dexrazoxane is injected IP in the mice the more effective the therapy; efficacy decreases sharply six hours after the toxic insult with the anthracycline. Injection of dexrazoxane directly into the wound site does not improve outcome over that achieved with systemic administration. The non-clinical studies did not establish a clear dose effect. The most effective most effective schedule in mice was IP injections of 62.5 mg/kg (187.5 mg/m^2) at time = 0, 3 and 6 hours (562.5 mg/m^2 total dose) after the subcutaneous injection of anthracycline. The next most effective schedule was a single IP injection of 250 mg/kg (750 mg/m^2) immediately after the anthracycline injection. Dexrazoxane does not ameliorate the damage caused by radical generators such as hydrogen peroxide, suggesting that it is not a radical scavenger. Neither does it appear to work by chelating metal cations such as iron.

Non-clinical safety issues relevant to clinical use

Dexrazoxane is clastogenic to human lymphocytes *in vitro* and to mouse bone marrow erythrocytes *in vivo* (micronucleus test). No one has yet done carcinogenicity studies of pure dexrazoxane and for this indication, none are unnecessary. Nevertheless, a study by the National Cancer Institute has reported that long term dosing with razoxane (the racemic mixture of dexrazoxane, ICRF-187, and its enantiomer ICRF-186) is associated with the development of malignancies in rats and possibly in mice. Thus, dexrazoxane is likely carcinogenic.

The possible adverse effects of dexrazoxane on the fertility of humans and experimental animals, male or female, have not been adequately studied. Dosing was associated with testicular atrophy at doses as low as 30 mg/kg weekly for 6 weeks in rats (1/6 the human dose on a mg/m^2 basis) and as low as 20 mg/kg weekly for 13 weeks in dogs (approximately half the human dose on a mg/m^2 basis).

In studies of reproductive toxicity in rats, dexrazoxane caused maternal toxicity at doses of 2 mg/kg (1.2 % of the human dose on a mg/m^2 basis). It was embryotoxic and teratogenic at 8 mg/kg (about 5% of the human dose on a mg/m^2 basis) when given daily to pregnant rats during the period of organogenesis. In rabbits, doses of 5 mg/kg (about 1/16 the human dose on a mg/m^2 basis) daily during organogenesis caused maternal toxicity. Doses of 20 mg/kg (1/4 the human dose on a mg/m^2 basis) were embryotoxic and teratogenic.

PHARMACOLOGY/TOXICOLOGY REVIEW

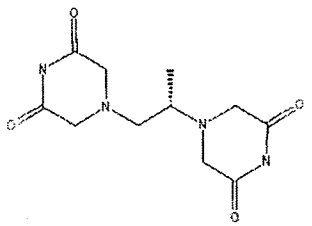
Introduction and Drug History

NDA number	22-025
Review	number 1
Submission	000
Information to sponsor	No
Sponsor	Topotarget A/S Copenhagen, Denmark
Reviewer name	W. David McGuinn, Jr., M.S., Ph. D., D.A.B.T.
Division name	Division of Oncology Drug Products
Review completion date	

Drug

	Trade name:	TOTECT™
	Generic name:	Dexrazoxane
	Code Name	ICRF-187
	Chemical Name	4-[1-(3,5-dioxopiperazin-1-yl)propan-2-yl]piperazine-2,6-dione
FW	268.269 g/mol	CAS 24584-09-6 $C_{11}H_{16}N_4O_4$

Structure



Relevant INDs & NDAs

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[redacted]

NDA 20212 Zinecard

Drug class	Cytotoxin Topoisomerase II inhibitor Immunosuppressive cytotoxin Metal ion chelating prodrug Cardiovascular protective
Intended clinical population	Treatment of anthracycline extravasation during chemotherapy
Clinical formulation	The proposed marketed pack for the TOTECT™ 500 mg powder and Solvent for Injection include: 10 vials of each 500 mg

	Dexrazoxane Hydrochloride Salt and 10 vials each 50 mL Sodium Lactate Injection component.
Route of administration	IV
Dose and schedule	1000 mg/m^2 (not to exceed 2000 mg) on the day of the extravasation, 1000 mg/m^2 (not to exceed 2000 mg) on day 2 and 500 mg/m^2 on day 3 (not to exceed 1000 mg)

Disclaimer: I have reconstructed all tabular and graphical information directly from the sponsor's paper submission unless otherwise specified. The original reports for many of the studies of efficacy in animals did not contain the line-listed data for the individual animals. We asked the sponsor to send this information as SAS transfer files. They complied via email, but as yet, they have not submitted these files officially to the document room. I have used this information in my review. We have requested the sponsor to submit the files to the document room. They have agreed to do so.

I calculated the percentage differences in physiological parameters in tables as:

(value in exposed animal – value in control) ÷ (value in control)

I have used some information from the reviews of NDA 20212 by Dr. Wendy Schmidt and Dr. A. W. Coulter (April 1992) in my summaries. I scanned Dr. Schmidt's and Dr. Coulter's reviews into PDF files and will upload them into DFS under the current NDA and NDA 22-212 for future referral.

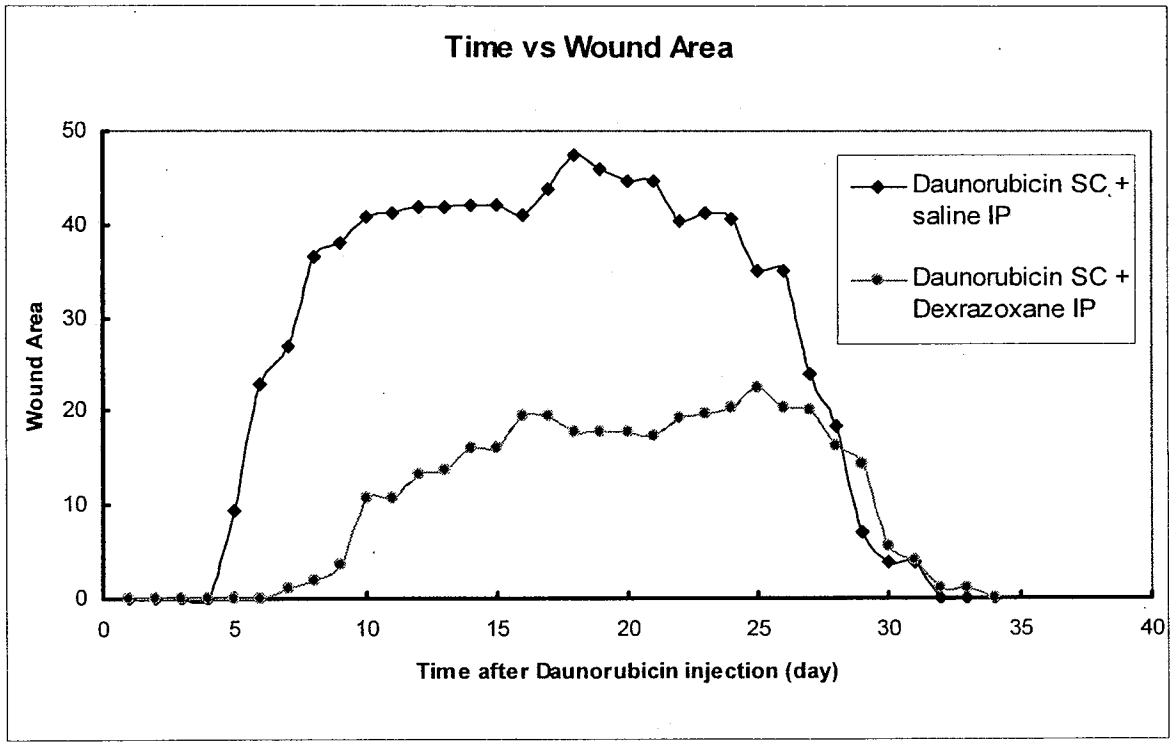
Pharmacology

Pharmacology summary

When an investigator injects a single dose of an anthracycline such as doxorubicin or daunorubicin under the skin of a mouse, a wound usually develops in the overlying skin and in the underlying tissue over the course of four to five days. The wound forms an eschar and heals over the course of 20 to 40 days depending on its size. The initial injection of anthracycline is painful, so in all the studies of dexrazoxane efficacy the investigators anesthetized the mice before dosing. A dose of 3 mg/kg (9 mg/m^2) of daunorubicin usually causes a wound with a surface area of 110 mm^2 or less. Larger doses caused excessive mortality and morbidity. In the numerous non-clinical experiments with mice that the sponsor did to establish the pharmacological activity of dexrazoxane, the investigators measured the size of the wounds that formed after anthracycline dosing in two directions. They multiplied these measurements to obtain an approximate wound surface area. They measured the wounds daily to form a graph of wound area verses time, a graph that demonstrates wound formation and resolution. By adding all the daily wound areas or by using the trapezoidal method they obtained an area under the wound-area verses time curve, wound AUC. They used this AUC metric as a measure of dexrazoxane efficacy, under the assumption that diminished AUC compared to controls demonstrates a treatment effect. They also compared the number of mice that formed wounds in the treatment group relative to controls as an indicator of efficacy. The size of the wound increases with increasing anthracycline dose. A dose of 3 mg/kg of daunorubicin results in

wound AUC values of about 1200 mm^2*day while a dose of 1 mg/kg results in wound AUC values about one third as large, 426 mm^2*day.

The following graph is an example of a typical time verses wound area plot. In this case as in most, the daunorubicin dose (3 mg/kg) was given SC and the dexrazoxane dose (250 mg/kg) or saline control was given IP. The points represent the means of the wound area for seven mice under each experimental condition (experiment SL064).



In this experiment, a single dose of 250 mg/kg dexrazoxane given IP immediately after a dose of 3 mg/kg daunorubicin SC decreased the wound area AUC from 1050 mm^2*day in controls to 433 mm^2*day in treated animals. This dose did not decrease wound incidence. A higher single IP dose of dexrazoxane (375 mg/kg) in combination with daunorubicin was lethal to three of seven mice. Thus, 250 mg/kg dexrazoxane was an MTD in these experiments. When investigators gave a dose of 250 mg/kg of dexrazoxane three hours after the anthracycline dose, it also decreases wound severity but not as much as the dose given immediately after the daunorubicin. In this experiment (SL077), dexrazoxane at t=0 decreased the AUC to 26% of that seen in saline controls while at t=3 hr it decreased it to 30% of control. But, the same dose given 6 hours after daunorubicin dosing caused a decrease in wound AUC to only 66% that of saline control and the difference was not statistically significant. Treatment four or more days after the insult provided no protection at all (SL238). Thus, dexrazoxane efficacy diminishes rapidly as the time after the anthracycline insult increases.

When investigators injected 30, 100 or 250 mg/kg of dexrazoxane directly into the same subcutaneous site as the daunorubicin immediately after the anthracycline challenge the results were no better than when they gave the drug systemically at a dose of 250 mg/kg IP (SL087 and SL223). IV administration of the dexrazoxane dose (250 mg/kg) actually resulted in larger wound AUC values than those obtained after IP injection of the same dose though the difference did not achieve statistical significance (SL159). With daunorubicin (SL173 and SL185),

treatment with 62.5, 125 or 250 mg/kg at t = 0, 3, and 6 hours (three doses) was statistically no better than a single dose of 250 mg/kg at t = 0, but in other experiments this regimen was frequently superior. Thus, these individual experiments with daunorubicin showed that multiple doses were usually no more effective than a single dose immediately after the toxic insult.

A single dose of 62.5 mg/kg of dexrazoxane IP given immediately after 3 mg/kg of doxorubicin provided the same protection as 125 mg/kg and 250 mg/kg given at t = 0 (SL174). A single dose of 125 mg/kg dexrazoxane given IP immediately after a dose of 2 mg/kg doxorubicin SC decreased wound incidence from 6 of 7 in the control group to 1 of 7 in the treated group (SL069). None of the animals given a single IP dose of 250 or 375 mg/kg of dexrazoxane (6 of 6 and 7 of 7 respectively) developed lesions. In this case, logistic regression analysis demonstrated some evidence of a dose response. Doxorubicin is a less potent vesicant than daunorubicin, consistently forming smaller and fewer lesions at an equivalent dose on a mg/kg basis. Dexrazoxane was consistently more effective at preventing or ameliorating wound formation by doxorubicin when compared to daunorubicin. Nevertheless, none of the mice treated with dexrazoxane (62.5 mg/kg q3hX3, total dose 187.5 mg/kg) after a dose of 3 mg/kg of daunorubicin or doxorubicin developed skin lesions (SL210). This regimen usually produced the best results. An ice pack placed over the SC injection site did not improve the results (SL207). Three doses of 62.5 mg/kg given at t=0, 3 and 6 hours of Zinecard and Cardioxane provided statistically equivalent protection against the formation of a skin wound after a single SC doses of daunorubicin or doxorubicin (3 mg/kg) (AT054, AT055 and SL248). The two commercial formulations of dexrazoxane are pharmacologically equivalent.

A dose of 0.05 mg/kg of idarubicin SC caused little wound formation. A dose of 0.25 mg/kg caused wounds in 4 of 9 mice treated with saline but only 1 of 9 mice treated with dexrazoxane (SL099). A dose of 0.75 mg/kg caused wounds in 9 of 9 mice treated with saline, but only 2 of 9 mice treated with dexrazoxane. The mean wound AUC in mice treated with saline was 419 ± 209 mm^2*day while only 119 ± 4.2 in mice treated with dexrazoxane. Thus, dexrazoxane diminishes wound formation caused by idarubicin in mice. The investigators do not state why they used doses of idarubicin so much lower than the doses of daunorubicin or doxorubicin they used in other experiments. The size of the wounds formed after a dose of 0.75 mg/kg idarubicin is larger that those that formed after a dose of 1 mg/kg daunorubicin. This suggests that idarubicin may be a more potent vesicant than daunorubicin on a mg/kg basis.

Epirubicin did not produce skin wounds as consistently as daunorubicin or doxorubicin nor were the wounds as severe as measured by wound AUC (about 1200 mm^2*days for daunorubicin and about 470 mm^2*days for epirubicin, both 3 mg/kg). IP treatment with dexrazoxane had no effect on the formation of wounds caused by epirubicin (SL237). A relatively high dose of 9 mg/kg of epirubicin consistently caused skin lesions. Treatment with single or repeat doses of dexrazoxane did not prevent the formation of wounds but the severity of the wounds decreased with dose and dose intensity as measured by AUC (SL246). Neither aclarubicin nor etoposide consistently caused skin wounds (SL114). Wounds formed in animals injected with 2.5 mg/kg mitoxantrone SC were clearly smaller in mice treated with dexrazoxane (62.5 mg/kg q3hX3) than in controls. The AUC in the controls was 1319 ± 427 verses 440 ± 318 mm^2*day in the treated animals. Mitoxantrone produced wounds more consistently than daunorubicin, suggesting that it is a more potent vesicant (SL249). A dose of 5 mg/kg of mitoxantrone produced wound AUCs almost twice as large as those caused by 2.5 mg/kg. Treatment with dexrazoxane diminished the mean wound AUC by half. While there is a clear treatment difference, this difference did not reach statistical significance due to the experimental variability (p = 0.06).

Hydrogen peroxide hydrolyzes to form active oxygen radicals that are severely destructive to tissue. To investigate further the mechanism of wound formation, investigators challenged mice with subcutaneous injections of compounds other than anthracyclines (SL114).

An SC dose of 0.05 mL of 30% hydrogen peroxide caused severe irritation in mice necessitating their destruction. An SC dose of 0.05 mL of 10% hydrogen peroxide caused skin lesions in four of four mice treated. The mean size of these wounds was about half that caused by a 3 mg/kg SC dose of daunorubicin. Dexrazoxane treatment had no effect on wound formation by hydrogen peroxide. This suggests that dexrazoxane does not scavenge oxygen radicals.

The sponsor has suggested that dexrazoxane may act by chelating iron ions released from cells after an anthracycline insult. They further suggest that these iron ions catalyze the formation of oxygen radicals, which would be the proximate cause of the tissue damage. EDTA chelates iron and other metal cations. In experiment SL193, investigators determined that EDTA given IP had no effect on wound formation after a single SC dose of daunorubicin. The experiment suggests that either EDTA does not act to chelate the available iron, perhaps because of its high water solubility, or that release of iron ions may not be the ultimate cause of progressive anthracycline damage. α-Tocopherol, amifostine and N-acetylcysteine are radical scavengers. None of these drugs given IP had an effect on wound formation after a single SC dose of daunorubicin (SL193 and SL198). These experiments all suggest that radicals do not mediate anthracycline damage. Indeed, EDTA or N-acetylcysteine injected SC at the wound site exacerbated wound formation (SL223).

Merbarone is a topoisomerase 2-α inhibitor. Investigators determined that this drug given IP had no effect on wound formation after a single SC dose of daunorubicin. The experiment suggests that inhibition of topoisomerase 2-α may not be the mechanism of action of dexrazoxane. Or, the dose of Merbarone may simply have been too low. It is also possible that dexrazoxane acts at a different site on topoisomerase from that of merbarone.

ADR-925, the major double ring-opened metabolite of dexrazoxane, neither caused skin lesions when injected subcutaneously nor prevented wounds induced by a subcutaneous injection of daunorubicin. Thus, the metabolite is probably not responsible for prevention of daunorubicin skin damage (SL224).

Pharmacology Review

Comment on the statistical analyses

In the analysis of the AUC values in all the following experiments, the sponsor calculated the mean values across treatment groups by excluding zero values, that is, they censored animals with no measurable wound. I consulted with Dr. Rajeshwari Sridhara, the supervisory statistician on this application, about the inclusion of the zero values in the calculation of means and she said the zero values should be included. I have done so in all calculations of means across treatment groups. Thus, the means I report in my statistical analysis using JMP, Microsoft Excel or GraphPad Prism are usually smaller than the means reported by the sponsor because the denominator is larger. In most of my ANOVA calculations below, I have treated wound AUC as a continuous variable and Dose as ordinal, but this is probably not strictly correct. Wound AUC is probably not a true continuous variable since there is a clear threshold for the formation of wounds. Healing processes confound a truly continuous response when it prevents underlying tissue damage from manifesting as a surface wound. Only some measure of the damage beneath the skin could be truly continuous. Nevertheless, I have included ANOVA of parametric means in my reviews because I think they aid in the understanding in the differences among treatments. Non-parametric means calculated by Kruskal-Wallis analysis suffer the same limitation but again are useful for the demonstration of treatment differences. In both cases, the reader should

interpret the calculated p values with these limitations in mind. I have also analyzed differences in wound incidence with Fisher's exact test. This test does not suffer the limitation of the threshold of wound formation; indeed, it is only possible because of it.

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1) Evaluation of the protection by a single dose of 250 or 375 mg/kg dexrazoxane against 3 mg/kg daunorubicin-induced skin necrosis in mice.

Major findings

A single dose of 250 mg/kg dexrazoxane given IP immediately after a dose of 3 mg/kg daunorubicin SC decreased the wound area AUC from 1050 mm^2*day in controls to 433 mm^2*day. This dose did not decrease wound incidence. A single IP dose of 375 mg/kg of dexrazoxane in combination with daunorubicin was lethal to three of seven mice. The sample size was too small to demonstrate a dose response but the control mean was different from the treated means with a p-value of 0.013 (ANOVA).

Study number	SL064, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	August 15, 1998
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam

Methods

Doses	See table below
Species	Female B6D2F1 mice
Number	7 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam All mice were injected at t = 0, SC with daunorubicin 3 mg/kg

Group	Skin wound induction	Treatment to prevent wound formation IP at t = 0
1	Daunorubicin 3 mg/kg SC	Isotonic saline
2	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane
3	Daunorubicin 3 mg/kg SC	375 mg/kg Dexrazoxane

Formulation	Isotonic saline
Methods	Mice were examined for skin wounds for 33 days The investigators plotted study day against wound size and calculated the area under this curve (AUC) The sponsor did statistical comparisons using Student's T-test. This is the value in the table below.

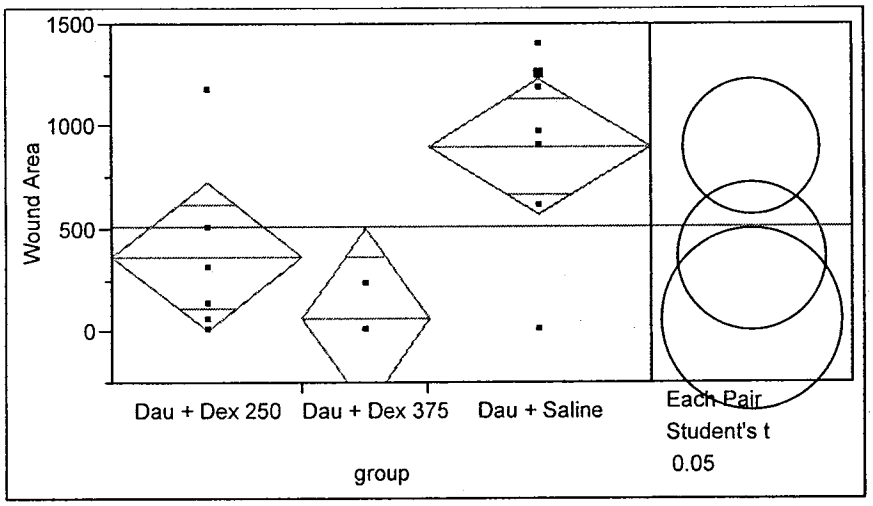
Results

One mouse in the group treated with 250 mg/kg dexrazoxane "died on day 4 of an unknown reason". Two mice in the group treated with 375 mg/kg dexrazoxane died on day 4 and another died on day 5 apparently from drug toxicity.

Group	Skin wound induction	Treatment to prevent wound formation IP at t = 0	Mean AUC mm^2*day	N Dead	P value compared to control	N without lesions	N with lesions
1	Daunorubicin 3 mg/kg SC	Isotonic saline	1050 ± 282	0		1	6
2	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane	433 ± 449	1	0.026	1	5
3	Daunorubicin 3 mg/kg SC	375 mg/kg Dexrazoxane	276	3		3	1

I analyzed the wound area as a function of dexrazoxane dose using JMP and GraphPad Prism. I used Student's t-test and the Kruskal-Wallis nonparametric test. The data was inadequate to demonstrate dose response by modeling.

One-way Analysis of Wound Area By group



Missing Rows
4 Excluded Rows
28

One-way Anova
Summary of Fit

R square	0.463031
Adjusted R square	0.386321
Root Mean Square Error	409.0946
Mean of Response	511.4118
Observations (or Sum Weights)	17

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Probability > F
group	2	2020394.5	1010197	6.0361	0.0129
Error	14	2343017.6	167358		
C. Total	16	4363412.1			

Means for One-way Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Dau + Dex 250	6	361.000	167.01	2.8	719.2
Dau + Dex 375	4	56.750	204.55	-382.0	495.5
Dau + Saline	7	900.143	154.62	568.5	1231.8

Std Error uses a pooled estimate of error variance

Means Comparisons
Comparisons for each pair using Student's t

t	Alpha
2.14479	0.05

Abs(Dif)-LSD	Dau + Saline	Dau + Dex 250	Dau + Dex 375
Dau + Saline	-469.00	50.99	293.44
Dau + Dex 250	50.99	-506.58	-262.12
Dau + Dex 375	293.44	-262.12	-620.43

Positive values show pairs of means that are significantly different.

Level			Mean
Dau + Saline	A		900.14286
Dau + Dex 250		B	361.00000
Dau + Dex 375		B	56.75000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	p-Value	Difference
Dau + Saline	Dau + Dex 375	843.3929	293.440	1393.345	0.0054	
Dau + Saline	Dau + Dex 250	539.1429	50.991	1027.295	0.0328	
Dau + Dex 250	Dau + Dex 375	304.2500	-262.123	870.623	0.2686	

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
Dau + Dex 250	6	49	8.1667	-0.458
Dau + Dex 375	4	17	4.2500	-2.121
Dau + Saline	7	87	12.4286	2.322

1-way Test, ChiSquare Approximation

Chi Square	DF	Probability >ChiSq
7.1036	2	0.0287

Small sample sizes. Refer to statistical tables for tests, rather than large-sample approximations.

GraphPad reported identical results from the Kruskal-Wallis test and the following table for Dunn's Multiple Comparison Test (a posttest of significance between groups).

Dunn's Multiple Comparison Test	Difference in rank sum	P value	Summary
Dau + Saline vs Dau + Dex 250	4.3	P > 0.05	ns
Dau + Saline vs Dau + Dex 375	8.2	P < 0.05	*
Dau + Dex 250 vs Dau + Dex 375	3.9	P > 0.05	ns

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2) Evaluation of the protection by a single dose of 125, 250 and 375 mg/kg dexrazoxane against 2 mg/kg Doxorubicin-induced skin necrosis in mice.

Major findings

A single dose of 125 mg/kg dexrazoxane given IP immediately after a dose of 2 mg/kg doxorubicin SC decreased wound incidence from 6 of 7 in the control group to 1 of 7 in the treated group. None of the animals given a single IP dose of 250 or 375 mg/kg of dexrazoxane (6 of 6 and 7 of 7 respectively) developed lesions. Logistic regression analysis demonstrated a dose response. ANOVA and Kruskal-Wallis analysis demonstrated that the mean value of the wound area was significantly different from the means of the treated groups.

Study number	SL069, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	October 7, 1998
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam

Methods

Doses	See table below
Species	Female B6D2F1 mice
Number	7 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam All mice were injected at t = 0, SC with daunorubicin 3 mg/kg

Group	Skin wound induction	Treatment to prevent wound formation IP at t = 0
1	Doxorubicin 2 mg/kg SC	Isotonic saline
2	Doxorubicin 2 mg/kg SC	125 mg/kg Dexrazoxane
3	Doxorubicin 2 mg/kg SC	250 mg/kg Dexrazoxane
4	Doxorubicin 2 mg/kg SC	375 mg/kg Dexrazoxane

Formulation	Isotonic saline
Methods	Mice were examined for skin wounds for 33 days The investigators plotted study day against wound size and calculated the area under this curve (AUC) I did the statistical comparisons of the number of animals with and without lesions using Fishers exact test (two tails).

Results

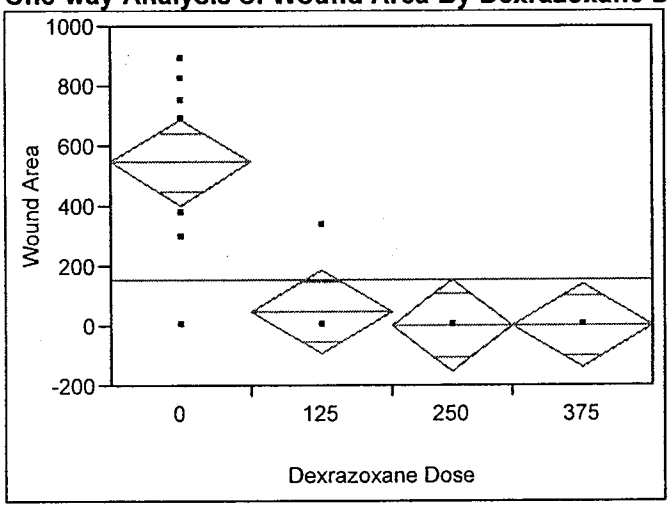
One mouse treated with 250 mg/kg dexrazoxane died during anesthesia "most likely due to hypothermia".

Group	Skin wound induction	Treatment to prevent wound formation IP at t = 0	Mean AUC mm^2*day	N Dead	P value compared to control	N without lesions	N with lesions
1	Doxorubicin 2 mg/kg SC	Isotonic saline	634 ± 244	0		1	6
2	Doxorubicin 2 mg/kg SC	125 mg/kg Dexrazoxane	334	0	0.029	6	1
3	Doxorubicin 2 mg/kg SC	250 mg/kg Dexrazoxane		1	0.0046	6	0
4	Doxorubicin 2 mg/kg SC	375 mg/kg Dexrazoxane		0	0.0046	7	0

The sponsor reported a p value of < 0.0001 by Fisher's exact test

I analyzed the data in JMP and wound incidence demonstrated a dose response by logistic regression ($p < 0.0001$, analysis not shown). Analysis of variance demonstrated that the mean of the control values was significantly different from the means of the treatment groups ($p < 0.0001$). Non-parametric analysis by Kruskal-Wallis also demonstrated a significant difference ($p < 0.0004$)

One-way Analysis of Wound Area By Dexrazoxane Dose



Missing Rows
1

One-way Anova
Summary of Fit

Rsquare	0.66216
Adj Rsquare	0.618094
Root Mean Square Error	179.282
Mean of Response	153.2593
Observations (or Sum Wgts)	27

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Dexrazoxane Dose	3	1448952.0	482984	15.0265	<.0001
Error	23	739267.1	32142		
C. Total	26	2188219.2			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
0	7	543.429	67.762	403.3	683.61
125	7	47.714	67.762	-92.5	187.89
250	6	0.000	73.192	-151.4	151.41
375	7	0.000	67.762	-140.2	140.18

Std Error uses a pooled estimate of error variance

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
0	7	156.500	22.3571	**4.164**
125	7	85.000	12.1429	-0.897
250	6	63.000	10.5000	-1.551
375	7	73.500	10.5000	-1.723

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
17.9645	3	**0.0004**

Dunn's Multiple Comparison Test	Difference in rank sum	P value	Summary
Dox 2 vs Dox 2 + Dex 125	10	P < 0.05	*
Dox 2 vs Dox 2 + Dex 250	12	P < 0.01	**
Dox 2 vs Dox 2 + Dex 375	12	P < 0.01	**
Dox 2 + Dex 125 vs Dox 2 + Dex 250	1.6	P > 0.05	ns
Dox 2 + Dex 125 vs Dox 2 + Dex 375	1.6	P > 0.05	ns
Dox 2 + Dex 250 vs Dox 2 + Dex 375	0	P > 0.05	ns

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3) Evaluation of the timing of a single dose of 250 mg/kg dexrazoxane in the protection against 3 mg/kg daunorubicin-induced skin wounds in mice.

Major findings

A single dose of 250 mg/kg dexrazoxane given IP immediately after a dose of 3 mg/kg daunorubicin SC did not decrease wound incidence compared to control but it did decrease the mean area of the wounds from 1397 mm^2*day in control to 326 mm^2*day. The wound area in animals given dexrazoxane three hours after the daunorubicin challenge was also less than control but the wound area in animals given dexrazoxane six hours after the daunorubicin challenge was statistically equal to that of the controls.

Study number	SL077, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	October 27, 1998
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam

Methods

Doses	See table below
Species	Female B6D2F1 mice
Number	7 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam All mice were injected at t = 0, SC with daunorubicin 3 mg/kg

Group	Skin wound induction	Treatment to prevent wound formation IP
1	Daunorubicin 3 mg/kg SC	Isotonic saline
2	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane at t=0
3	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane at t=1 hr
4	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane at t=3 hr
5	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane at t=6 hr

Formulation	Isotonic saline
Methods	Mice were examined for skin wounds for 33 days The investigators plotted study day against wound size and calculated the area under this curve (AUC) The sponsor analyzed the data using a Student's t-test between the control and each treatment group. The p values in the following table are those the sponsor calculated.

Results

Group	Skin wound induction	Treatment to prevent wound formation IP	Mean AUC MM^2*day	P value AUC	N without lesions	N with lesions
1	Daunorubicin 3 mg/kg SC	Isotonic saline	1397 ± 474		0	7
2	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane at t=0	326 ± 151	0.0006 vs control	0	7
3	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane at t=1 hr	Not reported [a]			
4	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane at t=3 hr	982 ± 253[b] 421 ± 161[c]	0.03 vs group 2	4	3
5	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane at t=6 hr	926 ± 431	0.02 vs group 2	0	7

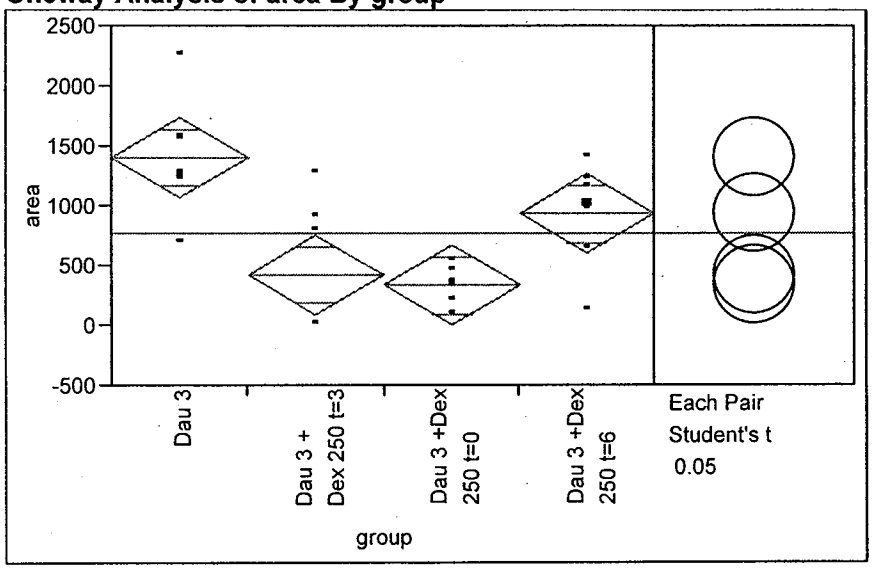
a = the sponsor excluded this group because the Hamilton syringe used for this group was defective resulting in dosing errors

b = reported by the sponsor

c = my calculation with pooled estimate of variance, see below.

I analyzed the data in JMP. One-way ANOVA demonstrated that the wound areas of animals treated three hours after daunorubicin challenge were less than controls. Additionally, Student's t-test and Kruskal-Wallis analysis also demonstrated a difference among the means.

Oneway Analysis of area By group



Oneway Anova
Summary of Fit

Rsquare	0.54
Adj Rsquare	0.48
Root Mean Square Error	427.25
Mean of Response	767.86
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
group	3	5158989.4	1719663	9.4207	0.0003
Error	24	4380990.0	182541		
C. Total	27	9539979.4			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Dau 3	7	1397.57	161.48	1064	1730.9
Dau 3 + Dex 250 t=3	7	420.86	161.48	88	754.1
Dau 3 +Dex 250 t=0	7	326.43	161.48	-6.8596	659.7
Dau 3 +Dex 250 t=6	7	926.57	161.48	593	1259.9

Std Error uses a pooled estimate of error variance

Means Comparisons
Comparisons for each pair using Student's t

t	Alpha
2.06390	0.05

Abs(Dif)-LSD	Dau 3	Dau 3 +Dex 250 t=6	Dau 3 + Dex 250 t=3	Dau 3 +Dex 250 t=0
Dau 3	-471.34	-0.34	505.37	599.80
Dau 3 +Dex 250 t=6	-0.34	-471.34	34.37	128.80
Dau 3 + Dex 250 t=3	505.37	34.37	-471.34	-376.91
Dau 3 +Dex 250 t=0	599.80	128.80	-376.91	-471.34

Positive values show pairs of means that are significantly different.

Level			Mean
Dau 3	A		1397.5714
Dau 3 +Dex 250 t=6	A		926.5714
Dau 3 + Dex 250 t=3		B	420.8571
Dau 3 +Dex 250 t=0		B	326.4286

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	p-Value	Difference
Dau 3	Dau 3 +Dex 250 t=0	1071.143	599.802	1542.483	<.0001	
Dau 3	Dau 3 + Dex 250 t=3	976.714	505.374	1448.055	0.0003	
Dau 3 +Dex 250 t=6	Dau 3 +Dex 250 t=0	600.143	128.802	1071.483	0.0147	
Dau 3 +Dex 250 t=6	Dau 3 + Dex 250 t=3	505.714	34.374	977.055	0.0365	
Dau 3	Dau 3 +Dex 250 t=6	471.000	-0.341	942.341	0.0502	
Dau 3 + Dex 250 t=3	Dau 3 +Dex 250 t=0	94.429	-376.912	565.769	0.6829	

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
Dau 3	7	162.000	23.1429	3.188
Dau 3 + Dex 250 t=3	7	64.000	9.1429	-1.966
Dau 3 +Dex 250 t=0	7	62.000	8.8571	-2.072
Dau 3 +Dex 250 t=6	7	118.000	16.8571	0.850

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
14.6091	3	0.0022

Dunn's Multiple Comparison Test	Difference in rank sum	P value	Summary
Daun 3 vs Daun 3 + Dex 250 t=0	14	P < 0.01	**
Daun 3 vs Daun 3 + Dex 250 t=3	14	P < 0.01	**
Daun 3 vs Daun 3 + Dex 250 t=6	6.3	P > 0.05	ns
Daun 3 + Dex 250 t=0 vs Daun 3 + Dex 250 t=3	-0.29	P > 0.05	ns
Daun 3 + Dex 250 t=0 vs Daun 3 + Dex 250 t=6	-8	P > 0.05	ns
Daun 3 + Dex 250 t=3 vs Daun 3 + Dex 250 t=6	-7.7	P > 0.05	ns

I used JMP to analyze the wound incidence data to determine the effect of the delay in treatment on response. Logistic regression analysis of this data did not demonstrate a clear time effect because of the low sample size.

Linear regression of the Wound AUC versus time after the daunorubicin dose did demonstrate a line with a non-zero slope ($p = 0.014$). While this is not the correct model for this type of data (the curve should be hyperbolic), the analysis does demonstrate that Wound AUC increases as the interval between the dose of daunorubicin and that of dexrazoxane increases.

4) **Evaluation of the effect of pre-treatment with a single dose of dexrazoxane 250 mg/kg intraperitoneally before 1 or 3 mg/kg experimental daunorubicin extravasation in mice.**

Major findings

Wounds that formed after a single SC injection of daunorubicin 3 mg/kg were about three times bigger than those that formed after a dose of 1 mg/kg in animals given IP saline. A dose of 250 mg/kg of dexrazoxane given immediately before a 3 mg/kg dose of daunorubicin did not provide statistically significant protection against wound formation (incidence) but it did significantly decrease wound area. This dose completely prevented wound formation after a 1 mg/kg dose of daunorubicin.

Study number	SL081, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	September 18, 1998
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam

Methods

Doses	See table below
Species	Female B6D2F1 mice
Number	7 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam All mice were injected at t = 0 with either isotonic saline or dexrazoxane 250 mg/kg. They were thin injected with either 1 or 3 mg/kg daunorubicin SC evidently immediately after the first injection.

Group	First treatment	Second treatment
1	Isotonic saline	Daunorubicin 3 mg/kg SC
2	Isotonic saline	Daunorubicin 1 mg/kg SC
3	250 mg/kg Dexrazoxane IP	Daunorubicin 3 mg/kg SC
4	250 mg/kg Dexrazoxane IP	Daunorubicin 1 mg/kg SC

Formulation	Isotonic saline
Methods	Mice were examined for skin wounds for 38 days The investigators plotted study day against wound size and calculated the area under this curve (AUC) The sponsor analyzed the AUC data using Student's t-test. The p values in the following table are those the sponsor calculated. I calculated the p-values for lesion incidence using Fisher's exact test

Results

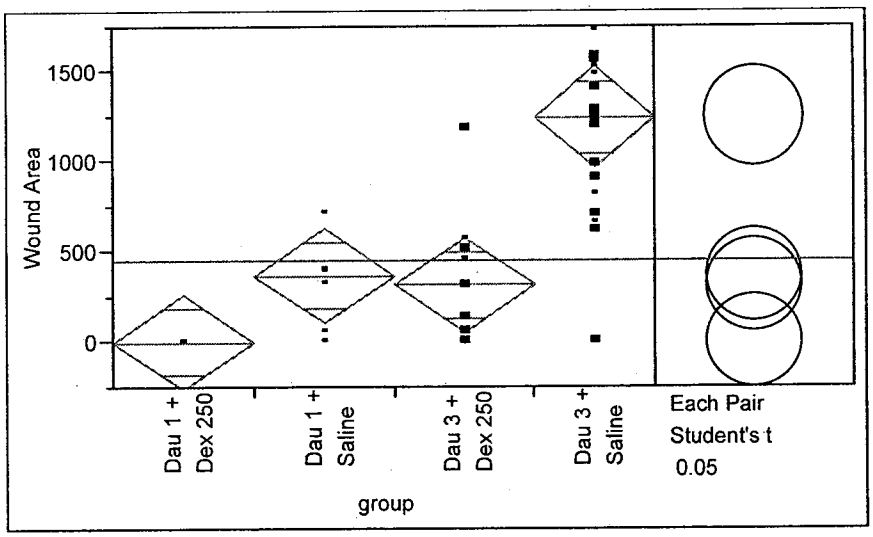
One animal in group-1 died without developing a wound. The sponsor reported no other information on this animal.

Group	Skin wound induction	Treatment to prevent wound formation IP at t = 0	Mean AUC MM^2*day	N Dead	P value Student's t-test	P value Fishers [a]	N without lesions	N with lesions
1	Daunorubicin 3 mg/kg SC	Isotonic saline	1239 ± 424	1			0	6
2	Daunorubicin 1 mg/kg SC	Isotonic saline	426 ± 274				1	6
3	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP	731 ± 391		0.56 [b]	0.07	4	3
4	Daunorubicin 1 mg/kg SC	250 mg/kg Dexrazoxane IP				0.005	7	0

a = Lesion formation compared to relevant control. The sponsor reported p = 0.003 for both groups.
b = AUC values compared to relevant control

I analyzed the data by ANOVA, Student's t-test and Kruskal-Wallis tests using JMP and GraphPad Prism and obtained the following results. All these tests demonstrated differences among the Wound area means for the various treatment groups.

One-way Analysis of Wound Area By group



Missing Rows
1Excluded Rows
49

One-way Anova
Summary of Fit

Rsquare	0.673754
Adj Rsquare	0.6312
Root Mean Square Error	335.2442
Mean of Response	451.3333
Observations (or Sum Wgts)	27

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
group	3	5338347.9	1779449	15.8330	<.0001
Error	23	2584940.1	112389		
C. Total	26	7923288.0			

Means for One-way Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Dau 1 + Dex 250	7	0.00	126.71	-262.1	262.1
Dau 1 + Saline	7	364.86	126.71	102.7	627.0
Dau 3 + Dex 250	7	313.57	126.71	51.5	575.7
Dau 3 + Saline	6	1239.50	136.86	956.4	1522.6

Std Error uses a pooled estimate of error variance

Means Comparisons
Comparisons for each pair using Student's t

t	Alpha
2.06866	0.05

Abs(Dif)-LSD	Dau 3 + Saline	Dau 1 + Saline	Dau 3 + Dex 250	Dau 1 + Dex 250
Dau 3 + Saline	-400.40	488.81	540.10	853.67
Dau 1 + Saline	488.81	-370.69	-319.41	-5.84
Dau 3 + Dex 250	540.10	-319.41	-370.69	-57.12
Dau 1 + Dex 250	853.67	-5.84	-57.12	-370.69

Positive values show pairs of means that are significantly different.

Level			Mean
Dau 3 + Saline	A		1239.5000
Dau 1 + Saline		B	364.8571
Dau 3 + Dex 250		B	313.5714
Dau 1 + Dex 250		B	0.0000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	p-Value	Difference
Dau 3 + Saline	Dau 1 + Dex 250	1239.500	853.669	1625.331	<.0001	
Dau 3 + Saline	Dau 3 + Dex 250	925.929	540.098	1311.759	<.0001	
Dau 3 + Saline	Dau 1 + Saline	874.643	488.812	1260.474	0.0001	
Dau 1 + Saline	Dau 1 + Dex 250	364.857	-5.837	735.551	0.0534	
Dau 3 + Dex 250	Dau 1 + Dex 250	313.571	-57.123	684.266	0.0935	
Dau 1 + Saline	Dau 3 + Dex 250	51.286	-319.409	421.980	0.7773	

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
Dau 1 + Dex 250	7	45.500	6.5000	-3.012
Dau 1 + Saline	7	105.500	15.0714	0.405
Dau 3 + Dex 250	7	84.000	12.0000	-0.782
Dau 3 + Saline	6	143.000	23.8333	3.572

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq

17.5703	3	0.0005

Dunn's Multiple Comparison Test	Difference in rank sum	P value	Summary
Dau 3 + Saline vs Dau 1 + Saline	8.8	P > 0.05	ns
Dau 3 + Saline vs Dau 3 + Dex 250	12	P < 0.05	*
Dau 3 + Saline vs Dau 1 + Dex 250	17	P < 0.001	***
Dau 1 + Saline vs Dau 3 + Dex 250	3.1	P > 0.05	ns
Dau 1 + Saline vs Dau 1 + Dex 250	8.6	P > 0.05	ns
Dau 3 + Dex 250 vs Dau 1 + Dex 250	5.5	P > 0.05	ns

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5) Evaluation of the effect of intralesional 100 mg/kg dexrazoxane injection and comparison with systemic 250 mg/kg dexrazoxane treatment on 3 mg/kg daunorubicin-induced skin wounds in mice.

Major findings

Dexrazoxane given directly into the lesion (100 mg/kg) after a dose of daunorubicin (3 mg/kg) decreased the mean wound size relative to control but this treatment was no better than giving 250 mg/kg dexrazoxane IP.

Study number	SL087, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	November 18, 1998
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam
Methods	
Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam All mice were injected at t = 0 with 3 mg/kg daunorubicin SC. They were then injected with either saline IL (intralesionally) or IP and with dexrazoxane according to the following table.

Group	First treatment (wound induction)	Second treatment
1	Daunorubicin 3 mg/kg SC	0.2 mL isotonic saline IP
2	Daunorubicin 3 mg/kg SC	0.05 mL isotonic saline IL 0.2 mL isotonic saline IP
3	Daunorubicin 3 mg/kg SC	100 mg/kg Dexrazoxane IL (0.05 mL) 0.2 mL isotonic saline IP
4	Daunorubicin 3 mg/kg SC	0.05 mL isotonic saline IL 250 mg/kg Dexrazoxane IP

Formulation Isotonic saline

Methods Mice were examined for skin wounds for 38 days
The investigators plotted study day against wound size and calculated the area under this curve (AUC)
The sponsor analyzed the AUC data using Student's t-test. I calculated the p-values for lesion incidence using Fisher's exact test.

Results

Group	Skin wound induction	Treatment to prevent wound formation at t = 0	Mean AUC mm^2*day	P value	N without lesions	N with lesions
1	Daunorubicin 3 mg/kg SC	0.2 mL isotonic saline IP	1131 ± 477		0	9
2	Daunorubicin 3 mg/kg SC	0.05 mL isotonic saline IL 0.2 mL isotonic saline IP	1290 ± 418	0.53 [a]	0	9
3	Daunorubicin 3 mg/kg SC	100 mg/kg Dexrazoxane IL (0.05 mL) 0.2 mL isotonic saline IP	518 ± 530	0.47 [b]	2	7
4	Daunorubicin 3 mg/kg SC	0.05 mL isotonic saline IL 250 mg/kg Dexrazoxane IP	631 ± 282	1 [b] 0.03 [a]	1	8

a Student's t-test relative to Group 1 (sponsor's)
b Fishers exact test on lesion formation relative to G2

I analyzed the data by ANOVA, Student's t-test and the Kruskal-Wallis test (with Dunn's post-test) using JMP and GraphPad Prism.

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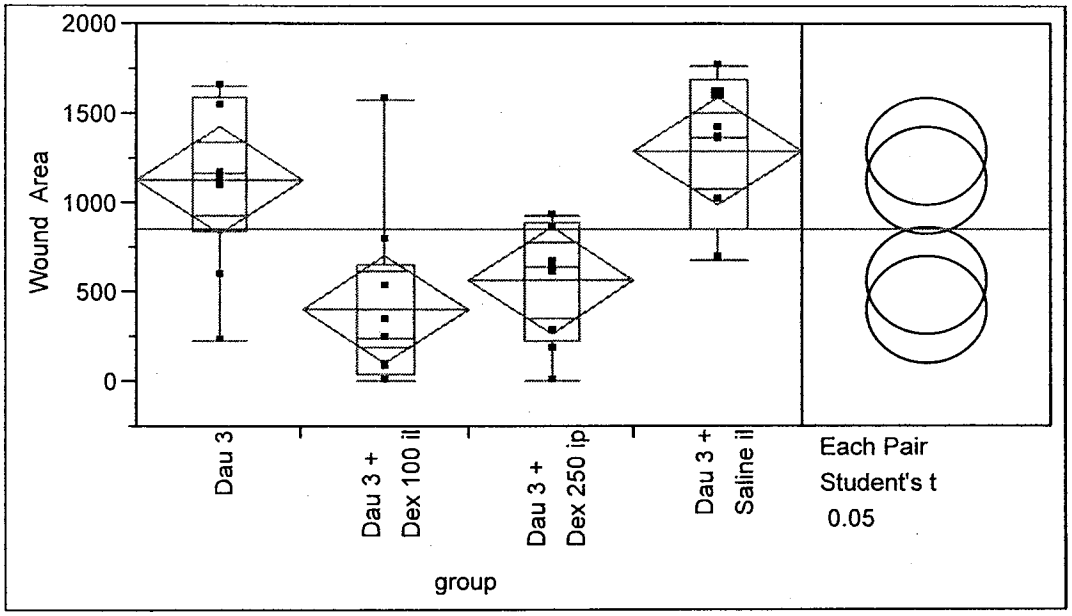
One-way Analysis of Wound Area By group



One-way Anova
Summary of Fit

Rsquare	0.44467
Adj Rsquare	0.392608
Root Mean Square Error	441.6071
Mean of Response	846.25
Observations (or Sum Wgts)	36

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
group	3	4997001	1665667	8.5411	0.0003
Error	32	6240537	195017		
C. Total	35	11237539			

Means for One-way Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Dau 3	9	1131.00	147.20	831.16	1430.8
Dau 3 + Dex 100 il	9	402.89	147.20	103.05	702.7
Dau 3 + Dex 250 ip	9	561.56	147.20	261.71	861.4
Dau 3 + Saline il	9	1289.56	147.20	989.71	1589.4

Std Error uses a pooled estimate of error variance

Means Comparisons
Comparisons for each pair using Student's t

t	Alpha
2.03693	0.05

Abs(Dif)-LSD	Dau 3 + Saline il	Dau 3	Dau 3 + Dex 250 ip	Dau 3 + Dex 100 il
Dau 3 + Saline il	-424.04	-265.48	303.96	462.63
Dau 3	-265.48	-424.04	145.40	304.07
Dau 3 + Dex 250 ip	303.96	145.40	-424.04	-265.37
Dau 3 + Dex 100 il	462.63	304.07	-265.37	-424.04

Positive values show pairs of means that are significantly different.

Level			Mean
Dau 3 + Saline il	A		1289
Dau 3	A		1131
Dau 3 + Dex 250 ip		B	561
Dau 3 + Dex 100 il		B	402

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	p-Value	Difference
Dau 3 + Saline il	Dau 3 + Dex 100 il	886.6667	462.627	1310.706	0.0002	
Dau 3	Dau 3 + Dex 100 il	728.1111	304.071	1152.151	0.0014	
Dau 3 + Saline il	Dau 3 + Dex 250 ip	728.0000	303.960	1152.040	0.0014	
Dau 3	Dau 3 + Dex 250 ip	569.4444	145.405	993.484	0.0101	
Dau 3 + Dex 250 ip	Dau 3 + Dex 100 il	158.6667	-265.373	582.706	0.4515	
Dau 3 + Saline il	Dau 3	158.5556	-265.484	582.595	0.4518	

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
Dau 3	9	214.000	23.7778	1.718
Dau 3 + Dex 100 il	9	91.000	10.1111	-2.741
Dau 3 + Dex 250 ip	9	119.000	13.2222	-1.718
Dau 3 + Saline il	9	242.000	26.8889	2.741

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
15.9392	3	0.0012

Dunn's Multiple Comparison Test	Difference in rank sum	P value	Summary
Dau 3 vs Dau 3 + Saline IL	-3.1	P > 0.05	ns
Dau 3 vs Dau 3 + Dex 100 IL	14	P < 0.05	*
Dau 3 vs Dau 3 + Dex 250 IP	11	P > 0.05	ns
Dau 3 + Saline IL vs Dau 3 + Dex 100 IL	17	P < 0.01	**
Dau 3 + Saline IL vs Dau 3 + Dex 250 IP	14	P < 0.05	*
Dau 3 + Dex 100 IL vs Dau 3 + Dex 250 IP	-3.1	P > 0.05	ns

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6) Evaluation of the effect of pretreatment with a single dose of dexrazoxane 250 mg/kg intraperitoneally before 1 or 3 mg/kg experimental daunorubicin extravasation in mice.

Major findings

The lesions that formed after an SC injection of 1 mg/kg daunorubicin were more than 4 times smaller than those that formed after an injection of 3 mg/kg. Prophylactic dexrazoxane (250 mg/kg IP, immediately before the daunorubicin dose) diminished the size of the wounds that formed after a 3 mg/kg dose of daunorubicin by more than four fold ($p < 0.001$) but did not affect the incidence of wound formation. Prophylactic dexrazoxane (250 mg/kg IP) did not diminish the size of the wounds that formed after an injection of 1 mg/kg daunorubicin but significantly decreased the incidence of wound formation (88% reduced to 22%).

Study number	SL094, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	October 16, 1998
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam

Methods

Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam All mice were injected at t = 0 with isotonic saline or dexrazoxane IP. They were then injected SC with 1 or 3 mg/kg daunorubicin. The investigators did not specify the time between the two injections but it appears the daunorubicin followed immediately after the saline or dexrazoxane.

Group	First treatment	Second treatment
1	Isotonic saline IP	Daunorubicin 3 mg/kg SC
2	Isotonic saline IP	Daunorubicin 1 mg/kg SC
3	250 mg/kg Dexrazoxane IP	Daunorubicin 3 mg/kg SC
4	250 mg/kg Dexrazoxane IP	Daunorubicin 1 mg/kg SC

Formulation	Isotonic saline
Methods	Mice were examined for skin wounds for 35 days The investigators plotted study day against wound size and calculated the area under this curve (AUC) The sponsor analyzed the AUC data using Student's t-test without the 0 values. I calculated the p-values for lesion incidence using Fisher's exact test.

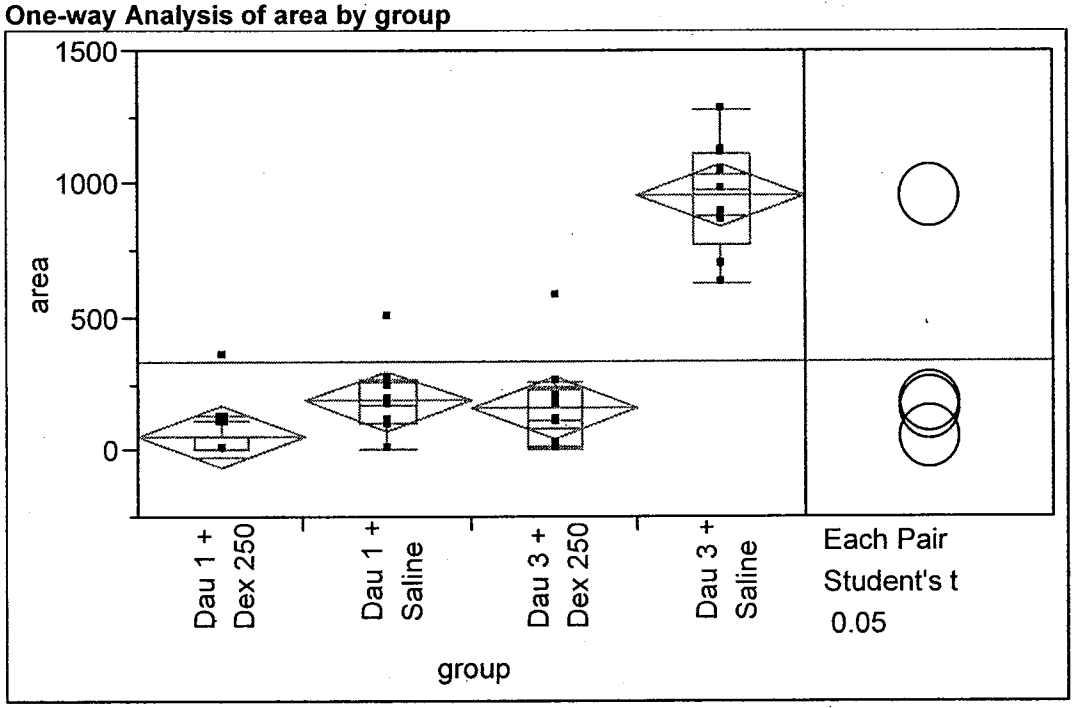
Results

Group	First treatment	Second treatment	Mean AUC MM^2*day	P value	N without lesions	N with lesions
1	Isotonic saline IP	Daunorubicin 3 mg/kg SC	955 ± 211		0	9
2	Isotonic saline IP	Daunorubicin 1 mg/kg SC	207 ± 134		1	8
3	250 mg/kg Dexrazoxane IP	Daunorubicin 3 mg/kg SC	203 ± 180	<0.001 [a] 0.47 [b]	2	7
4	250 mg/kg Dexrazoxane IP	Daunorubicin 1 mg/kg SC	226 ± 171	0.15 [b]	7	2

a Student's t-test for AUC (group 3 to group 1)
b Fisher's exact test for lesion incidence (group 3 to group 1; group 4 to group 2)

I analyzed the data by ANOVA, Student's t-test and the Kruskal-Wallis test (with Dunn's post-test) using JMP and GraphPad Prism.

One-way Analysis of area by group



One-way Anova
Summary of Fit

Rsquare	0.840679
Adj Rsquare	0.825743
Root Mean Square Error	166.448
Mean of Response	337.0556
Observations (or Sum Wgts)	36

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
group	3	4678042.3	1559347	56.2841	<.0001
Error	32	886557.6	27705		
C. Total	35	5564599.9			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Dau 1 + Dex 250	9	50.333	55.483	-62.7	163.3
Dau 1 + Saline	9	184.333	55.483	71.3	297.3
Dau 3 + Dex 250	9	158.222	55.483	45.2	271.2
Dau 3 + Saline	9	955.333	55.483	842.3	1068.3

Std Error uses a pooled estimate of error variance

Means Comparisons
Comparisons for each pair using Student's t

t	Alpha
2.03693	0.05

Abs(Dif)-LSD	Dau 3 + Saline	Dau 1 + Saline	Dau 3 + Dex 250	Dau 1 + Dex 250
Dau 3 + Saline	-159.83	611.17	637.28	745.17
Dau 1 + Saline	611.17	-159.83	-133.72	-25.83
Dau 3 + Dex 250	637.28	-133.72	-159.83	-51.94
Dau 1 + Dex 250	745.17	-25.83	-51.94	-159.83

Positive values show pairs of means that are significantly different.

Level			Mean
Dau 3 + Saline	A		955.3
Dau 1 + Saline		B	184.3
Dau 3 + Dex 250		B	158.2
Dau 1 + Dex 250		B	50.3

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	p-Value	Difference
Dau 3 + Saline	Dau 1 + Dex 250	905.0	745.173	1064.827	<.0001	
Dau 3 + Saline	Dau 3 + Dex 250	797.1	637.285	956.938	<.0001	
Dau 3 + Saline	Dau 1 + Saline	771.0	611.173	930.827	<.0001	
Dau 1 + Saline	Dau 1 + Dex 250	134.0	-25.827	293.827	0.0974	
Dau 3 + Dex 250	Dau 1 + Dex 250	107.9	-51.938	267.715	0.1787	
Dau 1 + Saline	Dau 3 + Dex 250	26.1	-133.715	185.938	0.7415	

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
Dau 1 + Dex 250	9	79.000	8.7778	-3.213
Dau 1 + Saline	9	158.500	17.6111	-0.277
Dau 3 + Dex 250	9	140.500	15.6111	-0.942
Dau 3 + Saline	9	288.000	32.0000	4.468

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
23.6878	3	<.0001

Dunn's Multiple Comparison Test	Difference in rank sum	P value	Summary
Dau 3 + Saline vs Dau 1 + Saline	14	P < 0.05	*
Dau 3 + Saline vs Dau 3 + Dex 250	16	P < 0.01	**
Dau 3 + Saline vs Dau 1 + Dex 250	23	P < 0.001	***
Dau 1 + Saline vs Dau 3 + Dex 250	2	P > 0.05	ns
Dau 1 + Saline vs Dau 1 + Dex 250	8.8	P > 0.05	ns
Dau 3 + Dex 250 vs Dau 1 + Dex 250	6.8	P > 0.05	ns

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7) Evaluation of the effect of a single dose of 250 mg/kg dexrazoxane on skin wounds induced with 0.05, 0.25 or 0.75 mg/kg Idarubicin.

Major findings

A dose of 0.05 mg/kg of Idarubicin SC caused little wound formation. A dose of 0.25 mg/kg caused wounds in 4 of 9 mice treated with saline but only 1 of 9 mice treated with dexrazoxane. A dose of 0.75 mg/kg caused wounds in 9 of 9 mice treated with saline, but only 2 of 9 mice treated with dexrazoxane. Logistic regression analysis and orthogonal regression of the control data both demonstrated that wound formation increased with anthracycline dose. The mean wound area in mice treated with saline was 419 mm^2*day while only 44 mm^2*day in mice treated with dexrazoxane. Dexrazoxane diminishes wound formation caused by Idarubicin.

Study number	SL099, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	November 11, 1998
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam
Methods	
Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam All mice were injected at t = 0 with isotonic saline or dexrazoxane IP. They were then injected SC with doses of Idarubicin.

Group	Wound induction	Treatment
1	Idarubicin 0.05 mg/kg SC	Isotonic saline IP
2	Idarubicin 0.05 mg/kg SC	250 mg/kg Dexrazoxane IP
3	Idarubicin 0.25 mg/kg SC	Isotonic saline IP
4	Idarubicin 0.25 mg/kg SC	250 mg/kg Dexrazoxane IP
5	Idarubicin 0.75 mg/kg SC	Isotonic saline IP
6	Idarubicin 0.75 mg/kg SC	250 mg/kg Dexrazoxane IP

Formulation	Isotonic saline
Methods	Mice were examined for skin wounds for 36 days The investigators plotted study day against wound size and calculated the area under this curve (AUC) The sponsor did not provide AUC values for individual animals. I calculated the p-values for lesion incidence using Fisher's exact test.

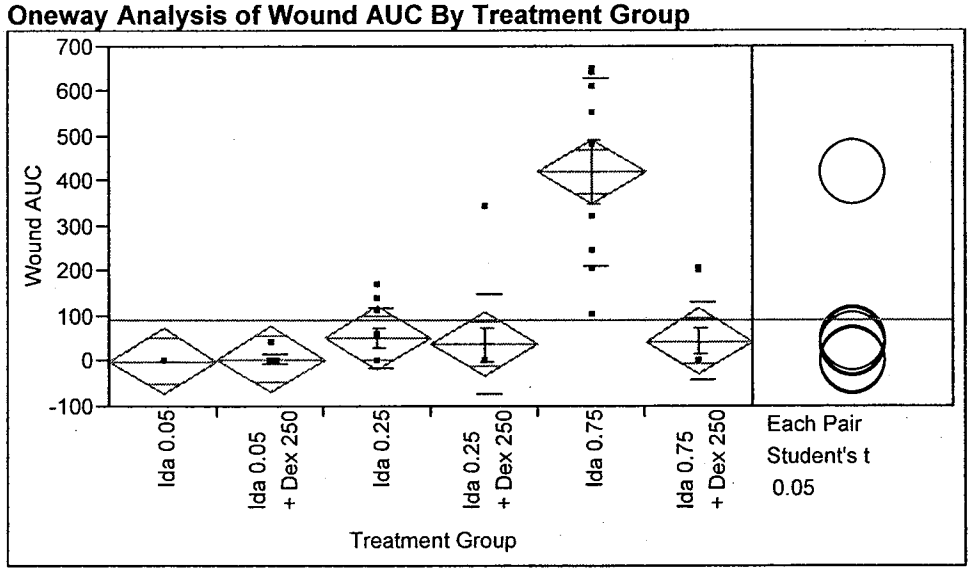
Results

Group	Wound induction SC injection	Treatment	Mean AUC mm^2*day	P value	N without lesions	N with lesions
1	Idarubicin 0.05 mg/kg	Isotonic saline IP	No wounds		9	0
2	Idarubicin 0.05 mg/kg	250 mg/kg Dexrazoxane IP	38		8	1
3	Idarubicin 0.25 mg/kg	Isotonic saline IP	116 ± 47		5	4
4	Idarubicin 0.25 mg/kg	250 mg/kg Dexrazoxane IP	339	0.29 [b]	8	1
5	Idarubicin 0.75 mg/kg	Isotonic saline IP	419 ± 209		0	9
6	Idarubicin 0.75 mg/kg	250 mg/kg Dexrazoxane IP	119 ± 4.2[a]	0.001 [c]	7	2

a The sponsor did not report Student's t-test p values for AUC (group 3 to group 1)
b Fisher's exact test for lesion formation (group 4 to group 3
c Fisher's exact test for lesion formation (group 6 to group 5

I analyzed the sponsor's AUC data and wound incidence using JMP and GraphPad's (Prism) Kruskal-Wallis statistics routine with Dunn's posttest. The analysis demonstrated that there was a significant difference among the means. Dunn's posttest showed that the difference between animals treated with 0.05 mg/kg or 0.25 mg/kg of Idarubicin and those treated with these doses plus dexrazoxane did not reach significance. This is because these low doses of Idarubicin did not cause much wound formation absent dexrazoxane. The 0.75 mg/kg dose of Idarubicin did cause significant wound formation and dexrazoxane provided significant protection against this damage (significant differences highlighted in blue).

Oneway Analysis of Wound AUC By Treatment Group



Oneway Anova
Summary of Fit

Rsquare	0.682689
Adj Rsquare	0.649635
Root Mean Square Error	106.6666
Mean of Response	92.90741
Observations (or Sum Wgts)	54

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment Group	5	1174992.3	234998	20.6542	<.0001
Error	48	546132.2	11378		
C. Total	53	1721124.5			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Ida 0.05	9	0.000	35.556	-71.5	71.49
Ida 0.05 + Dex 250	9	4.222	35.556	-67.3	75.71
Ida 0.25	9	51.444	35.556	-20.0	122.93
Ida 0.25 + Dex 250	9	37.667	35.556	-33.8	109.16
Ida 0.75	9	419.889	35.556	348.4	491.38
Ida 0.75 + Dex 250	9	44.222	35.556	-27.3	115.71

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
Ida 0.05	9	0.000	0.000	0.000	0.0	0.00
Ida 0.05 + Dex 250	9	4.222	12.667	4.222	-5.5	13.96
Ida 0.25	9	51.444	67.434	22.478	-0.3895	103.28
Ida 0.25 + Dex 250	9	37.667	113.000	37.667	-49.2	124.53
Ida 0.75	9	419.889	207.575	69.192	260.3	579.45
Ida 0.75 + Dex 250	9	44.222	87.764	29.255	-23.2	111.68

Means Comparisons
Comparisons for each pair using Student's t

t	Alpha
2.01063	0.05

Abs(Dif)-LSD	Ida 0.75	Ida 0.25	Ida 0.75 + Dex 250	Ida 0.25 + Dex 250	Ida 0.05 + Dex 250	Ida 0.05
Ida 0.75	-101.10	267.34	274.57	281.12	314.57	318.79
Ida 0.25	267.34	-101.10	-93.88	-87.32	-53.88	-49.66
Ida 0.75 + Dex 250	274.57	-93.88	-101.10	-94.55	-61.10	-56.88
Ida 0.25 + Dex 250	281.12	-87.32	-94.55	-101.10	-67.66	-63.43
Ida 0.05 + Dex 250	314.57	-53.88	-61.10	-67.66	-101.10	-96.88
Ida 0.05	318.79	-49.66	-56.88	-63.43	-96.88	-101.10

Positive values show pairs of means that are significantly different.

Level			Mean
Ida 0.75	A		419.88889
Ida 0.25		B	51.44444
Ida 0.75 + Dex 250		B	44.22222
Ida 0.25 + Dex 250		B	37.66667
Ida 0.05 + Dex 250		B	4.22222

Ida 0.05		B	0.00000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	p-Value	Difference
Ida 0.75	Ida 0.05	419.8889	318.788	520.9898	<.0001	
Ida 0.75	Ida 0.05 + Dex 250	415.6667	314.566	516.7676	<.0001	
Ida 0.75	Ida 0.25 + Dex 250	382.2222	281.121	483.3232	<.0001	
Ida 0.75	Ida 0.75 + Dex 250	375.6667	274.566	476.7676	<.0001	
Ida 0.75	Ida 0.25	368.4444	267.344	469.5454	<.0001	
Ida 0.25	Ida 0.05	51.4444	-49.656	152.5454	0.3114	
Ida 0.25	Ida 0.05 + Dex 250	47.2222	-53.879	148.3232	0.3524	
Ida 0.75 + Dex 250	Ida 0.05	44.2222	-56.879	145.3232	0.3835	
Ida 0.75 + Dex 250	Ida 0.05 + Dex 250	40.0000	-61.101	141.1009	0.4302	
Ida 0.25 + Dex 250	Ida 0.05	37.6667	-63.434	138.7676	0.4575	
Ida 0.25 + Dex 250	Ida 0.05 + Dex 250	33.4444	-67.656	134.5454	0.5092	
Ida 0.25	Ida 0.25 + Dex 250	13.7778	-87.323	114.8787	0.7853	
Ida 0.25	Ida 0.75 + Dex 250	7.2222	-93.879	108.3232	0.8864	
Ida 0.75 + Dex 250	Ida 0.25 + Dex 250	6.5556	-94.545	107.6565	0.8968	
Ida 0.05 + Dex 250	Ida 0.05	4.2222	-96.879	105.3232	0.9334	

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
Ida 0.05	9	171.000	19.0000	-2.142
Ida 0.05 + Dex 250	9	190.000	21.1111	-1.606
Ida 0.25	9	260.000	28.8889	0.338
Ida 0.25 + Dex 250	9	201.000	22.3333	-1.296
Ida 0.75	9	441.000	49.0000	5.439
Ida 0.75 + Dex 250	9	222.000	24.6667	-0.704

1-way Test, ChiSquare Approximation

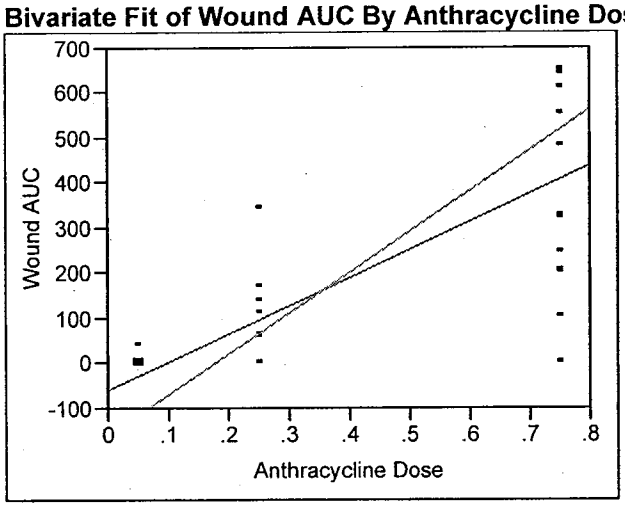
ChiSquare	DF	Prob>ChiSq
32.8006	5	<.0001

APPEARS THIS WAY ON ORIGINAL

Kruskal-Wallis test			
P value	P<0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups		6	
Kruskal-Wallis statistic		33	
Dunn's Multiple Comparison Test	Difference in rank sum	P value	Summary
Ida 0.05 vs Ida 0.05 + Dex	-2.1	P > 0.05	ns
Ida 0.05 vs Ida 0.25	-9.9	P > 0.05	ns
Ida 0.05 vs Ida 0.25 + Dex	-3.3	P > 0.05	ns
Ida 0.05 vs Ida 0.75	-30	P < 0.001	***
Ida 0.05 vs Ida 0.75 + Dex	-5.7	P > 0.05	ns
Ida 0.05 + Dex vs Ida 0.25	-7.8	P > 0.05	ns
Ida 0.05 + Dex vs Ida 0.25 + Dex	-1.2	P > 0.05	ns
Ida 0.05 + Dex vs Ida 0.75	-28	P < 0.001	***
Ida 0.05 + Dex vs Ida 0.75 + Dex	-3.6	P > 0.05	ns
Ida 0.25 vs Ida 0.25 + Dex	6.6	P > 0.05	ns
Ida 0.25 vs Ida 0.75	-20	P < 0.05	*
Ida 0.25 vs Ida 0.75 + Dex	4.2	P > 0.05	ns
Ida 0.25 + Dex vs Ida 0.75	-27	P < 0.001	***
Ida 0.25 + Dex vs Ida 0.75 + Dex	-2.3	P > 0.05	ns
Ida 0.75 vs Ida 0.75 + Dex	24	P < 0.01	**

The following is a liner regression and orthogonal regression analysis of the control data. Wound AUC data from those animals treated only with anthracycline, no dexrazoxane, is regressed to show a dose response. While one would not expect a dose response to be linear, it may be pseudo-linear within a given region. The analysis does demonstrate that Wound AUC increases with idarubicin dose. Curiously, the orthogonal regression predicts what one might interpret as a threshold. This may be a real effect since wound formation does involve a threshold response.

Bivariate Fit of Wound AUC By Anthracycline Dose



Linear Fit

Wound AUC = -62.05769 + 626.19658 Anthracycline Dose

Summary of Fit

RSquare	0.692403
RSquare Adj	0.680099
Root Mean Square Error	127.691
Mean of Response	157.1111
Observations (or Sum Wgts)	27

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	917565.9	917566	56.2751
Error	25	407624.8	16305	Prob > F
C. Total	26	1325190.7		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-62.05769	38.17672	-1.63	0.1166
Anthracycline Dose	626.19658	83.47422	7.50	<.0001

Orthogonal Regression

Variable	Mean	Std Dev	Variance Ratio	Correlation
Anthracycline Dose	0.35	0.3	0	0.8321
Wound AUC	157.1111	225.7629		

Intercept	Slope
-159.423	904.3818

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8) Evaluation of the effect of timing of a single-dose of 250 mg/kg dexrazoxane in the protection against 3 mg/kg doxorubicin-induced skin necrosis.

Major findings

The AUC of lesions caused by SC Doxorubicin increased as the time of injection of IP dexrazoxane increased from t=0 to t=6 hours. After an injection at t=6 hours the wound AUC was twice as large as that which occurred after an injection at t=0 (101 vs 45 mm^2*days). The wound AUC was not significantly different from control by non-parametric analysis. Thus, dexrazoxane given 6 hours after extravasation does not protect against wound formation as well as giving the drug immediately or within 3 hours.

Study number	SL101, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	October 28, 1998
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam

Methods

Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam All mice were injected SC at t = 0 with Doxorubicin then at various times with 250 mg/kg dexrazoxane.

Group	Wound induction	Treatment
1	Doxorubicin 2 mg/kg	Isotonic saline IP
2	Doxorubicin 2 mg/kg	T = 0
3	Doxorubicin 2 mg/kg	T = 3 hr
4	Doxorubicin 2 mg/kg	T = 6 hr

Formulation	Isotonic saline
Methods	Mice were examined for skin wounds for 36 days The investigators plotted study day against wound size and calculated the area under this curve (AUC)

Results

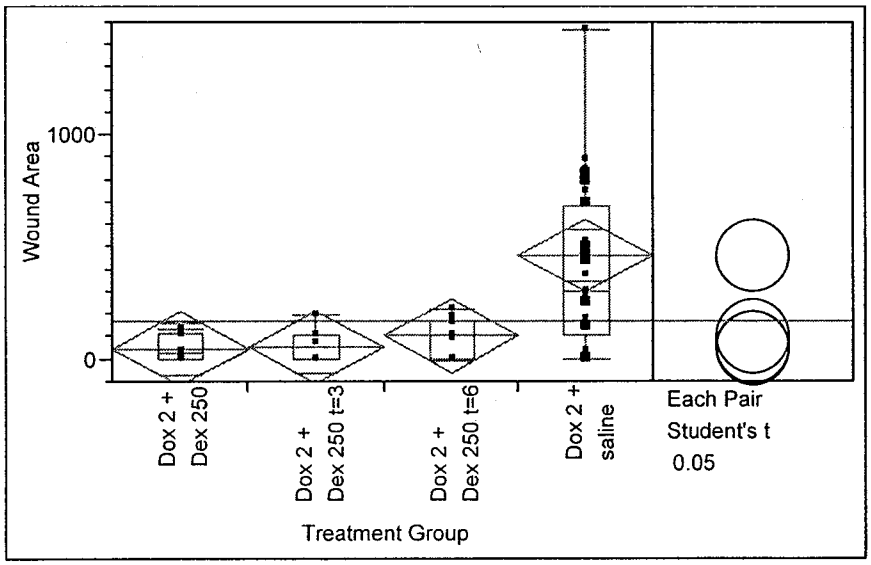
Group	Wound induction SC injection	Treatment	Mean AUC MM^2*day	P value VS control[A]	N without lesions	N with lesions
1	Doxorubicin 2 mg/kg	Isotonic saline IP	514±456		1	8
2	Doxorubicin 2 mg/kg	T = 0	81 ± 51	0.002	4	5
3	Doxorubicin 2 mg/kg	T = 3 hr	116 ± 52	0.04	5	4
4	Doxorubicin 2 mg/kg	T = 6 hr	151 ± 50	0.07	3	6

a Student's t-test p values for AUC calculated by the sponsor

APPEARS THIS WAY ON ORIGINAL

The sponsor reported doing a linear regression analysis of time of injection vs AUC (N=15) and reported a p value in the regression model of 0.03. Since they did not include 0 values in their other calculation I would infer that they did not include them in this regression. When I did the regression including the zero values, I determined an r^2 value of 0.1. The slope of the regression line was not significantly different from 0 ($p = 0.104$). When I excluded the zero values I confirmed that the slope was significantly different from zero ($p = 0.03$) with a correlation coefficient of 0.31. Irrespective of the model and inadequate sample size, it is clear that wound area increases as the time of the dexrazoxane dose increases. Delay of treatment is a bad thing. I analyzed the data with JMP using parametric and nonparametric statistical methods and obtained the following.

Oneway Analysis of Wound Area By Treatment Group



Excluded Rows
179

Oneway Anova
Summary of Fit

Rsquare	0.366546
Adj Rsquare	0.307159
Root Mean Square Error	238.2711
Mean of Response	163.8611
Observations (or Sum Wgts)	36

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment Group	3	1051249.0	350416	6.1722	0.0020
Error	32	1816739.3	56773		
C. Total	35	2867988.3			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Dox 2 + Dex 250	9	45.000	79.424	-116.8	206.78
Dox 2 + Dex 250 t=3	9	51.778	79.424	-110.0	213.56
Dox 2 + Dex 250 t=6	9	101.222	79.424	-60.6	263.00
Dox 2 + saline	9	457.444	79.424	295.7	619.23

Std Error uses a pooled estimate of error variance

Means Comparisons
Comparisons for each pair using Student's t

t	Alpha
2.03693	0.05

Abs(Dif)-LSD	Dox 2 + saline	Dox 2 + Dex 250 t=6	Dox 2 + Dex 250 t=3	Dox 2 + Dex 250
Dox 2 + saline	-228.79	127.43	176.87	183.65
Dox 2 + Dex 250 t=6	127.43	-228.79	-179.35	-172.57
Dox 2 + Dex 250 t=3	176.87	-179.35	-228.79	-222.01
Dox 2 + Dex 250	183.65	-172.57	-222.01	-228.79

Positive values show pairs of means that are significantly different.

Level			Mean
Dox 2 + saline	A		457.44444
Dox 2 + Dex 250 t=6		B	101.22222
Dox 2 + Dex 250 t=3		B	51.77778
Dox 2 + Dex 250		B	45.00000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	p-Value	Difference
Dox 2 + saline	Dox 2 + Dex 250	412.4444	183.652	641.2370	0.0009	
Dox 2 + saline	Dox 2 + Dex 250 t=3	405.6667	176.874	634.4592	0.0010	
Dox 2 + saline	Dox 2 + Dex 250 t=6	356.2222	127.430	585.0148	0.0033	
Dox 2 + Dex 250 t=6	Dox 2 + Dex 250	56.2222	-172.570	285.0148	0.6201	
Dox 2 + Dex 250 t=6	Dox 2 + Dex 250 t=3	49.4444	-179.348	278.2370	0.6627	
Dox 2 + Dex 250 t=3	Dox 2 + Dex 250	6.7778	-222.015	235.5703	0.9523	

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
Dox 2 + Dex 250	9	123.500	13.7222	-1.590
Dox 2 + Dex 250 t=3	9	122.500	13.6111	-1.628
Dox 2 + Dex 250 t=6	9	169.000	18.7778	0.075
Dox 2 + saline	9	251.000	27.8889	3.144

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
11.4818	3	0.0094

I analyzed the sponsor's AUC data using GraphPad's (Prism) Kruskal-Wallis statistics routine with Dunn's posttest. The analysis demonstrated that there was a significant difference among the means. Dunn's posttests demonstrated that wound AUC in the t=0 and t=3 hour groups was different from saline control but that the AUC in the t=6 hour group was not.

Kruskal-Wallis test				
P value		0.0094		
Exact or approximate P value?	Gaussian Approximation			
P value summary	**			
Do the medians vary signif. (P < 0.05)	Yes			
Number of groups		4		
Kruskal-Wallis statistic		11		
Dunn's Multiple Comparison Test	Difference in rank sum		P value	Summary
Dox 2 + Saline vs Dox 2 + Dex 250		14	P < 0.05	*
Dox 2 + Saline vs Dox 2 + Dex 250 t+3		14	P < 0.05	*
Dox 2 + Saline vs Dox 2 + Dex 250 t+6		9.1	P > 0.05	ns
Dox 2 + Dex 250 vs Dox 2 + Dex 250 t+3		0.11	P > 0.05	ns
Dox 2 + Dex 250 vs Dox 2 + Dex 250 t+6		-5.1	P > 0.05	ns
Dox 2 + Dex 250 t+3 vs Dox 2 + Dex 250 t+6		-5.2	P > 0.05	ns

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9) Evaluation of possible protection of a single dose of 10 or 20 mg/kg aclarubicin against daunorubicin-induced skin necrosis and comparison with the effect of a single dose of 250 mg/kg dexrazoxane in mice.

Major findings

Aclarubicin at doses of 10 or 20 mg/kg IP does not protect against formation of wounds after an SC injection of daunorubicin.

Study number	SL102, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	January 27, 1999
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam
Methods	
Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam

All mice were injected SC at t = 0 with daunorubicin then immediately with dexrazoxane or Aclarubicin.

Group	Wound induction	Treatment for wound protection
1	Daunorubicin 3 mg/kg SC	Isotonic saline IP Control
2	Daunorubicin 3 mg/kg SC	Aclarubicin 10 mg/kg IP
3	Daunorubicin 3 mg/kg SC	Aclarubicin 20 mg/kg IP
4	Daunorubicin 3 mg/kg SC	Dexrazoxane 250 mg/kg IP

Formulation Isotonic saline
Methods Mice were examined for skin wounds for 36 days
The investigators plotted study day against wound size and calculated the area under this curve (AUC)
The sponsor did not provide AUC values for individual animals.

Results

One mouse died of causes unknown on day 8 in the group treated with 20 mg/kg aclarubicin. Another mouse died on the day of treatment with 250 mg/kg dexrazoxane "most likely due to development of hypothermia during anesthesia".

Group	Wound induction	Treatment for wound protection	Mean AUC mm^2*day	P value vs control[a]	N without lesions	N with lesions
1	Daunorubicin 3 mg/kg SC	Isotonic saline IP Control	1118 ± 387		0	9
2	Daunorubicin 3 mg/kg SC	Aclarubicin 10 mg/kg IP	1150 ± 286	0.74	0	9
3	Daunorubicin 3 mg/kg SC	Aclarubicin 10 mg/kg IP	1280 ± 435	0.46	0	8
4	Daunorubicin 3 mg/kg SC	Dexrazoxane 250 mg/kg IP	186 ± 127	<0.0001	6	2

a Student's t-test p values for AUC calculated by the sponsor

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10) Investigation of the possible vesicant effect of up to 30% H_2O_2, 1, 3 or 6 mg/kg aclarubicin and 1, 4 or 8 mg/kg etoposide in B6DF1 mice.

Major findings

An SC dose of 0.05 mL of 30% H_2O_2 caused severe irritation in mice necessitating their destruction. An SC dose of 0.05 mL of 10% H_2O_2 caused skin lesions in four of four mice treated. The mean size of these wounds was about half that caused by a 3 mg/kg SC dose of daunorubicin. Neither Aclarubicin nor Etoposide consistently caused skin wounds.

Study number SL114, Volume 1
Conducting laboratory TopoTarget A/S, Copenhagen, Denmark
Date of study initiation February 18, 1999
GLP compliance No
QA report No

Drug H_2O_2, aclarubicin and etoposide
Dexrazoxane was not used in this experiment

Methods

Doses See table below
Species Female B6D2F1 mice
Number 4 per treatment group
Schedule Mice were anaesthetized with fentanyl, fluanison and midazolam
Various vesicants SC as shown in the following table.

Group	Wound induction
1	H_2O_2 30% solution, 0.05 mL
2	H_2O_2 10% solution, 0.05 mL
3	H_2O_2 1% solution, 0.05 mL
4	Etoposide 1 mg/kg
5	Etoposide 4 mg/kg
6	Etoposide 8 mg/kg
7	Aclarubicin 1 mg/kg
8	Aclarubicin 3 mg/kg
9	Aclarubicin 6 mg/kg

Formulation Isotonic saline
Methods Mice were examined for skin wounds for 28 days
The investigators plotted study day against wound size and calculated the area under this curve (AUC)
The sponsor did not provide AUC values for individual animals.
Results

All mice in group 1 were killed immediately after the injection of 0.05 mL of 30% H_2O_2 because this dose caused "severe irritation."

Group	Wound induction	Mean AUC mm^2*day	N without lesions	N with lesions
1	H_2O_2 30% solution, 0.05 mL	lethal		
2	H_2O_2 10% solution, 0.05 mL	624±334	0	4
3	H_2O_2 1% solution, 0.05 mL	ND	4	0
4	Etoposide 1 mg/kg	ND	4	0
5	Etoposide 4 mg/kg	ND	3	1
6	Etoposide 8 mg/kg	ND	3	1
7	Aclarubicin 1 mg/kg	ND	4	0
8	Aclarubicin 3 mg/kg	ND	3	1
9	Aclarubicin 6 mg/kg	ND	4	0

a Student's t-test p values for AUC calculated by the sponsor
ND = not determined

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11) Evaluation of the effect of dexrazoxane 250 mg/kg on 0.75 or 1.5 mg/kg experimental Idarubicin extravasation.

Major findings

Wound formation by Idarubicin increased with increasing dose and dexrazoxane treatment appeared to diminish the size of these wounds. The effect did not reach statistical significance because of the small sample size. Nevertheless, dexrazoxane does not appear to protect against wound formation caused by Idarubicin as well as it does against daunorubicin.

Study number	SL118, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	February 21, 1999
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam
Methods	
Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam The mice were injected SC with a dose of Idarubicin (see table below) followed by a dose of Isotonic saline or dexrazoxane IP.

Group	Wound induction	Wound prevention regimen
1	Idarubicin 0.75 mg/kg SC	Isotonic saline 0.2 mL IP
2	Idarubicin 0.75 mg/kg SC	250 mg/kg Dexrazoxane IP
3	Idarubicin 1.5 mg/kg SC	Isotonic saline 0.2 mL IP
4	Idarubicin 1.5 mg/kg SC	250 mg/kg Dexrazoxane IP

Formulation	Isotonic saline
Methods	Mice were examined for skin wounds for 29 days The investigators plotted study day against wound size and calculated the area under this curve (AUC) The sponsor did not provide AUC values for individual animals.
Results	

One mouse in group 4 died as a consequence of the anesthesia procedure.

Group	Wound induction	Wound prevention regimen	Mean AUC mm^2*day	P value [a]	N without lesions	N with lesions
1	Idarubicin 0.75 mg/kg SC	Isotonic saline 0.2 mL IP	294 ± 242		3	6
2	Idarubicin 0.75 mg/kg SC	250 mg/kg Dexrazoxane IP	118 ± 96	0.19[b]	6	3
3	Idarubicin 1.5 mg/kg SC	Isotonic saline 0.2 mL IP	454 ± 246		2	7
4	Idarubicin 1.5 mg/kg SC	250 mg/kg Dexrazoxane IP	243 ± 124	0.59[c]	2	6

a Student's t-test p values for AUC calculated by the sponsor
b Group 2 compared to group 1
c Group 4 compared to group 3

I analyzed the sponsor's AUC data using GraphPad's (Prism) Kruskal-Wallis statistics routine with Dunn's posttest. The analysis demonstrated that there was not a significant difference among the means.

Kruskal-Wallis test			
P value		0.0666	
Exact or approximate P value?	Gaussian Approximation		
P value summary	ns		
Do the medians vary signif. (P < 0.05)	No		
Number of groups		4	
Kruskal-Wallis statistic		7.2	
Dunn's Multiple Comparison Test	Difference in rank sum	P value	Summary
Ida 0.75 + saline vs Ida 0.75 + Dex 250		7.3 P > 0.05	ns
Ida 0.75 + saline vs Ida 1.5 + saline		-5.1 P > 0.05	ns
Ida 0.75 + saline vs Ida 1.5 + Dex 250		-0.56 P > 0.05	ns
Ida 0.75 + Dex 250 vs Ida 1.5 + saline		-12 P < 0.05	*
Ida 0.75 + Dex 250 vs Ida 1.5 + Dex 250		-7.9 P > 0.05	ns
Ida 1.5 + saline vs Ida 1.5 + Dex 250		4.6 P > 0.05	ns

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12) Evaluation of the effect of dexrazoxane 250 mg/kg intraperitoneally on experimental extravasation of 1, 2, or 3 mg/kg doxorubicin in mice.

Major findings

A dose of 1 mg/kg of Doxorubicin SC did not cause skin wounds in mice. Doses of 2 or 3 mg/kg caused wounds in most treated mice. The area of these wounds in saline treated controls was twice as great in mice treated with 3 mg/kg as it was in mice treated with 2 mg/kg. None of the mice treated with 250 mg/kg dexrazoxane IP after any Doxorubicin dose up to 3 mg/kg developed skin lesions.

Study number	SL119, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	March 8, 1999
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam
Methods	
Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam The mice were injected SC with a dose of Doxorubicin (see table below) followed by a dose of isotonic saline or dexrazoxane IP.

Group	Wound induction	Wound prevention regimen
1	Doxorubicin 1 mg/kg SC	Isotonic saline 0.2 mL IP
2	Doxorubicin 2 mg/kg SC	Isotonic saline 0.2 mL IP
3	Doxorubicin 3 mg/kg SC	Isotonic saline 0.2 mL IP
4	Doxorubicin 1 mg/kg SC	250 mg/kg Dexrazoxane IP
5	Doxorubicin 2 mg/kg SC	250 mg/kg Dexrazoxane IP
6	Doxorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP

Formulation Isotonic saline

Methods Mice were examined for skin wounds for 31 days
The investigators plotted study day against wound size and calculated the area under this curve (AUC)
The sponsor did not provide AUC values for individual animals.

Results

One mouse in group 4 died as a consequence of the anesthesia procedure.

Group	Wound induction	Wound prevention regimen	Mean AUC mm^2*day	P value [a]	N without lesions	N with lesions
1	Doxorubicin 1 mg/kg SC	Isotonic saline 0.2 mL IP			9	0
2	Doxorubicin 2 mg/kg SC	Isotonic saline 0.2 mL IP	511 ± 246		1	8
3	Doxorubicin 3 mg/kg SC	Isotonic saline 0.2 mL IP	1040 ± 433		1	8
4	Doxorubicin 1 mg/kg SC	250 mg/kg Dexrazoxane IP			9	0
5	Doxorubicin 2 mg/kg SC	250 mg/kg Dexrazoxane IP		0.0004[b]	9	0
6	Doxorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP		0.0004[c]	8	0

a I calculated these p values using Fisher's exact test
b Group 5 compared to group 2
c Group 6 compared to group 3

I analyzed the sponsor's AUC data using GraphPad's (Prism) Kruskal-Wallis statistics routine with Dunn's posttest. The analysis demonstrated that there was a significant difference among the means. Dexrazoxane protects against doses of Doxorubicin as high as 3 mg/kg.

Kruskal-Wallis test			
P value	P<0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups		6	
Kruskal-Wallis statistic		44	
Dunn's Multiple Comparison Test	Difference in rank sum	P value	Summary
Dox 1 + saline vs Dox 2 + saline		-22 P < 0.01	**
Dox 1 + saline vs Dox 3 + saline		-26 P < 0.001	***
Dox 1 + saline vs Dox 1 + Dex 250		0 P > 0.05	ns
Dox 1 + saline vs Dox 2 + Dex 250		0 P > 0.05	ns
Dox 1 + saline vs Dox 3 + Dex 250		0 P > 0.05	ns
Dox 2 + saline vs Dox 3 + saline		-4.9 P > 0.05	ns
Dox 2 + saline vs Dox 1 + Dex 250		22 P < 0.01	**
Dox 2 + saline vs Dox 2 + Dex 250		22 P < 0.01	**
Dox 2 + saline vs Dox 3 + Dex 250		22 P < 0.01	**
Dox 3 + saline vs Dox 1 + Dex 250		26 P < 0.001	***
Dox 3 + saline vs Dox 2 + Dex 250		26 P < 0.001	***
Dox 3 + saline vs Dox 3 + Dex 250		26 P < 0.001	***
Dox 1 + Dex 250 vs Dox 2 + Dex 250		0 P > 0.05	ns
Dox 1 + Dex 250 vs Dox 3 + Dex 250		0 P > 0.05	ns
Dox 2 + Dex 250 vs Dox 3 + Dex 250		0 P > 0.05	ns

13) Evaluation of the effect of dexrazoxane 250 mg/kg intraperitoneally on skin wounds induced with subcutaneous injection of up to 10% H_2O_2 and 10 mg/kg etoposide in mice.

Major findings

A dose of 10 mg/kg etoposide SC was insufficient to cause skin lesions, as was a 0.05 mL dose of 3% H_2O_2 in mice. A 0.05 mL dose of 6% H_2O_2 caused lesions in 16 of 18 treated mice; dexrazoxane had no appreciable effect on this trauma. All mice treated with a 0.05 mL dose of 10% H_2O_2 developed skin lesions. Again, dexrazoxane treatment did not prevent wound formation or diminish the size of the wounds. This experiment suggests that dexrazoxane does not prevent wound formation by a mechanism involving radicals.

Study number	SL132 and 136, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	March 3, 1999
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam

Methods

Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam The mice were then injected SC with a dose of H_2O_2 or etoposide (see table below) followed by a dose of isotonic saline or dexrazoxane IP or no treatment.

Exp	Group	Wound induction	Wound prevention regimen
SL132	1	10% H_2O_2 SC	250 mg/kg Dexrazoxane IP
SL132	2	10% H_2O_2 SC	None
SL132	3	10 mg/kg etoposide SC	250 mg/kg Dexrazoxane IP
SL132	4	10 mg/kg etoposide SC	None
SL136	1	3% H_2O_2 SC	Isotonic saline 0.2 mL IP
SL136	2	3% H_2O_2 SC	250 mg/kg Dexrazoxane IP
SL136	3	6% H_2O_2 SC	Isotonic saline 0.2 mL IP
SL136	4	6% H_2O_2 SC	250 mg/kg Dexrazoxane IP

Formulation	Isotonic saline
Methods	Mice were examined for skin wounds for 26 days The investigators plotted study day against wound size and calculated the area under this curve (AUC) The sponsor did not provide AUC values for individual animals.

Results

One mouse in group 1 and two mice in group 2 of experiment SL132 died prematurely. The investigators did not state a cause for these deaths.

Group	Wound induction	Wound prevention regimen	Mean AUC mm^2*day	P value [a]	N without lesions	N with lesions
1	10% H_2O_2 SC	250 mg/kg Dexrazoxane IP	668 ± 230		0	8
2	10% H_2O_2 SC	None	589 ± 135	0.7[b]	0	7
3	10 mg/kg etoposide SC	250 mg/kg Dexrazoxane IP			9	0
4	10 mg/kg etoposide SC	None			9	0
1	3% H_2O_2 SC	Isotonic saline 0.2 mL IP	429 ± 117		7	2
2	3% H_2O_2 SC	250 mg/kg Dexrazoxane IP	137		8	1
3	6% H_2O_2 SC	Isotonic saline 0.2 mL IP	435 ± 327		0	9
4	6% H_2O_2 SC	250 mg/kg Dexrazoxane IP	431 ± 185	0.7[c]	2	7

a The investigators calculated these p values using Student's t-test
b Group 2 compared to group 1
c Group 6 compared to group 5

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14) Evaluation of the effect of dexrazoxane 250 mg/kg intraperitoneally on skin wounds induced by subcutaneous injection of 15 or 30 mg/kg etoposide.

Major findings

15 mg/kg etoposide SC did not produce skin lesions with or without dexrazoxane. 30 mg/kg etoposide alone produced relatively small lesions in 2 of 8 mice. Dexrazoxane treatment had no significant effect on the formation of lesions by 30 mg/kg etoposide.

Study number	SL137, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	March 17, 1999
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam

Methods

Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam The mice were then injected SC with a dose etoposide (see table below) followed by a dose of isotonic saline or dexrazoxane IP.

Group	Wound induction	Wound prevention regimen
1	Etoposide 15 mg/kg SC	0.2 mL isotonic saline IP
2	Etoposide 15 mg/kg SC	250 mg/kg Dexrazoxane IP
3	Etoposide 30 mg/kg SC	0.2 mL isotonic saline IP
4	Etoposide 30 mg/kg SC	250 mg/kg Dexrazoxane IP

Formulation	Isotonic saline
Methods	Mice were examined for skin wounds for 26 days

The investigators plotted study day against wound size and calculated the area under this curve (AUC)

Results

One mouse died after treatment in groups 3 and 4. The investigators did not state a cause for these deaths.

Group	Wound induction	Wound prevention regimen	Mean AUC mm^2*day	P value [a]	N without lesions	N with lesions
1	Etoposide 15 mg/kg SC	0.2 mL isotonic saline IP			9	0
2	Etoposide 15 mg/kg SC	250 mg/kg Dexrazoxane IP			9	0
3	Etoposide 30 mg/kg SC	0.2 mL isotonic saline IP	193 & 150		6	2
4	Etoposide 30 mg/kg SC	250 mg/kg Dexrazoxane IP	270		7	1

a The investigators calculated these p values using Student's t-test
b Group 2 compared to group 1
c Group 6 compared to group 5

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15) Comparison of the effect of administering 250 mg/kg dexrazoxane intraperitoneally or intravenously, and evaluation of the effect of injecting dexrazoxane intralesionally on daunorubicin-induced skin wounds.

Major findings

After wound induction, treatment with dexrazoxane IP is equivalent if not slightly better than treatment with the same dose IV, though this difference does not reach statistical significance. Treatment with an intralesional dose of either 30 or 250 mg/kg dexrazoxane concurrent with the daunorubicin challenge provided protection equivalent to the systemic dose of 250 mg/kg. There was no statistically significant evidence for a dose effect after intralesional dosing. To the contrary, dosing with 250 mg/kg dexrazoxane concomitant with daunorubicin provided less protection than dosing with 30 mg/kg.

Study number	SL159, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	July 29, 1999
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam
Methods	
Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam The mice were then injected with a 3 mg/kg daunorubicin then treated as the following table shows.

Group	Wound induction	Wound prevention regimen
1	Daunorubicin 3 mg/kg SC	0.2 mL isotonic saline IP
2	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IV
3	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP
4	Mixed Daunorubicin 3 mg/kg plus 30 mg/kg Dexrazoxane SC	
5	Mixed Daunorubicin 3 mg/kg plus 250 mg/kg Dexrazoxane SC	

Formulation Isotonic saline

Methods Mice were examined for skin wounds for 34 days
The investigators plotted study day against wound size and calculated the area under this curve (AUC)

Results

One mouse died after treatment in groups 3. The investigators did not state a cause for these deaths. The following table shows my calculation of the mean AUC values, standard deviations and the statistical differences between them. I used Student's t-test and assumed that the variances were not equal. I also included (at the advice of Dr. Shenghui Tang, the statistician for this NDA) the animals that did not form wounds, scoring the AUC as 0.

	G1	G2	G3	G4	G5
	Dau3 SC + Saline IP	Dau3 SC + Dex IV	Dau3 SC +Dex IP	Dau3+Dex 30 SC	Dau3+Dex 250 SC
	1758	1092	814	956	568
	1460	708	518	902	341
	1407	486	236	826	1009
	2284	727	213	1688	682
	1078	974	225	423	629
	2035	323	611	0	880
	2100	0	428	0	123
	2103	0	0	0	236
	0	0		0	923
average	**1581**	**479**	**381**	**533**	**599**
sd	713	425	262	600	313
	p values	G2 to G1	G3 to G1	G4 to G1	G5 to G1
	one tail	0.0008	0.0004	0.0020	0.0015
	two tail	**0.0016**	**0.0008**	**0.0040**	**0.0031**
			G3 to G2	G4 to G2	G5 to G2
	two tail		0.57	0.65	0.66
					G5 < G4
	one tail				0.39

The table shows that all of the groups treated with dexrazoxane were statistically different from saline control. Giving the drug IV versus IP made not difference and in this experiment, there was no significant dose response. Most importantly, giving the dexrazoxane directly into the lesion SC at the daunorubicin injection site was no different from giving systemically. The investigators in the study did not include the AUC=0 values in their calculations. This decreases the p value because it decreases the standard deviation. Nevertheless, their results and the interpretation thereof were the same.

It is somewhat odd that in this case there was no dose effect between group 4 and group 5 especially sense other experiments do show a dose effect and there is such a large difference between the doses. This may be a real effect of dosing SC. The local concentration of the 30 mg/kg dose may approximate that achieved with the systemic dose of 250 mg/kg, and the achievable effect reaches a plateau such that the higher SC dose affords no further protection. Or it may be a consequence of the variability of the data. A Scatter diagram of this data shows a linear but this trend does not reach statistical significance (probability > F = 0.105).

My analysis by the Kruskal-Wallis nonparametric test with Dunn's posttest also demonstrated that 250 mg/kg of dexrazoxane given concomitantly with daunorubicin provided less protection against wound formation than did 30 mg/kg.

Kruskal-Wallis test			
P value	0.0077		
Exact or approximate P value?	Gaussian Approximation		
P value summary	**		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	5		
Kruskal-Wallis statistic	14		
Dunn's Multiple Comparison Test	Difference in rank sum	P value	Summary
Dau3 SC + Saline IP vs Dau3 SC + Dex IV	17	P < 0.05	*
Dau3 SC + Saline IP vs Dau3 SC + Dex IP	20	P < 0.05	*
Dau3 SC + Saline IP vs Dau3 + Dex 30 SC	18	P < 0.05	*
Dau3 SC + Saline IP vs Dau3 + Dex 250 SC	14	P > 0.05	ns
Dau3 SC + Dex IV vs Dau3 SC + Dex IP	2.7	P > 0.05	ns
Dau3 SC + Dex IV vs Dau3 + Dex 30 SC	0.33	P > 0.05	ns
Dau3 SC + Dex IV vs Dau3 + Dex 250 SC	-3.6	P > 0.05	ns
Dau3 SC + Dex IP vs Dau3 + Dex 30 SC	-2.4	P > 0.05	ns
Dau3 SC + Dex IP vs Dau3 + Dex 250 SC	-6.3	P > 0.05	ns
Dau3 + Dex 30 SC vs Dau3 + Dex 250 SC	-3.9	P > 0.05	ns

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16) Comparison of the effect of administering 250 mg/kg dexrazoxane intraperitoneally or intravenously, and evaluation of the effect of injecting dexrazoxane intralesionally, on doxorubicin-induced skin wounds.

Major findings

Doxorubicin is probably a less potent vesicant than daunorubicin. IV dosing of dexrazoxane may be less effective than IP dosing but the difference did not reach statistical significance. SC dosing (intralesional) dosing with 30 mg/kg of dexrazoxane was as effective as IP dosing with 250 mg/kg. Intralesional dosing with 250 mg/kg of dexrazoxane provided no protection against wound formation compared to saline control.

Study number	SL167, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	July 29, 1999
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam

Methods

Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam The mice were then injected with a 3 mg/kg Doxorubicin then treated as the following table shows.

Group	Wound induction	Wound prevention regimen
1	Doxorubicin 3 mg/kg SC	0.2 mL isotonic saline IP
2	Doxorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IV
3	Doxorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP
4	Mixed Doxorubicin 3 mg/kg plus 30 mg/kg Dexrazoxane SC	
5	Mixed Doxorubicin 3 mg/kg plus 250 mg/kg Dexrazoxane SC	

Formulation	Isotonic saline
Methods	Mice were examined for skin wounds for 34 days The investigators plotted study day against wound size and calculated the area under this curve (AUC)

Results

The investigators did not state a cause for these deaths. The following table shows my calculation of the mean AUC values, standard deviations and the statistical differences between them. I used Student's t-test and assumed that the variances were not equal. I also included the animals that did not form wounds, scoring the AUC as 0.

	G1	G2	G3	G4	G5
	Dox3 SC + Saline IP	Dox3 SC + Doxra IV	Dox3 SC +Doxra IP	Dox3+Dexra 30 SC	Dox3+Dexra 250 SC
	848	63	0	0	130
	529	90	0	0	186
	672	0	0	0	431
	175	0	0	0	313
	0	0	0	0	224
	0	0	0	0	77
	0	0	0	0	159
	0	0	0	0	0
	0	0	0	0	0
average	247	17	0	0	169
sd	341	34	0	0	141
	p values	G2 to G1	G3 to G1	G4 to G1	G5 to G1
	one tail	0.0391	0.0308	0.0308	0.2693
	two tail	0.0782	0.0616	0.0616	0.5386
			G3 to G2	G4 to G2	G5 to G2
	two tail		0.18	0.18	0.01
					G5 < G4
	one tail				0.00

The most striking feature of this data is that Doxorubicin given at a dose of 3 mg/kg SC is not as potent a vesicant as daunorubicin. Only four animals in the saline control group form lesions, making statistical comparisons with the rest of the treatment groups difficult. Nevertheless, there is a clear treatment effect in groups 2, 3, and 4. The difference in incidence between groups 1 and 3 and 1 and 4 reaches statistical significance ($p = 0.04$, Fisher's exact test).

IV dosing is again visually if not statistically less effective than IP dosing. If this effect is real, it is probably a result of the slow steady increase in systemic concentration associated with IP dosing. But 250 mg/kg dexrazoxane SC after Doxorubicin dosing actually increases the number of lesions though this increase does not reach statistical significance by Fisher's exact test. With no known mechanism of action for dexrazoxane, it is difficult to account for this result.

- Non-parametric analysis by the Kruskal-Wallis test with Dunn's post test provides similar results.

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17) Evaluation of the effect of dexrazoxane administered as single doses, repeated doses, or as a single 250 mg/kg dose at different times after experimental extravasation of 3 mg/kg of doxorubicin.

Major findings

All of the groups in this experiment that received treatment with some regimen of dexrazoxane had smaller wounds as measured by AUC and a lower incidence of wounds than did saline controls. Treatment with 62.5, 125 or 250 mg/kg at t = 0, 3, and 6 hours provided no better protection from wound formation than a single dose of 250 mg/kg at t = 0. A single dose of 63.5 mg/kg of dexrazoxane IP given immediately after 3 mg/kg of doxorubicin provided the same protection as 125 mg/kg and 250 mg/kg given at t = 0. Likewise, three doses at t = 0, 3 and 6 hours provided no better protection than a single dose at t = 0. Some evidence suggested that delaying the treatment for six hours after the Doxorubicin insult provided less protection than giving the dexrazoxane immediately.

Study number	SL173 and SL174, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	September 21, 1999
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam
Methods	
Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam The mice were then injected with a 3 mg/kg doxorubicin then treated as the following table shows.

Group	Exp	Wound induction	Wound prevention regimen
1	173	Doxorubicin 3 mg/kg SC	Isotonic saline IP t=0
2	173	Doxorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP t=0
3	173	Doxorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP t=3
4	173	Doxorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP t=6
1	174	Doxorubicin 3 mg/kg SC	Isotonic saline IP t=0
2	174	Doxorubicin 3 mg/kg SC	62.5 mg/kg Dexrazoxane IP t=0
3	174	Doxorubicin 3 mg/kg SC	125 mg/kg Dexrazoxane IP t=0
4	174	Doxorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP t=0
5	174	Doxorubicin 3 mg/kg SC	62.5 mg/kg Dexrazoxane IP t=0, 3, and 6 hr total dose 187.5 mg/kg
6	174	Doxorubicin 3 mg/kg SC	125 mg/kg Dexrazoxane IP t=0, 3 and 6 hr total dose 375 mg/kg
7	174	Doxorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP t=0 d0, d1, and d2 total dose 750 mg/kg

Formulation Isotonic saline

Methods Mice were examined for skin wounds for 34 days
The investigators plotted study day against wound size and calculated the area under this curve (AUC). The mean AUC values in the table below are those calculated by the investigators. They do not include 0 values. The sponsor combined data from identical treatment groups. I have done the same since the experiments were done together.

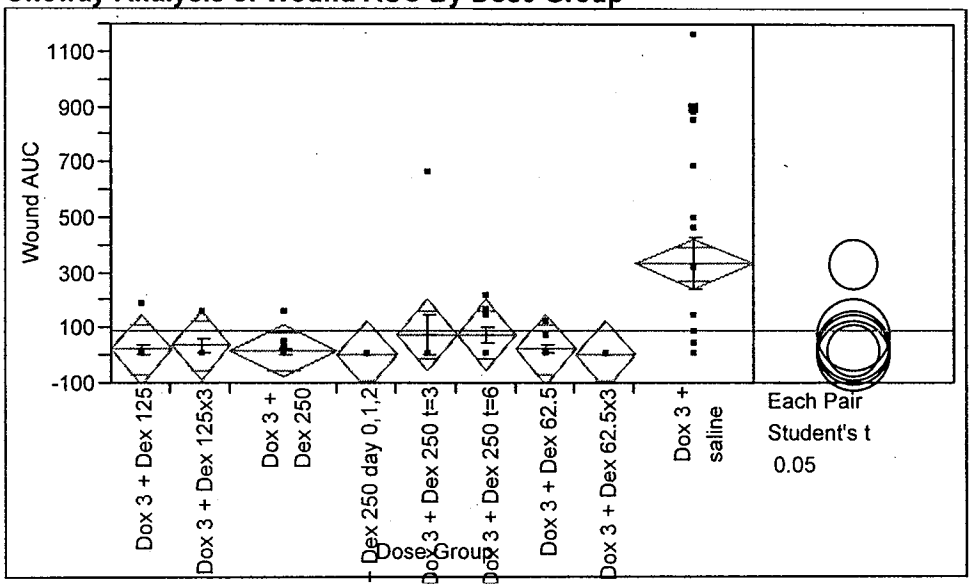
Results

Two mice died in treatment group 4 during anesthesia. The investigators consider these deaths likely due to hypothermia during the procedure. The following table shows the mean AUC values reported by the sponsor. Again, they differ from my calculated means because I included the 0 values on the advice of our statisticians.

Group	Wound induction	Wound prevention regimen	Mean AUC MM^2*day	N without lesions	N with lesions
1 SL173 & 1 SL174	Doxorubicin 3 mg/kg SC	Isotonic saline IP t=0	541 ± 376	7	11
2 SL173 & 4 SL174	Doxorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP t=0	73 ± 76.7	13	3
3 SL173	Doxorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP t=3	657	8	1
4 SL173	Doxorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP t=6	165 ± 31	5	4
2 SL174	Doxorubicin 3 mg/kg SC	62.5 mg/kg Dexrazoxane IP t=0	92 ± 33	7	2
3 SL174	Doxorubicin 3 mg/kg SC	125 mg/kg Dexrazoxane IP t=0	182	8	1
5 SL174	Doxorubicin 3 mg/kg SC	62.5 mg/kg Dexrazoxane IP t=0, 3, and 6 hr total dose 187.5 mg/kg	No wounds	9	0
6 SL174	Doxorubicin 3 mg/kg SC	125 mg/kg Dexrazoxane IP t=0, 3 and 6 hr total dose 375 mg/kg	154 ± 0	7	2
7 SL174	Doxorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP t=0 d0, d1, and d2 total dose 750 mg/kg	No wounds	9	0

I analyzed the data from this experiment using JMP. The following table shows the mean values for the AUCs including 0 values.

Oneway Analysis of Wound AUC By Dose Group



Oneway Anova
Summary of Fit

Rsquare	0.303208
Adj Rsquare	0.239863
Root Mean Square Error	191.2995
Mean of Response	84.23711
Observations (or Sum Wgts)	97

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Dose Group	8	1401353.0	175169	4.7866	<.0001
Error	88	3220402.6	36595		
C. Total	96	4621755.5			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Dox 3 + Dex 125	9	20.222	63.766	-106.5	146.94
Dox 3 + Dex 125x3	9	34.222	63.766	-92.5	160.94
Dox 3 + Dex 250	16	13.750	47.825	-81.3	108.79
Dox 3 + Dex 250 day 0,1,2	9	0.000	63.766	-126.7	126.72
Dox 3 + Dex 250 t=3	9	73.000	63.766	-53.7	199.72
Dox 3 + Dex 250 t=6	9	73.444	63.766	-53.3	200.17
Dox 3 + Dex 62.5	9	20.556	63.766	-106.2	147.28
Dox 3 + Dex 62.5x3	9	0.000	63.766	-126.7	126.72
Dox 3 + saline	18	331.000	45.090	241.4	420.61

Std Error uses a pooled estimate of error variance

Means Comparisons
Comparisons for each pair using Student's t

t	Alpha
1.98729	0.05

Abs(Dif)-LSD	Dox 3 + saline	Dox 3 + Dex 250 t=6	Dox 3 + Dex 250 t=3	Dox 3 + Dex 125x3	Dox 3 + Dex 62.5	Dox 3 + Dex 125	Dox 3 + Dex 250	Dox 3 + Dex 250 day 0,1,2	Dox 3 + Dex 62.5x3
Dox 3 + saline	-126.72	102.35	102.80	141.58	155.24	155.58	186.63	175.80	175.80
Dox 3 + Dex 250 t=6	102.35	-179.21	-178.77	-139.99	-126.32	-125.99	-98.71	-105.77	-105.77
Dox 3 + Dex 250 t=3	102.80	-178.77	-179.21	-140.43	-126.77	-126.43	-99.15	-106.21	-106.21
Dox 3 + Dex 125x3	141.58	-139.99	-140.43	-179.21	-165.55	-165.21	-137.93	-144.99	-144.99
Dox 3 + Dex 62.5	155.24	-126.32	-126.77	-165.55	-179.21	-178.88	-151.60	-158.66	-158.66
Dox 3 + Dex 125	155.58	-125.99	-126.43	-165.21	-178.88	-179.21	-151.93	-158.99	-158.99
Dox 3 + Dex 250	186.63	-98.71	-99.15	-137.93	-151.60	-151.93	-134.41	-144.65	-144.65
Dox 3 + Dex 250 day 0,1,2	175.80	-105.77	-106.21	-144.99	-158.66	-158.99	-144.65	-179.21	-179.21
Dox 3 + Dex 62.5x3	175.80	-105.77	-106.21	-144.99	-158.66	-158.99	-144.65	-179.21	-179.21

Positive values show pairs of means that are significantly different.

Level			Mean
Dox 3 + saline	A		331.00000
Dox 3 + Dex 250 t=6		B	73.44444
Dox 3 + Dex 250 t=3		B	73.00000
Dox 3 + Dex 125x3		B	34.22222
Dox 3 + Dex 62.5		B	20.55556
Dox 3 + Dex 125		B	20.22222
Dox 3 + Dex 250		B	13.75000
Dox 3 + Dex 250 day 0,1,2		B	0.00000
Dox 3 + Dex 62.5x3		B	0.00000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	p-Value	Difference
Dox 3 + saline	Dox 3 + Dex 250 day 0,1,2	331.0000	175.797	486.2027	<.0001	
Dox 3 + saline	Dox 3 + Dex 62.5x3	331.0000	175.797	486.2027	<.0001	
Dox 3 + saline	Dox 3 + Dex 250	317.2500	186.627	447.8726	<.0001	
Dox 3 + saline	Dox 3 + Dex 125	310.7778	155.575	465.9805	0.0001	
Dox 3 + saline	Dox 3 + Dex 62.5	310.4444	155.242	465.6472	0.0001	
Dox 3 + saline	Dox 3 + Dex 125x3	296.7778	141.575	451.9805	0.0003	
Dox 3 + saline	Dox 3 + Dex 250 t=3	258.0000	102.797	413.2027	0.0014	
Dox 3 + saline	Dox 3 + Dex 250 t=6	257.5556	102.353	412.7583	0.0014	
Dox 3 + Dex 250 t=6	Dox 3 + Dex 250 day 0,1,2	73.4444	-105.768	252.6571	0.4176	
Dox 3 + Dex 250 t=6	Dox 3 + Dex 62.5x3	73.4444	-105.768	252.6571	0.4176	
Dox 3 + Dex 250 t=3	Dox 3 + Dex 250 day 0,1,2	73.0000	-106.213	252.2127	0.4204	
Dox 3 + Dex 250 t=3	Dox 3 + Dex 62.5x3	73.0000	-106.213	252.2127	0.4204	
Dox 3 + Dex 250 t=6	Dox 3 + Dex 250	59.6944	-98.709	218.0976	0.4559	
Dox 3 + Dex 250 t=3	Dox 3 + Dex 250	59.2500	-99.153	217.6531	0.4593	
Dox 3 + Dex 250 t=6	Dox 3 + Dex 125	53.2222	-125.990	232.4349	0.5566	
Dox 3 + Dex 250 t=6	Dox 3 + Dex 62.5	52.8889	-126.324	232.1016	0.5591	
Dox 3 + Dex 250 t=3	Dox 3 + Dex 125	52.7778	-126.435	231.9904	0.5599	
Dox 3 + Dex 250 t=3	Dox 3 + Dex 62.5	52.4444	-126.768	231.6571	0.5624	
Dox 3 + Dex 250 t=6	Dox 3 + Dex 125x3	39.2222	-139.990	218.4349	0.6647	
Dox 3 + Dex 250 t=3	Dox 3 + Dex 125x3	38.7778	-140.435	217.9904	0.6682	
Dox 3 + Dex 125x3	Dox 3 + Dex 250 day 0,1,2	34.2222	-144.990	213.4349	0.7052	
Dox 3 + Dex 125x3	Dox 3 + Dex 62.5x3	34.2222	-144.990	213.4349	0.7052	
Dox 3 + Dex 62.5	Dox 3 + Dex 250	20.5556	-158.657	199.7682	0.8202	

	day 0,1,2					
Dox 3 + Dex 62.5	Dox 3 + Dex 62.5x3	20.5556	-158.657	199.7682	0.8202	
Dox 3 + Dex 125x3	Dox 3 + Dex 250	20.4722	-137.931	178.8753	0.7979	
Dox 3 + Dex 125	Dox 3 + Dex 250 day 0,1,2	20.2222	-158.990	199.4349	0.8231	
Dox 3 + Dex 125	Dox 3 + Dex 62.5x3	20.2222	-158.990	199.4349	0.8231	
Dox 3 + Dex 125x3	Dox 3 + Dex 125	14.0000	-165.213	193.2127	0.8770	
Dox 3 + Dex 250	Dox 3 + Dex 250 day 0,1,2	13.7500	-144.653	172.1531	0.8634	
Dox 3 + Dex 250	Dox 3 + Dex 62.5x3	13.7500	-144.653	172.1531	0.8634	
Dox 3 + Dex 125x3	Dox 3 + Dex 62.5	13.6667	-165.546	192.8793	0.8799	
Dox 3 + Dex 62.5	Dox 3 + Dex 250	6.8056	-151.598	165.2087	0.9322	
Dox 3 + Dex 125	Dox 3 + Dex 250	6.4722	-151.931	164.8753	0.9355	
Dox 3 + Dex 250 t=6	Dox 3 + Dex 250 t=3	0.4444	-178.768	179.6571	0.9961	
Dox 3 + Dex 62.5	Dox 3 + Dex 125	0.3333	-178.879	179.5460	0.9971	
Dox 3 + Dex 62.5x3	Dox 3 + Dex 250 day 0,1,2	0.0000	-179.213	179.2127	1.0000	

The following table shows that the mean for the saline control group was significantly different from all the other means using non-parametric analysis (under the assumption that the distribution was not normal). The values for the Kruskal-Wallis statistic (last column) is positive for both the Doxorubicin + saline control and the Doxorubicin + dexrazoxane 250 mg/kg at t=6 hours dose groups indicating that the score mean for these groups is significantly different from that of the other groups.

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
Dox 3 + Dex 125	9	383.000	42.5556	-0.944
Dox 3 + Dex 125x3	9	427.000	47.4444	-0.222
Dox 3 + Dex 250	16	715.000	44.6875	-0.879
Dox 3 + Dex 250 day 0,1,2	9	333.000	37.0000	-1.765
Dox 3 + Dex 250 t=3	9	388.000	43.1111	-0.862
Dox 3 + Dex 250 t=6	9	521.000	57.8889	1.305
Dox 3 + Dex 62.5	9	415.000	46.1111	-0.419
Dox 3 + Dex 62.5x3	9	333.000	37.0000	-1.765
Dox 3 + saline	18	1238.00	68.7778	4.355

1-way Test, ChiSquare Approximation

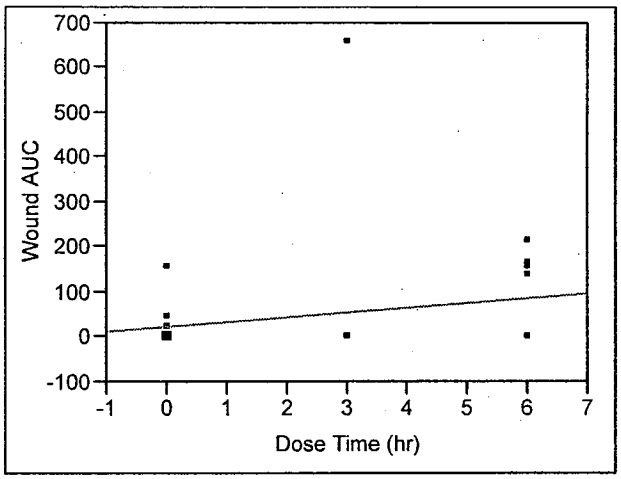
ChiSquare	DF	Prob>ChiSq
25.1349	8	0.0015

The following analysis in GraphPad shows which pairs of treatment groups are different by Dunn's post-test.

Kruskal-Wallis test			
P value	0.0015		
Exact or approximate P value?	Gaussian Approximation		
P value summary	**		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	9		
Kruskal-Wallis statistic	25		
Dunn's Multiple Comparison Test	Difference in rank sum	P value	Summary
Dox 3 + saline vs Dox 3 + Dex 250	24	P < 0.05	*
Dox 3 + saline vs Dox 3 + Dex 250 t=3	26	P > 0.05	ns
Dox 3 + saline vs Dox 3 + Dex 250 t=6	11	P > 0.05	ns
Dox 3 + saline vs Dox 3 + Dex 62.5	23	P > 0.05	ns
Dox 3 + saline vs Dox 3 + Dex 125	26	P > 0.05	ns
Dox 3 + saline vs Dox 3 + Dex 62.5x3	32	P < 0.01	**
Dox 3 + saline vs Dox 3 + Dex 125x3	21	P > 0.05	ns
Dox 3 + saline vs Dox 3 + Dex 250 day 012	32	P < 0.01	**
Dox 3 + Dex 250 vs Dox 3 + Dex 250 t=3	1.6	P > 0.05	ns
Dox 3 + Dex 250 vs Dox 3 + Dex 250 t=6	-13	P > 0.05	ns
Dox 3 + Dex 250 vs Dox 3 + Dex 62.5	-1.4	P > 0.05	ns
Dox 3 + Dex 250 vs Dox 3 + Dex 125	2.1	P > 0.05	ns
Dox 3 + Dex 250 vs Dox 3 + Dex 62.5x3	7.7	P > 0.05	ns
Dox 3 + Dex 250 vs Dox 3 + Dex 125x3	-2.8	P > 0.05	ns
Dox 3 + Dex 250 vs Dox 3 + Dex 250 day 012	7.7	P > 0.05	ns
Dox 3 + Dex 250 t=3 vs Dox 3 + Dex 250 t=6	-15	P > 0.05	ns
Dox 3 + Dex 250 t=3 vs Dox 3 + Dex 62.5	-3	P > 0.05	ns
Dox 3 + Dex 250 t=3 vs Dox 3 + Dex 125	0.56	P > 0.05	ns
Dox 3 + Dex 250 t=3 vs Dox 3 + Dex 62.5x3	6.1	P > 0.05	ns
Dox 3 + Dex 250 t=3 vs Dox 3 + Dex 125x3	-4.3	P > 0.05	ns
Dox 3 + Dex 250 t=3 vs Dox 3 + Dex 250 day 012	6.1	P > 0.05	ns
Dox 3 + Dex 250 t=6 vs Dox 3 + Dex 62.5	12	P > 0.05	ns
Dox 3 + Dex 250 t=6 vs Dox 3 + Dex 125	15	P > 0.05	ns
Dox 3 + Dex 250 t=6 vs Dox 3 + Dex 62.5x3	21	P > 0.05	ns
Dox 3 + Dex 250 t=6 vs Dox 3 + Dex 125x3	10	P > 0.05	ns
Dox 3 + Dex 250 t=6 vs Dox 3 + Dex 250 day 012	21	P > 0.05	ns
Dox 3 + Dex 62.5 vs Dox 3 + Dex 125	3.6	P > 0.05	ns
Dox 3 + Dex 62.5 vs Dox 3 + Dex 62.5x3	9.1	P > 0.05	ns
Dox 3 + Dex 62.5 vs Dox 3 + Dex 125x3	-1.3	P > 0.05	ns
Dox 3 + Dex 62.5 vs Dox 3 + Dex 250 day 012	9.1	P > 0.05	ns
Dox 3 + Dex 125 vs Dox 3 + Dex 62.5x3	5.6	P > 0.05	ns
Dox 3 + Dex 125 vs Dox 3 + Dex 125x3	-4.9	P > 0.05	ns
Dox 3 + Dex 125 vs Dox 3 + Dex 250 day 012	5.6	P > 0.05	ns
Dox 3 + Dex 62.5x3 vs Dox 3 + Dex 125x3	-10	P > 0.05	ns
Dox 3 + Dex 62.5x3 vs Dox 3 + Dex 250 day 012	0	P > 0.05	ns
Dox 3 + Dex 125x3 vs Dox 3 + Dex 250 day 012	10	P > 0.05	ns

Fitting the data for the three groups in which dexrazoxane was given at different times by linear regression produced a non-significant linear correlation (below). Nevertheless, I consider this evidence taken together strongly suggestive that dexrazoxane is best given immediately after the anthracycline insult. This is consistent with the results of other experiments (above and below).

Bivariate Fit of Wound AUC By Dose Time (hr)



Linear Fit

Wound AUC = 19.696629 + 10.719933 Dose Time (hr)

Summary of Fit

RSquare	0.048696
RSquare Adj	0.018967
Root Mean Square Error	121.9634
Mean of Response	45.23529
Observations (or Sum Wgts)	34

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	24365.78	24365.8	1.6380
Error	32	476002.34	14875.1	Prob > F
C. Total	33	500368.12		0.2098

Parameter Estimates

Term		Estimate	Std Error	t Ratio	Prob>|t|
Intercept		19.696629	28.9081	0.68	0.5005
Dose Time (hr)		10.719933	8.3759	1.28	0.2098

I analyzed the data to see if there was a relationship between dose and AUC or wound incidence. There was none in this experiment, 63.5 mg/kg of dexrazoxane IP given immediately after 3 mg/kg of Doxorubicin provided the same protection as 125 mg/kg and 250 mg/kg. Likewise, three doses at t = 0, 3 and six hours provided no better protection than a single dose at t=0.

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18) Evaluation of the effect of dexrazoxane administered as single doses or repeated doses on the experimental extravasation of 3 mg/kg of daunorubicin.

Major findings

Group	Wound induction	Wound prevention regimen
1	Daunorubicin 3 mg/kg SC	Isotonic saline IP t=0
2	Daunorubicin 3 mg/kg SC	62.5 mg/kg Dexrazoxane IP t=0
3	Daunorubicin 3 mg/kg SC	125 mg/kg Dexrazoxane IP t=0
4	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP t=0
5	Daunorubicin 3 mg/kg SC	62.5 mg/kg Dexrazoxane IP t=0, 3, and 6 hr total dose 187.5 mg/kg
6	Daunorubicin 3 mg/kg SC	125 mg/kg Dexrazoxane IP t=0, 3 and 6 hr total dose 375 mg/kg
7	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP t=0 d0, d1, and d2 total dose 750 mg/kg

Formulation Isotonic saline

Methods Mice were examined for skin wounds for 35 days
The investigators plotted study day against wound size and calculated the area under this curve (AUC). The mean AUC values in the table below are those calculated by the investigators. They do not include 0 values.

Results

One mouse died in treatment group 7 during anesthesia. The investigators consider these deaths likely due to hypothermia during the procedure.

Group	Wound induction	Wound prevention regimen	Mean AUC mm^2*day	N without lesions	N with lesions
1	Daunorubicin 3 mg/kg SC	Isotonic saline IP t=0	1546 ± 566	0	9
2	Daunorubicin 3 mg/kg SC	62.5 mg/kg Dexrazoxane IP t=0	745 ± 664	1	8
3	Daunorubicin 3 mg/kg SC	125 mg/kg Dexrazoxane IP t=0	669 ± 446	2	7
4	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP t=0	727 ± 452	3	6
5	Daunorubicin 3 mg/kg SC	62.5 mg/kg Dexrazoxane IP t=0, 3, and 6 hr total dose 187.5 mg/kg	No Wounds	9	0
6	Daunorubicin 3 mg/kg SC	125 mg/kg Dexrazoxane IP t=0, 3 and 6 hr total dose 375 mg/kg	252 ± 40	7	2
7	Daunorubicin 3 mg/kg SC	250 mg/kg Dexrazoxane IP t=0 d0, d1, and d2 total dose 750 mg/kg	170 ± 66	2	6

I analyzed the AUC data using JMP and obtained the following results.

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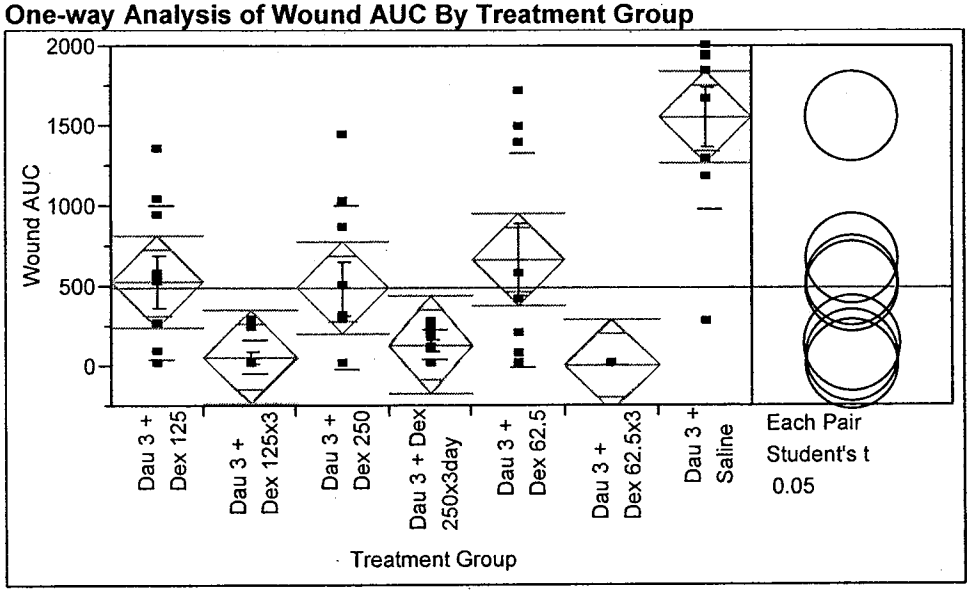
One-way Analysis of Wound AUC By Treatment Group



Means Comparisons

Comparisons for each pair using Student's t

t	Alpha
2.00404	0.05

Abs(Dif)-LSD	Dau3	Dau3+ dex62,5	Dau3+ dex125	Dau3+ dex250	Dau3+dex 250x3da	Dau3+ dex125 x3	Dau3+ dex62,5x 3
Dau3	-408.2	476.1	618.1	653.6	997.8	1082.1	1138.1
Dau3+dex62,5	476.1	-408.2	-266.2	-230.8	113.5	197.8	253.8
Dau3+dex125	618.1	-266.2	-408.2	-372.8	-28.5	55.8	111.8
Dau3+dex250	653.6	-230.8	-372.8	-408.2	-64.0	20.4	76.4
Dau3+dex250x3da	997.8	113.5	-28.5	-64.0	-433.0	-349.0	-293.0
Dau3+dex125x3	1082.1	197.8	55.8	20.4	-349.0	-408.2	-352.2
Dau3+dex62,5x3	1138.1	253.8	111.8	76.4	-293.0	-352.2	-408.2

Positive values show pairs of means that are significantly different.

Level					Mean
Dau3	A				1546.3333
Dau3+dex62,5		B			662.0000
Dau3+dex125		B	C		520.0000
Dau3+dex250		B	C		484.5556
Dau3+dex250x3da			C	D	127.7500
Dau3+dex125x3				D	56.0000
Dau3+dex62,5x3				D	0.0000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	p-Value	Difference
Dau3	Dau3+dex62,5x3	1546.333	1138.13	1954.538	<.0001	
Dau3	Dau3+dex125x3	1490.333	1082.13	1898.538	<.0001	
Dau3	Dau3+dex250x3d	1418.583	997.82	1839.351	<.0001	

	a					
Dau3	Dau3+dex250	1061.778	653.57	1469.982	<.0001	
Dau3	Dau3+dex125	1026.333	618.13	1434.538	<.0001	
Dau3	Dau3+dex62,5	884.333	476.13	1292.538	<.0001	
Dau3+dex62,5	Dau3+dex62,5x3	662.000	253.80	1070.204	0.0020	
Dau3+dex62,5	Dau3+dex125x3	606.000	197.80	1014.204	0.0043	
Dau3+dex62,5	Dau3+dex250x3da	534.250	113.48	955.017	0.0138	
Dau3+dex125	Dau3+dex62,5x3	520.000	111.80	928.204	0.0135	
Dau3+dex250	Dau3+dex62,5x3	484.556	76.35	892.760	0.0209	
Dau3+dex125	Dau3+dex125x3	464.000	55.80	872.204	0.0266	
Dau3+dex250	Dau3+dex125x3	428.556	20.35	836.760	0.0400	
Dau3+dex125	Dau3+dex250x3da	392.250	-28.52	813.017	0.0671	
Dau3+dex250	Dau3+dex250x3da	356.806	-63.96	777.573	0.0949	
Dau3+dex62,5	Dau3+dex250	177.444	-230.76	585.649	0.3875	
Dau3+dex62,5	Dau3+dex125	142.000	-266.20	550.204	0.4887	
Dau3+dex250x3days	Dau3+dex62,5x3	127.750	-293.02	548.517	0.5454	
Dau3+dex250x3days	Dau3+dex125x3	71.750	-349.02	492.517	0.7339	
Dau3+dex125x3	Dau3+dex62,5x3	56.000	-352.20	464.204	0.7844	
Dau3+dex125	Dau3+dex250	35.444	-372.76	443.649	0.8625	

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
Dau3	9	494.000	54.8889	4.324
Dau3+dex125	9	322.000	35.7778	0.782
Dau3+dex125x3	9	160.000	17.7778	-2.532
Dau3+dex250	9	306.000	34.0000	0.453
Dau3+dex250x3days	8	208.000	26.0000	-0.941
Dau3+dex62,5	9	350.500	38.9444	1.369
Dau3+dex62,5x3	9	112.500	12.5000	-3.510

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
35.3144	6	<.0001

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19) Evaluation of the effect of systemic treatment with EDTA or amifostine on experimental extravasation of daunorubicin.

EDTA is an iron-chelating drug. Amifostine is a radical scavenger. In this experiment, investigators determined that these drugs given IP had no effect on wound formation after a single SC dose of daunorubicin. The experiment suggests that iron generated superoxide anion or other radicals do not mediate wound formation after daunorubicin injection. The lack of effect by EDTA is equivocal since this compound does not cross cell membranes.

Study number	SL193, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	December 3, 1999
GLP compliance	No

QA report	No
Drug	EDTA 62.5, 125 or 250 mg/kg or Amifostine 100 or 200 mg/kg
Methods	
Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam The mice were then injected with a 3 mg/kg daunorubicin then treated with EDTA or amifostine IP.

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20) Evaluation of the effect of systemic treatment with N-acetylcysteine or alpha-tocopherol IP on daunorubicin induced skin wounds.

Alpha-tocopherol and N-acetylcysteine are radical scavengers. In this experiment, investigators determined that these drugs given IP had no effect on wound formation after a single SC dose of daunorubicin. The experiment suggests that radicals do not mediate wound formation after daunorubicin injection.

Study number	SL198, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	January 19, 2000
GLP compliance	No
QA report	No
Drug	N-acetylcysteine 1000 or 5000 mg/kg or alpha-tocopherol 62.5, 125 mg/kg
Methods	
Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam The mice were then injected with a 3 mg/kg daunorubicin then treated with alpha-tocopherol or N-acetylcysteine IP.

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21) Evaluation of the effect of 50 or 100 mg/kg single dose Merbarone IP on daunorubicin induced skin wounds in mice.

Merbarone is a topoisomerase 2-alpha inhibitor. In this experiment, investigators determined that this drug given IP had no effect on wound formation after a single SC dose of daunorubicin. The experiment suggests that competitive inhibition of topoisomerase 2 may not be the mechanism of action of dexrazoxane or that merbarone and dexrazoxane have significantly

different pharmacokinetics and metabolism. Or the dose of merbarone may simply have been too low.

Study number	SL207, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	February 4, 2000
GLP compliance	No
QA report	No
Drug	Merbarone 50 or 100 mg/kg
Methods	
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam The mice were then injected with a 3 mg/kg daunorubicin then treated with Merbarone IP.

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22) Evaluation of the protection by three injections of dexrazoxane against 3 mg/kg doxorubicin or daunorubicin-induced skin necrosis in mice.

Major findings

The results in this experiment are sufficiently striking as not to require statistical analysis. All the mice given daunorubicin or doxorubicin followed by saline (controls) developed skin wounds. The mean of the wound area in the animals injected with daunorubicin was nearly three times greater than that in animals injected with doxorubicin. None of the animals treated with 62.5 mg/kg dexrazoxane IP at t=0, 3, and 6 hr (total dose 187.5 mg/kg) after injection with daunorubicin or doxorubicin developed skin wounds (N=18 in each group).

Study number	SL210, Volume 1
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	March 2, 2000
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam
Methods	
Doses	See table below
Species	Female B6D2F1 mice
Number	5 per saline control group, 18 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam The mice were then injected with a 3 mg/kg daunorubicin or Doxorubicin then treated as the following table shows

Group	Wound induction	Wound prevention regimen
1	Doxorubicin 3 mg/kg SC	Isotonic saline IP t=0
2	Daunorubicin 3 mg/kg SC	Isotonic saline IP t=0 IP t=0
3	Doxorubicin 3 mg/kg SC	62.5 mg/kg Dexrazoxane IP t=0, 3, and 6 hr total dose 187.5 mg/kg
4	Daunorubicin 3 mg/kg SC	62.5 mg/kg Dexrazoxane IP t=0, 3, and 6 hr total dose 187.5 mg/kg

Formulation Isotonic saline

Methods Mice were examined for skin wounds for 34 days
The investigators plotted study day against wound size and calculated the area under this curve (AUC). The mean AUC values in the table below are those calculated by the investigators. They do not include 0 values.

Results

Group	Wound induction	Wound prevention regimen	Mean AUC MM^2*day	N without lesions	N with lesions
1	Doxorubicin 3 mg/kg SC	Isotonic saline IP t=0	519 ± 215	0	5
2	Daunorubicin 3 mg/kg SC	Isotonic saline IP t=0	1366 ± 504	0	5
3	Doxorubicin 3 mg/kg SC	62.5 mg/kg Dexrazoxane IP t=0, 3, and 6 hr total dose 187.5 mg/kg	No Wounds	18	0
4	Daunorubicin 3 mg/kg SC	62.5 mg/kg Dexrazoxane IP t=0, 3, and 6 hr total dose 187.5 mg/kg	No Wounds	18	0

When I compared the wound incidence with Fisher's exact test, the treated groups were statistically different from their respective controls with a p value of < 0.00003.

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23) Evaluation of the cooling on skin wounds induced by subcutaneous daunorubicin injection in mice.

Major findings

None of the mice treated with dexrazoxane (62.5 mg/kg q3hX3, total dose 187.5 mg/kg) and an ice pack developed skin wounds. All of the mice treated only with an ice pack developed skin wounds at the daunorubicin SC injection site. The maximum area of the wounds ranged between 36 and 100 mm^2. The wounds reached their maximum severity at about day seven and began to resolve about day 23. All wounds healed by day 32.

Study number SL214, Volume 2
Conducting laboratory TopoTarget A/S, Copenhagen, Denmark
Date of study initiation March 2, 2000
GLP compliance No
QA report No
Drug Dexrazoxane hydrochloride, Batch "not available"

Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam At time 0, mice were injected SC with daunorubicin 3 mg/kg After daunorubicin treatment the four groups were injected immediately afterwards at the initial injection site with Control – isotonic saline Group 2 – dexrazoxane 50 mg/kg Group 3 – Na_2EDTA 50 mg/kg Group 4 – N-acetylcysteine 200 mg/kg
Formulation	Isotonic saline, 1.2 mg/mL
Methods	Mice were examined for skin wounds for 37 days The investigators plotted study day against wound size and calculated the area under this curve (AUC)

Results

Group	Treatment	Mean AUC mm^2*days	Number without wounds	Number with wounds
Control	Isotonic saline	1154 ± 293	0	9
2	Dexrazoxane 50 mg/kg SC	241 ± 210	5	4
3	EDTA 50 mg/kg	1345 ± 546	0	9
4	N-acetylcysteine 200 mg/kg	1360 ± 594	0	9

I analyzed the wound incidence by Fisher's exact test. The incidence in the dexrazoxane treated group differs from that in the saline control with a p value of < 0.015.

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25) Evaluation of the effects of the double ring-opened derivate (sic) of dexrazoxane, ADR-925, intralesionally and systemically on daunorubicin-induced skin necrosis in mice.

Major findings

ADR-925 the major double ring-opened metabolite of dexrazoxane neither caused skin lesions when injected subcutaneously nor prevented wounds induced by a subcutaneous injection of daunorubicin. Thus, the metabolite is probably not responsible for prevention of daunorubicin skin damage. But the results are somewhat equivocal since this metabolite probably does not cross cell membranes.

Study number	SL224, Volume 2
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	May 9, 2000
GLP compliance	No
QA report	No
Drug	ADR-925, Batch "not available"
Methods	
Doses	see below
Species	Female B6D2F1 mice

Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam Except in group 2 (ADR-925 control), all mice were injected at At time 0, SC with daunorubicin 3 mg/kg After daunorubicin treatment the four groups were injected immediately afterwards with Control – isotonic saline Group 2 – ADR-925 SC 50 mg/kg (no daunorubicin) Group 3 – ADR-925 50 mg/kg in the lesion site Group 4 – ADR-925 62.5 mg/kg IP at t = 0 and 3 hr and at t = 6 hr with 250 mg/kg Group 5 – ADR-925 250 mg/kg IP at t = 0
Formulation	Isotonic saline, 1.2 mg/mL
Methods	Mice were examined for skin wounds for 38 days The investigators plotted study day against wound size and calculated the area under this curve (AUC)

Results

Group	Treatment	Mean AUC mm^2*days	Number without wounds	Number with wounds
Control	Isotonic saline	1238 ± 428	0	9
2	ADR-925 control	No wounds	9	0
3	ADR-925 50 mg/kg	1296 ± 513	0	9
4	ADR-925 repeat dose	1323 ± 359	0	9
5	ADR-925 250 mg/kg	1436 ± 437	0	9

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26) Evaluation of the effects of different doses of dexrazoxane on experimental extravasation of 3 mg/kg or 5 mg/kg of epirubicin in mice.

Major findings

Epirubicin did not produce skin wounds as consistently as did daunorubicin nor were the wounds as severe as measured by area under the curve (about 1200 mm^2*days for daunorubicin and about 400 mm^2*days for epirubicin). IP treatment with dexrazoxane had no statistically significant effect on the formation of wounds caused by epirubicin.

Study number	SL237, Volume 2
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	November 15, 2000
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam
Methods	
Doses	See below
Species	Female B6D2F1 mice

Number 9 per treatment group
Schedule Mice were anaesthetized with fentanyl, fluanison and midazolam
All mice were injected at t = 0, SC with epirubicin, 3 or 5 mg/kg

Group	Skin wound injection of Epirubicin mg/kg	Treatment	Time of injections
1	3	Isotonic saline, IP	T = 0
2	5	Isotonic saline, IP	T = 0
3	3	62.5 mg/kg Dexrazoxane IP	T = 0, 3 hr, 6 hr
4	3	125 mg/kg Dexrazoxane IP	T = 0, 3 hr, 6 hr
5	5	62.5 mg/kg Dexrazoxane IP	T = 0, 3 hr, 6 hr
6	5	125 mg/kg Dexrazoxane IP	T = 0, 3 hr, 6 hr

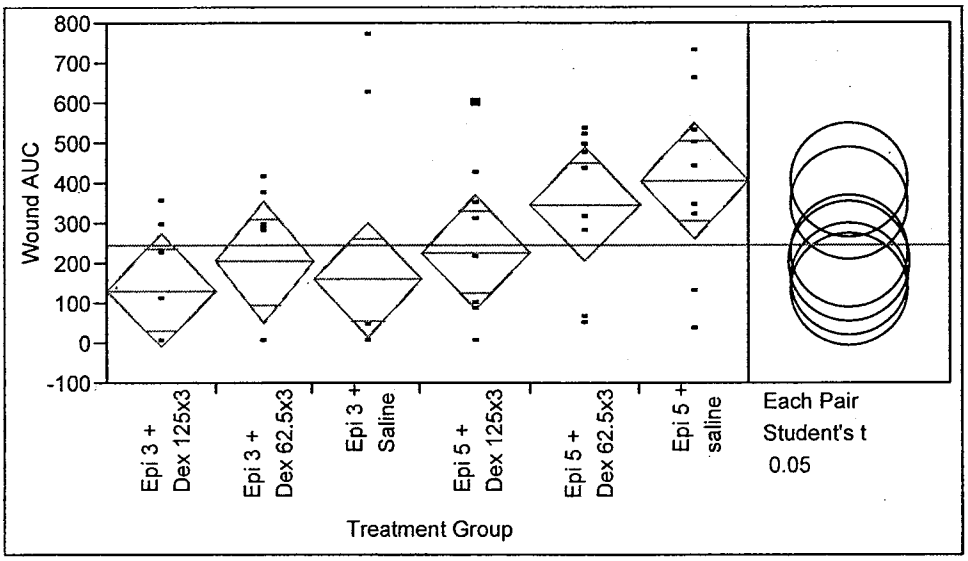
Formulation Isotonic saline, 1.2 mg/mL
Methods Mice were examined for skin wounds for 38 days
The investigators plotted study day against wound size and calculated the area under this curve (AUC)

Results

Group	Epirubicin MG/KG	Treatment	Time of injections	Mean AUC MM^2*days	N without lesions	N with lesions
1	3	Isotonic saline	T = 0	474 ± 385	6	3
2	5	Isotonic saline	T = 0	405 ± 229	0	9
3	3	62.5 mg/kg Dexrazoxane	T = 0, 3 hr, 6 hr	326 ± 59	3	5
4	3	125 mg/kg Dexrazoxane	T = 0, 3 hr, 6 hr	238 ± 91	4	5
5	5	62.5 mg/kg Dexrazoxane	T = 0, 3 hr, 6 hr	347 ± 189	0	9
6	5	125 mg/kg Dexrazoxane	T = 0, 3 hr, 6 hr	292 ± 182	2	7

My analysis of this data in JMP showed no statistically significant differences between a treatment group and its appropriate control.

Oneway Analysis of Wound AUC By Treatment Group



Missing Rows

1

Oneway Anova Summary of Fit

Rsquare	0.194912
Adj Rsquare	0.109264
Root Mean Square Error	214.2937
Mean of Response	246.4717
Observations (or Sum Wgts)	53

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment Group	5	522530.1	104506	2.2757	0.0621
Error	47	2158325.1	45922		
C. Total	52	2680855.2			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Epi 3 + Dex 125x3	9	132.444	71.431	-11.3	276.15
Epi 3 + Dex 62.5x3	8	204.000	75.764	51.6	356.42
Epi 3 + Saline	9	158.222	71.431	14.5	301.92
Epi 5 + Dex 125x3	9	227.222	71.431	83.5	370.92
Epi 5 + Dex 62.5x3	9	347.111	71.431	203.4	490.81
Epi 5 + saline	9	405.111	71.431	261.4	548.81

Std Error uses a pooled estimate of error variance

Means Comparisons
Comparisons for each pair using Student's t

t	Alpha
2.01174	0.05

Abs(Dif)-LSD	Epi 5 + saline	Epi 5 + Dex 62.5x3	Epi 5 + Dex 125x3	Epi 3 + Dex 62.5x3	Epi 3 + Saline	Epi 3 + Dex 125x3
Epi 5 + saline	-203.22	-145.22	-25.34	-8.37	43.66	69.44
Epi 5 + Dex 62.5x3	-145.22	-203.22	-83.34	-66.37	-14.34	11.44
Epi 5 + Dex 125x3	-25.34	-83.34	-203.22	-186.26	-134.22	-108.45
Epi 3 + Dex 62.5x3	-8.37	-66.37	-186.26	-215.55	-163.70	-137.92
Epi 3 + Saline	43.66	-14.34	-134.22	-163.70	-203.22	-177.45
Epi 3 + Dex 125x3	69.44	11.44	-108.45	-137.92	-177.45	-203.22

Positive values show pairs of means that are significantly different.

Level				Mean
Epi 5 + saline	A			405.11111
Epi 5 + Dex 62.5x3	A	B		347.11111
Epi 5 + Dex 125x3	A	B	C	227.22222
Epi 3 + Dex 62.5x3	A	B	C	204.00000
Epi 3 + Saline		B	C	158.22222
Epi 3 + Dex 125x3			C	132.44444

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	p-Value	Difference
Epi 5 + saline	Epi 3 + Dex 125x3	272.6667	69.443	475.8908	0.0096	
Epi 5 + saline	Epi 3 + Saline	246.8889	43.665	450.1130	0.0183	
Epi 5 + Dex 62.5x3	Epi 3 + Dex 125x3	214.6667	11.443	417.8908	0.0389	
Epi 5 + saline	Epi 3 + Dex 62.5x3	201.1111	-8.367	410.5897	0.0595	
Epi 5 + Dex 62.5x3	Epi 3 + Saline	188.8889	-14.335	392.1130	0.0677	

Level	- Level	Difference	Lower CL	Upper CL	p-Value	Difference
Epi 5 + saline	Epi 5 + Dex 125x3	177.8889	-25.335	381.1130	0.0848	
Epi 5 + Dex 62.5x3	Epi 3 + Dex 62.5x3	143.1111	-66.367	352.5897	0.1758	
Epi 5 + Dex 62.5x3	Epi 5 + Dex 125x3	119.8889	-83.335	323.1130	0.2413	
Epi 5 + Dex 125x3	Epi 3 + Dex 125x3	94.7778	-108.446	298.0019	0.3529	
Epi 3 + Dex 62.5x3	Epi 3 + Dex 125x3	71.5556	-137.923	281.0342	0.4953	
Epi 5 + Dex 125x3	Epi 3 + Saline	69.0000	-134.224	272.2241	0.4979	
Epi 5 + saline	Epi 5 + Dex 62.5x3	58.0000	-145.224	261.2241	0.5686	
Epi 3 + Dex 62.5x3	Epi 3 + Saline	45.7778	-163.701	255.2564	0.6622	
Epi 3 + Saline	Epi 3 + Dex 125x3	25.7778	-177.446	229.0019	0.7997	
Epi 5 + Dex 125x3	Epi 3 + Dex 62.5x3	23.2222	-186.256	232.7008	0.8245	

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
Epi 3 + Dex 125x3	9	172.500	19.1667	-1.677
Epi 3 + Dex 62.5x3	8	188.500	23.5625	-0.679
Epi 3 + Saline	9	168.000	18.6667	-1.785
Epi 5 + Dex 125x3	9	238.000	26.4444	-0.108
Epi 5 + Dex 62.5x3	9	319.000	35.4444	1.809
Epi 5 + saline	9	345.000	38.3333	2.432

1-way Test, Chi-Square Approximation

ChiSquare	DF	Prob>ChiSq
13.1800	5	0.0217

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27) Evaluation of the effects of late dexrazoxane treatment on day 4 or 6 or 8 after experimental daunorubicin extravasation in mice.

Major findings

Dexrazoxane given IP to mice 4, 6, or 8 days after SC daunorubicin treatment did not prevent or ameliorate the formation of skin lesions in mice.

Study number	SL238, Volume 2
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	October 12, 2000
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam
Methods	
Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam All mice were injected at t = 0, SC with daunorubicin 3 mg/kg

Group	Treatment with Dexrazoxane	Time of injections
1	Control isotonic saline, IP	Day 4
2	62.5 mg/kg q3hX3	Day 4
3	62.5 mg/kg q3hX3	Day 6
4	62.5 mg/kg q3hX3	Day 8

Formulation Isotonic saline, 1.2 mg/mL
Methods Mice were examined for skin wounds for 40 days
The investigators plotted study day against wound size and calculated the area under this curve (AUC)

Results

Group	Treatment with Dexrazoxane	Time of injections	Mean AUC mm^2*day	N without lesions	N with lesions
1	Control isotonic saline, IP	Day 4	868 ± 136	0	9
2	62.5 mg/kg q3hX3	Day 4	668 ± 116	0	7
3	62.5 mg/kg q3hX3	Day 6	749 ± 248	0	9
4	62.5 mg/kg q3hX3	Day 8	685 ± 191	0	9

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28) Evaluation of the effects of late dexrazoxane administered in different doses and schedules on experimental extravasation of 9 mg/kg epirubicin in mice.

Major findings

A relatively high dose of 9 mg/kg of epirubicin consistently caused skin lesions. Treatment with single or repeat doses of dexrazoxane did not prevent the formation of wounds but the severity of the wounds decreased with dose and dose intensity as measured by AUC.

Study number SL246, Volume 2
Conducting laboratory TopoTarget A/S, Copenhagen, Denmark
Date of study initiation March 6, 2001
GLP compliance No
QA report No
Drug Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam

Methods
Doses See table below
Species Female B6D2F1 mice
Number 9 per treatment group
Schedule Mice were anaesthetized with fentanyl, fluanison and midazolam
All mice were injected at t = 0, SC with epirubicin 9 mg/kg

Group	Treatment with Dexrazoxane	Time of injections
1	Control isotonic saline, IP	Day 0, immediately after injection
2	125 mg/kg IP	Day 0, immediately after injection
3	250 mg/kg IP	Day 0, immediately after injection
4	125 mg/kg IP	Day 0, t=0, 4 hr and 7 hr 375 mg/kg total dose
5	125 mg/kg IP	Day 0, t=0, 4 hr and 7 hr 8 AM, 12 AM, and 3 PM on d1 & d2 1125 mg/kg total dose

Formulation Isotonic saline, 1.2 mg/mL

Methods Mice were examined for skin wounds for 40 days
The investigators plotted study day against wound size and calculated the area under this curve (AUC)
Statistical comparisons were done with Student's T-test

Results

Group	Treatment with Dexrazoxane	Time of injections	Mean AUC MM^2*day	P value compared to control	N without lesions	N with lesions
1	Control isotonic saline, IP	Day 0, immediately after injection	900 ± 434		0	9
2	125 mg/kg IP	Day 0, immediately after injection	586 ± 201	0.27	0	9
3	250 mg/kg IP	Day 0, immediately after injection	562 ± 274	0.05	0	9
4	125 mg/kg IP	Day 0, t=0, 4 hr and 7 hr 375 mg/kg total dose	382 ± 200	0.01	1	8
5	125 mg/kg IP	Day 0, t=0, 4 hr and 7 hr 8 AM, 12 AM, and 3 PM on d1 & d2 1125 mg/kg total dose	304 ± 31	0.01	1	7

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29) Comparison of the effect of the dexrazoxane-containing drugs Cardioxane® and Zinecard® on Doxorubicin and daunorubicin-induced skin necrosis in mice.

Major findings

Cardioxane® and Zinecard® are marketed IV formulations of dexrazoxane in Europe and the United States respectively. Unfortunately, the results of this experiment were equivocal and do not contribute as much as one might hope to the body of evidence for the efficacy of dexrazoxane.

In the daunorubicin treated groups, there was no statistical difference in mean AUC between controls and animals treated with either Zinecard or Cardioxane, but this is due to the very large variability in the control group. The means are clearly different. There is no clear difference in the number of animals with wounds between the control group and the Cardioxane treated animals but there is a clear difference between controls and the Zinecard treated group. In

experiment SL214 (above) none of the dexrazoxane treated animals developed wounds. When compared to this first experiment with daunorubicin (SL214 above), the mean AUC obtained here is somewhat smaller and the variability is larger (1177 ± 406 vs 894 ± 521) though there is clearly no statistical difference between the two values. The investigators did not measure the wound size every day as in most of the previous experiments. This accounts for the decrease in AUC values.

In the Doxorubicin treated animals, the animals treated with Zinecard and Cardioxane clearly suffer less damage than the controls ($p = 0.04$, Student's t) as measured by AUC, but the number of animals that develop wounds is not statistically different ($p = 0.4$, Fisher's exact test). I suspect the efficacy may be affected by the formulation of the different drugs, but ultimately the problem here is experimental variability and two few animals in each treatment group.

Study number	SL248, Volume 2
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	May 14, 2001
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "not available" obtained as Cardioxane®, Chiron BV Amsterdam and as Zinecard, Pharmacia SP, Albuquerque, NM
Methods	
Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam All mice were injected at t = 0, SC with daunorubicin or Doxorubicin 3 mg/kg, then treated with saline, Zinecard Cardioxane IP at time = 0, 3hr and 6 hr (three injections)

Group	Skin wound induction	Treatment to prevent wound formation IP
1	3 mg/kg Daunorubicin SC	Isotonic saline
2	3 mg/kg Daunorubicin SC	62.5 mg/kg Zinecard, total dose 187.5 mg/kg
3	3 mg/kg Daunorubicin SC	62.5 mg/kg Cardioxane, total dose 187.5 mg/kg
4	3 mg/kg Doxorubicin SC	Isotonic saline
5	3 mg/kg Doxorubicin SC	62.5 mg/kg Zinecard, total dose 187.5 mg/kg
6	3 mg/kg Doxorubicin SC	62.5 mg/kg Cardioxane, total dose 187.5 mg/kg

Formulation	Isotonic saline, 1.2 mg/mL
Methods	Mice were examined for skin wounds for 40 days The investigators plotted study day against wound size and calculated the area under this curve (AUC) Statistical comparisons were done with Student's T-test
Results	

Group	Skin Wound Induction	Treatment to prevent wound formation	Mean AUC mm^2*day	P value compared to control	N without lesions	N with lesions
1	3 mg/kg Daunorubicin SC	Isotonic saline	895 ± 521		1	8
2	3 mg/kg Daunorubicin SC	62.5 mg/kg Zinecard, total dose 187.5 mg/kg	251 ± 120	0.94	5	4
3	3 mg/kg Daunorubicin SC	62.5 mg/kg Cardioxane, total dose 187.5 mg/kg	252 ± 68	0.94	2	7
4	3 mg/kg Doxorubicin SC	Isotonic saline	663 ± 249		2	9
5	3 mg/kg Doxorubicin SC	62.5 mg/kg Zinecard, total dose 187.5 mg/kg	246 ± 152	0.04	3	6
6	3 mg/kg Doxorubicin SC	62.5 mg/kg Cardioxane, total dose 187.5 mg/kg	145 ± 142	0.04	3	6

The sponsor has not submitted the AUC data for this experiment so I cannot do an independent analysis. The statistics above are those of the sponsor.

Zinecard formulation from the drug label

ZINECARD is available in 250 mg and 500 mg single use only vials. Each 250 mg vial contains dexrazoxane hydrochloride equivalent to 250 mg dexrazoxane. Hydrochloric Acid, NF is added for pH adjustment. When reconstituted as directed with the 25 mL vial of 0.167 Molar (M/6) Sodium Lactate Injection, USP diluent provided, each mL contains: 10 mg dexrazoxane. The pH of the resultant solution is 3.5 to 5.5. Each 500 mg vial contains dexrazoxane hydrochloride equivalent to 500 mg dexrazoxane. Hydrochloric Acid, NF is added for pH adjustment. When reconstituted as directed with the 50 mL vial of 0.167 Molar (M/6) Sodium Lactate Injection, USP diluent provided, each mL contains: 10 mg dexrazoxane. The pH of the resultant solution is 3.5 to 5.5.

Cardioxane formulation

Cardioxane is also supplied as the hydrochloride salt; I could not find the exact formulation. The investigators in this study reconstituted both drugs in isotonic saline for injection, not the sodium lactate to a concentration of 6.25 mg/mL not the 10 mg/mL recommended in the Zinecard label. I cannot determine from this report what effect these changes made on the efficacy of Cardioxane or Zinecard.

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30) Study of the effect of dexrazoxane on 2.5 mg/kg or 5 mg/kg Mitoxantrone-induced skin wounds in mice.

Major findings

Wounds formed in animals injected with 2.5 mg/kg mitoxantrone SC were clearly smaller in mice treated with dexrazoxane (62.5 mg/kg q3hX3, total dose 187.5 mg/kg) than in controls (saline). The AUC in the controls was 1319 ± 427 verses 440 ± 318 mm^2*day in the treated animals ($p < 0.0001$). But, mitoxantrone produced wounds more consistently than daunorubicin (SL214 above); all the control animals developed wounds as with daunorubicin above, whereas 7 of 9 animals treated with both mitoxantrone and dexrazoxane developed

Group	Skin wound induction	Treatment to prevent wound formation IP at t = 0, 3 hr and 6 hr	Mean AUC mm^2*day	P value compared to control	N without lesions	N with lesions
1	2.5 mg/kg Mitoxantrone SC	Isotonic saline	1319 ± 427		0	9
2	2.5 mg/kg Mitoxantrone SC	62.5 mg/kg Dexrazoxane, total dose 187.5 mg/kg	440 ± 318	$p < 0.0001$	2	7
3	5 mg/kg Mitoxantrone SC	Isotonic saline	2140 ± 572		0	9
4	5 mg/kg Mitoxantrone SC	62.5 mg/kg Dexrazoxane, total dose 187.5 mg/kg	1386 ± 208	$p = 0.06$	0	9

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31) Study of the effect of dexrazoxane given on day 0 and 3 after 1 and 3 mg/kg experimental Mitoxantrone extravasation in mice.

Major findings

Three 62.5 mg/kg doses of dexrazoxane IP on day 0 and 3 (six doses total, 375 mg/kg) after 1 mg/kg of mitoxantrone SC did not significantly affect the incidence of wound formation but it did significantly diminish the size of the wounds. Three 62.5 mg/kg doses of dexrazoxane IP on day 0 and 3 (six doses total, 375 mg/kg) after 3 mg/kg of mitoxantrone SC provided no protection against wound formation.

Study number	SL270, Volume 2
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	November 16, 2001
GLP compliance	No
QA report	No
Drug	dexrazoxane hydrochloride, Batch TC01B23-2/7 obtained as Cardioxane®, Chiron BV Amsterdam

Methods

Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group
Schedule	Mice were anaesthetized with fentanyl, fluanison and midazolam All mice were injected at t = 0, SC with Mitoxantrone 1 mg/kg or 3 mg/kg

Group	Skin wound induction	Treatment to prevent wound formation IP at t = 0, 3 hr and 6 hr on days 0 and 3 (six doses)
1	1 mg/kg Mitoxantrone SC	Isotonic saline
2	1 mg/kg Mitoxantrone SC	62.5 mg/kg Dexrazoxane, total dose 375 mg/kg
3	3 mg/kg Mitoxantrone SC	Isotonic saline
4	3 mg/kg Mitoxantrone SC	62.5 mg/kg Dexrazoxane, total dose 375 mg/kg

Formulation	Isotonic saline, 1.2 mg/mL
Methods	Mice were examined for skin wounds for 40 days The investigators plotted study day against wound size and calculated the area under this curve (AUC)

Statistical comparisons were done with Student's T-test

Results

Group	Skin wound induction	Treatment to prevent wound formation IP at t = 0, 3 hr and 6 hr on days 0 and 3	Mean AUC mm^2*day	P value compared to control relative to saline control	N without lesions	N with lesions
1	1 mg/kg Mitoxantrone SC	Isotonic saline	329 ± 57		6	3
2	1 mg/kg Mitoxantrone SC	62.5 mg/kg Dexrazoxane, total dose 375 mg/kg	89 ± 21	p = 0.47[a] Sponsor's p = 0.017 mine	4	5
3	3 mg/kg Mitoxantrone SC	Isotonic saline	897 ± 554		0	9
4	3 mg/kg Mitoxantrone SC	62.5 mg/kg Dexrazoxane, total dose 375 mg/kg	700 ± 495	p = 0.37 Sponsor's 0.47 mine	1	8

a = I believe this is probably a typographical error

Dexrazoxane did not significantly change the incidence of wound formation after a dose of either 1 or 3 mg/kg (Fishers exact test p = 0.3, my calculation) though the data suggests a trend to greater wound formation with increased dose. But a 3 mg/kg dose definitely caused larger wounds than the 1 mg/kg dose (p = 0.011, my calculation).

32) Study of the effect of intralesional hydrocortisone or topical treatment with DMSO alone or combined with dexrazoxane on skin wounds caused by experimental daunorubicin extravasation in mice.

Major findings

The data suggested the possibility that topical DMSO or intralesional hydrocortisone provided some protection from wound formation by SC daunorubicin but the effects failed to reach statistical significance due to high variability and small sample size. Both intralesional hydrocortisone and topical DMSO appeared to antagonize the protection afforded by dexrazoxane. In the case of topical DMSO the difference in wound incidence was statistically significant (Fisher's exact test, p = 0.002, my calculation).

Study number	SL271, Volume 2
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	November 25, 2003
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch TC01B23-2/7 obtained as Cardioxane®, Chiron BV Amsterdam

Methods

Doses	See table below
Species	Female B6D2F1 mice
Number	9 per treatment group

Schedule Mice were anaesthetized with fentanyl, fluanison and midazolam
All mice were injected at t = 0, SC with 3 mg/kg daunorubicin

Group	Skin wound induction	Treatment to prevent wound formation
1	3 mg/kg Daunorubicin SC	62.5 mg/kg Dexrazoxane IP at t = 0, 3 hr and 6 hr on days 0, total dose 185.5 mg/kg
2	3 mg/kg Daunorubicin SC	Hydrocortisone 125 mg/kg SC within the space of the daunorubicin injection (intralesionally) at t=0
3	3 mg/kg Daunorubicin SC	99% DMSO topically tid on day 0, 1, 2 and 3 (cotton swab)
4	3 mg/kg Daunorubicin SC	Isotonic saline at t=0 IP
5	3 mg/kg Daunorubicin SC	0.05 ml isotonic saline intralesionally at t=0
6	3 mg/kg Daunorubicin SC	Isotonic saline topically tid on day 0, 1, 2, and 3 (cotton swab)
7	3 mg/kg Daunorubicin SC	62.5 mg/kg Dexrazoxane IP at t = 0, 3 hr and 6 hr on days 0, total dose 185.5 mg/kg plus 125 mg/kg hydrocortisone intralesionally at t=0
8	3 mg/kg Daunorubicin SC	62.5 mg/kg Dexrazoxane IP at t = 0, 3 hr and 6 hr on days 0, total dose 185.5 mg/kg plus 99% DMSO topically tid on days 0, 1, 2, and 3

Formulation Isotonic saline, 1.2 mg/mL
Methods Mice were examined for skin wounds for 40 days
The investigators plotted study day against wound size and calculated the area under this curve (AUC)
Statistical comparisons were done with Student's T-test

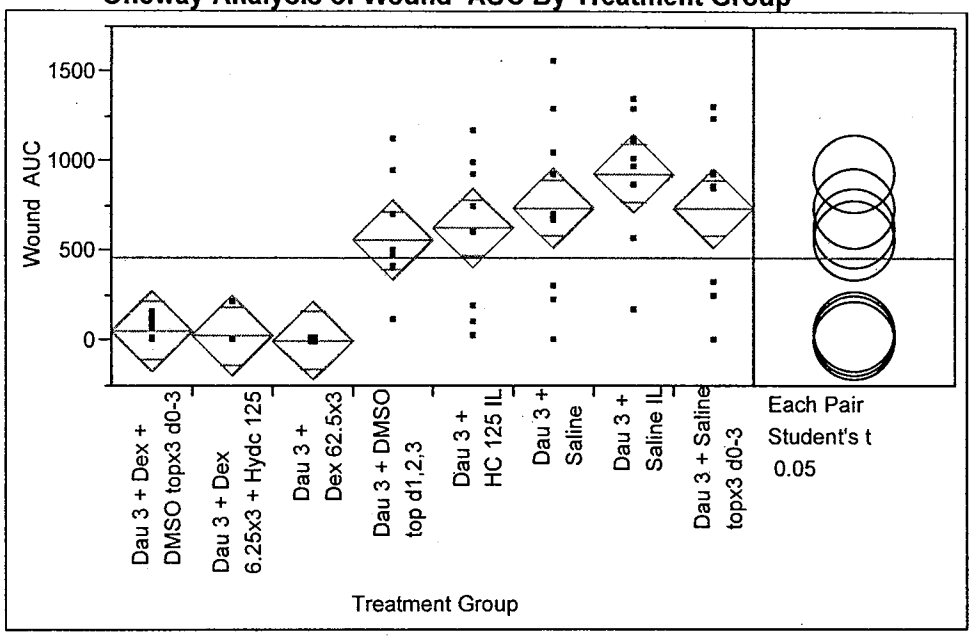
Results

Group	Skin wound induction	Treatment to prevent wound formation IP	Mean AUC mm^2*day	P value	N without lesions	N with lesions
1	3 mg/kg Daunorubicin SC	62.5 mg/kg Dexrazoxane	No wounds		9	0
2	3 mg/kg Daunorubicin SC	Hydrocortisone 125 mg/kg SC	630±431	0.11[a]	0	9
3	3 mg/kg Daunorubicin SC	99% DMSO topically	557±307	0.41[b]	0	9
4	3 mg/kg Daunorubicin SC	Isotonic saline at t=0 IP	831±462		1	8
5	3 mg/kg Daunorubicin SC	0.05 ml isotonic saline intralesionally at t=0	933±371		0	9
6	3 mg/kg Daunorubicin SC	Isotonic saline topically tid on day 0, 1, 2, and 3	827±378		1	8
7	3 mg/kg Daunorubicin SC	62.5 mg/kg Dexrazoxane IP plus 125 mg/kg hydrocortisone intralesionally at t=0	204		8	1
8	3 mg/kg Daunorubicin SC	62.5 mg/kg Dexrazoxane IP plus 99% DMSO topically tid	71±56	0.0001[c]	2	7

a = compared with control group 5
b = compared with control group 6
c = compared to treatment group 3

I did the following analysis in JMP. It shows that all the groups treated with dexrazoxane had smaller wound AUC means than the groups treated with dexrazoxane. There were no statistically significant differences among the means of the treated groups.

Oneway Analysis of Wound AUC By Treatment Group



Oneway Anova
Summary of Fit

Rsquare	0.553869
Adj Rsquare	0.505073
Root Mean Square Error	333.5511
Mean of Response	458.9792
Observations (or Sum Wgts)	72

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment Group	7	8839932	1262847	11.3508	<.0001
Error	64	7120405	111256		
C. Total	71	15960337			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Dau 3 + Dex + DMSO topx3 d0-3	9	55.333	111.18	-166.8	277.4
Dau 3 + Dex 6.25x3 + Hydc 125	9	22.722	111.18	-199.4	244.8
Dau 3 + Dex 62.5x3	9	0.000	111.18	-222.1	222.1
Dau 3 + DMSO top d1,2,3	9	556.667	111.18	334.6	778.8
Dau 3 + HC 125 IL	9	629.778	111.18	407.7	851.9
Dau 3 + Saline	9	738.833	111.18	516.7	960.9
Dau 3 + Saline IL	9	933.278	111.18	711.2	1155.4
Dau 3 + Saline topx3 d0-3	9	735.222	111.18	513.1	957.3

Std Error uses a pooled estimate of error variance

Means Comparisons
Comparisons for each pair using Student's t

t Alpha
1.99773 0.05

Abs(Dif)-LSD	Dau 3 + Saline IL	Dau 3 + Saline	Dau 3 + Saline topx3 d0-3	Dau 3 + HC 125 IL	Dau 3 + DMSO top d1,2,3	Dau 3 + Dex + DMSO topx3 d0-3	Dau 3 + Dex 6.25x3 + Hydc 125	Dau 3 + Dex 62.5x3

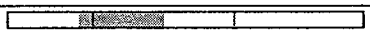
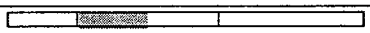
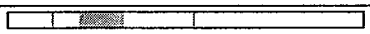
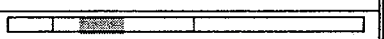
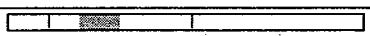
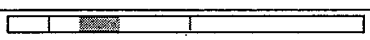
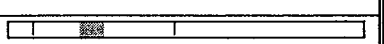
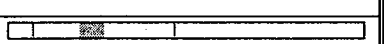
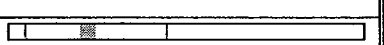
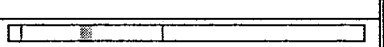
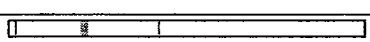
Dau 3 + Saline IL	-314.12	-119.67	-116.06	-10.62	62.49	563.83	596.44	619.16
Dau 3 + Saline	-119.67	-314.12	-310.51	-205.06	-131.95	369.38	401.99	424.72
Dau 3 + Saline topx3 d0-3	-116.06	-310.51	-314.12	-208.67	-135.56	365.77	398.38	421.10
Dau 3 + HC 125 IL	-10.62	-205.06	-208.67	-314.12	-241.01	260.33	292.94	315.66
Dau 3 + DMSO top d1,2,3	62.49	-131.95	-135.56	-241.01	-314.12	187.22	219.83	242.55
Dau 3 + Dex + DMSO topx3 d0-3	563.83	369.38	365.77	260.33	187.22	-314.12	-281.51	-258.78
Dau 3 + Dex 6.25x3 + Hydc 125	596.44	401.99	398.38	292.94	219.83	-281.51	-314.12	-291.40
Dau 3 + Dex 62.5x3	619.16	424.72	421.10	315.66	242.55	-258.78	-291.40	-314.12

Positive values show pairs of means that are significantly different.

Level				Mean
Dau 3 + Saline IL	A			933.27778
Dau 3 + Saline	A	B		738.83333
Dau 3 + Saline topx3 d0-3	A	B		735.22222
Dau 3 + HC 125 IL	A	B		629.77778
Dau 3 + DMSO top d1,2,3		B		556.66667
Dau 3 + Dex + DMSO topx3 d0-3			C	55.33333
Dau 3 + Dex 6.25x3 + Hydc 125			C	22.72222
Dau 3 + Dex 62.5x3			C	0.00000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	p-Value	Difference
Dau 3 + Saline IL	Dau 3 + Dex 62.5x3	933.2778	619.160	1247.396	<.0001	
Dau 3 + Saline IL	Dau 3 + Dex 6.25x3 + Hydc 125	910.5556	596.438	1224.674	<.0001	
Dau 3 + Saline IL	Dau 3 + Dex + DMSO topx3 d0-3	877.9444	563.826	1192.062	<.0001	
Dau 3 + Saline	Dau 3 + Dex 62.5x3	738.8333	424.715	1052.951	<.0001	
Dau 3 + Saline topx3 d0-3	Dau 3 + Dex 62.5x3	735.2222	421.104	1049.340	<.0001	
Dau 3 + Saline	Dau 3 + Dex 6.25x3 + Hydc 125	716.1111	401.993	1030.229	<.0001	
Dau 3 + Saline topx3 d0-3	Dau 3 + Dex 6.25x3 + Hydc 125	712.5000	398.382	1026.618	<.0001	

Dau 3 + Saline	Dau 3 + Dex + DMSO topx3 d0-3	683.5000	369.382	997.618	<.0001	
Dau 3 + Saline topx3 d0-3	Dau 3 + Dex + DMSO topx3 d0-3	679.8889	365.771	994.007	<.0001	
Dau 3 + HC 125 IL	Dau 3 + Dex 62.5x3	629.7778	315.660	943.896	0.0002	
Dau 3 + HC 125 IL	Dau 3 + Dex 6.25x3 + Hydc 125	607.0556	292.938	921.174	0.0003	
Dau 3 + HC 125 IL	Dau 3 + Dex + DMSO topx3 d0-3	574.4444	260.326	888.562	0.0005	
Dau 3 + DMSO top d1,2,3	Dau 3 + Dex 62.5x3	556.6667	242.549	870.785	0.0008	
Dau 3 + DMSO top d1,2,3	Dau 3 + Dex 6.25x3 + Hydc 125	533.9444	219.826	848.062	0.0012	
Dau 3 + DMSO top d1,2,3	Dau 3 + Dex + DMSO topx3 d0-3	501.3333	187.215	815.451	0.0022	
Dau 3 + Saline IL	Dau 3 + DMSO top d1,2,3	376.6111	62.493	690.729	0.0195	
Dau 3 + Saline IL	Dau 3 + HC 125 IL	303.5000	-10.618	617.618	0.0580	
Dau 3 + Saline IL	Dau 3 + Saline topx3 d0-3	198.0556	-116.062	512.174	0.2124	
Dau 3 + Saline IL	Dau 3 + Saline	194.4444	-119.674	508.562	0.2207	
Dau 3 + Saline	Dau 3 + DMSO top d1,2,3	182.1667	-131.951	496.285	0.2509	
Dau 3 + Saline topx3 d0-3	Dau 3 + DMSO top d1,2,3	178.5556	-135.562	492.674	0.2604	
Dau 3 + Saline	Dau 3 + HC 125 IL	109.0556	-205.062	423.174	0.4905	
Dau 3 + Saline topx3 d0-3	Dau 3 + HC 125 IL	105.4444	-208.674	419.562	0.5049	
Dau 3 + HC 125 IL	Dau 3 + DMSO top d1,2,3	73.1111	-241.007	387.229	0.6435	
Dau 3 + Dex + DMSO topx3 d0-3	Dau 3 + Dex 62.5x3	55.3333	-258.785	369.451	0.7261	
Dau 3 + Dex +	Dau 3 + Dex	32.6111	-281.507	346.729	0.8364	

DMSO topx3 d0-3	6.25x3 + Hydc 125					
Dau 3 + Dex 6.25x3 + Hydc 125	Dau 3 + Dex 62.5x3	22.7222	-291.396	336.840	0.8856	
Dau 3 + Saline	Dau 3 + Saline topx3 d0-3	3.6111	-310.507	317.729	0.9817	

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
Dau 3 + Dex + DMSO topx3 d0-3	9	206.000	22.8889	-2.104
Dau 3 + Dex 6.25x3 + Hydc 125	9	122.000	13.5556	-3.552
Dau 3 + Dex 62.5x3	9	99.000	11.0000	-3.948
Dau 3 + DMSO top d1,2,3	9	402.000	44.6667	1.259
Dau 3 + HC 125 IL	9	419.000	46.5556	1.552
Dau 3 + Saline	9	432.000	48.0000	1.776
Dau 3 + Saline IL	9	515.000	57.2222	3.207
Dau 3 + Saline topx3 d0-3	9	433.000	48.1111	1.793

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
46.9070	7	<.0001

---

33) Comparison of a one-day and three-day treatment schedule with 62.5 mg/kg dexrazoxane given three times with three hours interval of (*sic*) a large 6 mg/kg doxorubicin experimental extravasation in mice.

Major findings

Giving three treatments with 62.5 mg/kg dexrazoxane three hours apart on the day of dosing and the day after provided no greater protection against wound formation than did treatment on the day of dosing alone.

Study number	AT214, Volume 2
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	September 27, 2004
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch ADR064A obtained as Zinecard®, Pharmacia, SP, Albuquerque NM

Methods

Doses	See table below
Species	Female B6D2F1 mice
Number	25 per treatment group

Schedule Mice were anaesthetized with fentanyl, fluanison and midazolam
All mice were injected at t = 0, SC with 6 mg/kg Doxorubicin

Group	Skin wound induction	Treatment to prevent wound formation
1	6 mg/kg doxorubicin SC	0.2 mL isotonic Saline IP q3h X3 on day 0 (day of dosing)
2	6 mg/kg doxorubicin SC	Dexrazoxane 62.5 mg/kg IP q3h X3 on day 0 (day of dosing)
3	6 mg/kg doxorubicin SC	Dexrazoxane 62.5 mg/kg IP q3h X3 on day 0 & 1 (day of dosing & next day)

Formulation Isotonic saline, 1.2 mg/mL
Methods Mice were examined for skin wounds for 40 days
The investigators plotted study day against wound size and calculated the area under this curve (AUC)
Statistical comparisons were done with ANOVA on log transformed AUC values
Results

One mouse died in each of the treatment groups on day 1 of the experiment. The investigators do not describe these deaths or suggest a cause.

Group	Skin wound induction	Treatment to prevent wound formation IP	Mean AUC mm^2*day	P value	N without lesions	N with lesions	% with lesions
1	6 mg/kg doxorubicin SC	0.2 mL isotonic Saline IP q3h X3	1005±416		4	21	84
2	6 mg/kg doxorubicin SC	Dexrazoxane IP q3h X3 on day 0	150±160	< 0.001	22	2	8
3	6 mg/kg doxorubicin SC	Dexrazoxane IP q3h X3 on day 0 & 1	138±138	< 0.001	21	3	12

The treatment groups were different from saline control when compared by the Chi-square test $p < 0.0001$

The dataset the sponsor provided for this experiment was incomplete or incorrect. I could not do an analysis for this experiment.

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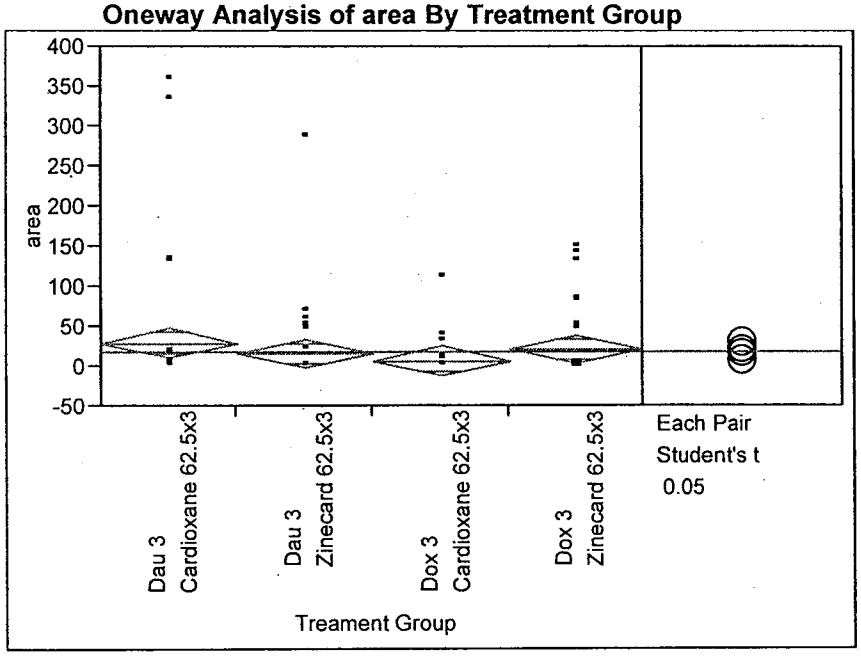
34) Effect *in vivo* (bioequivalence) of Zinecard and Cardioxane on daunorubicin and doxorubicin-induced skin wounds in mice.

Major findings

Three doses of 62.5 mg/kg (given t=0, 3 and 6 hours) of Zinecard and Cardioxane provided statistically equivalent protection against the formation of a skin wound after a single SC doses of daunorubicin or Doxorubicin (3 mg/kg).

Study number AT054 (daunorubicin) and AT055 (Doxorubicin), Volume 2
Conducting laboratory TopoTarget A/S, Copenhagen, Denmark
Date of study initiation February 6, 2003



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1

Oneway Anova
Summary of Fit

Rsquare	0.022897
Adj Rsquare	0.001183
Root Mean Square Error	54.93077
Mean of Response	17.23022
Observations (or Sum Wgts)	139

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treament Group	3	9545.54	3181.85	1.0545	0.3708
Error	135	407347.59	3017.39		
C. Total	138	416893.13			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Dau 3 Cardioxane 62.5x3	35	28.4571	9.2850	10.09	46.820
Dau 3 Zinecard 62.5x3	35	15.0286	9.2850	-3.33	33.391
Dox 3 Cardioxane 62.5x3	35	5.6143	9.2850	-12.75	23.977
Dox 3 Zinecard 62.5x3	34	19.8971	9.4205	1.27	38.528

Std Error uses a pooled estimate of error variance

Means Comparisons
Comparisons for each pair using Student's t

t	Alpha
1.97769	0.05

Abs(Dif)-LSD	Dau 3 Cardioxane 62.5x3	Dox 3 Zinecard 62.5x3	Dau 3 Zinecard 62.5x3	Dox 3 Cardioxane 62.5x3
Dau 3 Cardioxane 62.5x3	-25.969	-17.599	-12.540	-3.126
Dox 3 Zinecard 62.5x3	-17.599	-26.348	-21.291	-11.876
Dau 3 Zinecard 62.5x3	-12.540	-21.291	-25.969	-16.555
Dox 3 Cardioxane 62.5x3	-3.126	-11.876	-16.555	-25.969

Positive values show pairs of means that are significantly different.

Level		Mean
Dau 3 Cardioxane 62.5x3	A	28.457143
Dox 3 Zinecard 62.5x3	A	19.897059
Dau 3 Zinecard 62.5x3	A	15.028571
Dox 3 Cardioxane 62.5x3	A	5.614286

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	p-Value	Difference
Dau 3 Cardioxane 62.5x3	Dox 3 Cardioxane 62.5x3	22.84286	-3.1262	48.81187	0.0842	
Dox 3 Zinecard 62.5x3	Dox 3 Cardioxane 62.5x3	14.28277	-11.8765	40.44203	0.2822	
Dau 3 Cardioxane 62.5x3	Dau 3 Zinecard 62.5x3	13.42857	-12.5404	39.39758	0.3083	
Dau 3 Zinecard 62.5x3	Dox 3 Cardioxane 62.5x3	9.41429	-16.5547	35.38330	0.4746	
Dau 3 Cardioxane 62.5x3	Dox 3 Zinecard 62.5x3	8.56008	-17.5992	34.71935	0.5186	
Dox 3 Zinecard 62.5x3	Dau 3 Zinecard 62.5x3	4.86849	-21.2908	31.02775	0.7134	

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
Dau 3 Cardioxane 62.5x3	35	2548.00	72.8000	0.696
Dau 3 Zinecard 62.5x3	35	2414.00	68.9714	-0.253
Dox 3 Cardioxane 62.5x3	35	2317.00	66.2000	-0.945
Dox 3 Zinecard 62.5x3	34	2451.00	72.0882	0.508

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
1.2861	3	0.7324

---

35) Study of the extension of subcutaneous fibrosis in mouse skin 35 days after injection with 3 mg/kg daunorubicin subcutaneously and treatment with 250 mg/kg dexrazoxane or isotonic saline intraperitoneally.

Major findings

Treatment with dexrazoxane after a subcutaneous injection of daunorubicin greatly diminished the extent of microscopically observable fibrosis at the injection site (1.4 mm in the dexrazoxane treated group compared to 4.1 mm in the control). It also decreased the fibrosis grade observed in the underlying panniculus muscularis.

Study number	SL181, Volume 2
Conducting laboratory	TopoTarget A/S, Copenhagen, Denmark
Date of study initiation	October 1, 1999
GLP compliance	No
QA report	No
Drug	Dexrazoxane hydrochloride, Batch "Not available" obtained as Cardioxane®, Chiron BV, Amsterdam

Methods

Doses See table below
Species Female B6D2F1 mice
Number 9 controls, 18 treatment
Schedule Mice were anaesthetized with fentanyl, fluanison and midazolam
All mice were injected at t = 0, SC with 3 mg/kg daunorubicin

Group	Skin wound induction	Treatment to prevent wound formation
1	3 mg/kg Daunorubicin SC	Isotonic saline
2	3 mg/kg Daunorubicin SC	250 mg/kg Dexrazoxane IP

Formulation Isotonic saline, 1.2 mg/mL
Methods On day 33, the investigators removed the hair on the back of the mice chemically and assessed the skin lesions grossly. On day 35 they killed five mice from each group and assessed the wound for fibrosis microscopically, measuring its extent. The microscopic samples were blinded to the investigator assessing the damage. The investigator also assess the grade of fibrosis in the underlying panniculuus muscularis.

Results

Group	Skin wound induction	Treatment to prevent wound formation IP	Visible fibrosis on day 33	Extent of fibrosis (range in mm) determined in five mice from each group microscopically
1	3 mg/kg Daunorubicin SC	Isotonic saline	8/9	2.15 to 5.98
2	3 mg/kg Daunorubicin SC	250 mg/kg Dexrazoxane IP	0/18	0.68 to 2.02

	Panniculus muscularis fibrosis grade		
	0	1	2
Isotonic saline	0/5	2/5	3/5
250 mg/kg Dexrazoxane IP	2/5	3/5	0/5

The following analysis in JMP (mine) shows that the difference in the extent of fibrosis between treatment and control is highly significant.

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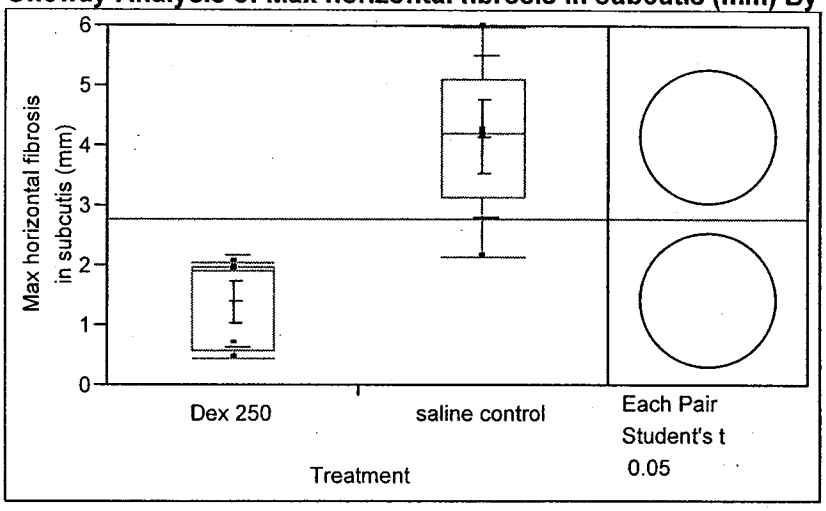
Oneway Analysis of Max horizontal fibrosis in subcutis (mm) By Treatment



Means and Std Deviations

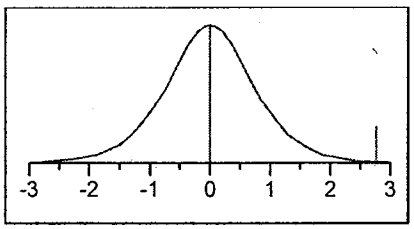
Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
Dex 250	5	1.39200	0.77056	0.34461	0.4352	2.3488
saline control	5	4.14400	1.35659	0.60668	2.4596	5.8284

t Test

saline control-Dex 250

Assuming unequal variances

Difference	2.75200	t Ratio	3.944248
Std Err Dif	0.69772	DF	6.337785
Upper CL Dif	4.43746	Prob > |t|	0.0068
Lower CL Dif	1.06654	Prob > t	0.0034
Confidence	0.95	Prob < t	0.9966



Means Comparisons

Comparisons for each pair using Student's t

t	Alpha
2.30600	0.05

Abs(Dif)-LSD	saline control	Dex 250
saline control	-1.6090	1.1430
Dex 250	1.1430	-1.6090

Positive values show pairs of means that are significantly different.

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
Dex 250	5	15.000	3.00000	-2.507
saline control	5	40.000	8.00000	2.507

2-Sample Test, Normal Approximation

S	Z	Prob>|Z|

S	Z	Prob>|Z|
40	2.50672	0.0122

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
6.8182	1	0.0090

Small sample sizes. Refer to statistical tables for tests, rather than large-sample approximations.

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Secondary Pharmacology

Secondary Pharmacology Summary

Doses of 50, 100 or 200 mg/kg (150, 300 or 600 mg/m^2) caused no behavioral or autonomic changes in mice in the Irwin standard test battery. A dose of 200 mg/kg of Cardioxane caused a moderate (28%) increase in hexobarbital-induced sleeping time. Lower doses caused no changes in sleeping time. This increase is possibly due to competitive metabolism. A dose of 50, 100 or 200 mg/kg of Cardioxane caused no significant changes in spontaneous locomotor activity over the first half of the one-hour observation period. The same doses caused no significant changes in motor coordination in trained mice (rotarod test) and no significant changes in intestinal motility. Doses of 50, 100 or 200 mg/kg caused no biologically significant changes in urine output relative to control. Neither did these single doses cause changes in urine electrolytes. Dexrazoxane caused no statistically or biologically significant changes in cardiac or respiratory parameters at single progressive doses of 25, 50 or 100 mg/kg (500, 1000, or 2000 mg/m^2) in anesthetized dogs. At the doses tested, dexrazoxane shows little secondary pharmacological activity.

Secondary Pharmacology Review

1) Assessment of the effects of using the Irwin dose-range in the mouse (*sic*).

Major findings

At doses of 50, 100 or 200 mg/kg (150, 300 or 600 mg/m^2) caused no behavioral or autonomic changes in mice.

Study number	EUC1/91577, Volume 2
Conducting laboratory	[redacted] b(4)
Date of study initiation	April 8, 1991
GLP compliance	Yes
QA report	Yes
Drug	Cardioxane®, Chiron BV, Amsterdam, Batch Q221289, purity >98%
Methods	
Doses	0, 50, 100 or 200 mg/kg (150, 300 or 600 mg/m^2)
Species	CD-1 mice
Number	4 per dose group
Schedule	single dose, IV tail vein, 1 mL/min, 10 mL/kg
Formulation	Isotonic saline
Methods	Observations for changes immediately after and at 15, 30, 60, 120, 240 min and 24 hours after injection for standard Irwin parameters
Results	No changes in any of the animals

2) ICF-187 (Cardioxane). Assessment of the effects on hexobarbital-induced sleeping time in the mouse.

Major findings

A dose of 200 mg/kg (600 mg/m^2) of Cardioxane caused a moderate (28%) increase in hexobarbital-induced sleeping time. Lower doses of 50 or 100 mg/kg caused no significant change in hexobarbital-induced sleeping time.

Study number	EUC2/91578, Volume 2
Conducting laboratory	[redacted] b(4)
Date of study initiation	April 16, 1991
GLP compliance	Yes
QA report	Yes
Drug	Cardioxane®, Chiron BV, Amsterdam, Batch Q221289, purity >98%
Methods	
Doses	0, 50, 100 or 200 mg/kg (150, 300 or 600 mg/m^2)
Species	CD-1 mice
Number	10 per dose group
Schedule	single dose, IV tail vein, 1 mL/min, 10 mL/kg
Formulation	Isotonic saline
Active Control	Chlorpromazine HCl
Hexobarbital	100 mg/kg IP
Methods	Sleeping time measured
Results	

Group		Group Mean Sleeping time (min ± SD)	p value relative to control
1	Vehicle	33 ± 11	
2	Dexrazoxane 50 mg/kg	37 ± 10	> 0.05
3	Dexrazoxane 100 mg/kg	35 ± 7	> 0.05
4	Dexrazoxane 200 mg/kg	43 ± 8	< 0.05
5	Chlorpromazine	56 ± 8	< 0.001

3) ICF-187 (Cardioxane). Assessment of the effects on spontaneous locomotor activity in the mouse

Major findings

A dose of 50, 100 or 200 mg/kg (150, 300 or 600 mg/m^2) of Cardioxane caused no significant changes in spontaneous locomotor activity over the first half of the one hour observation period. Nevertheless, there was a decrease in activity in the second half of the observation period. The decrease did not reach statistical significance and the investigators did not consider it biologically significant. Considering the unusual and unknown effects of this compound, I am not sure I agree. The decrease in activity shows a linear trend with time in all three-dose groups but not a dose effect. Dexrazoxane may cause some delayed sedation in mice.

Study number	EUC3/91519, Volume 2
Conducting laboratory	[redacted] b(4)
Date of study initiation	April 18, 1991

GLP compliance	Yes
QA report	Yes
Drug	Cardioxane®, Chiron BV, Amsterdam, Batch Q221289, purity >98%
Methods	
Doses	0, 50, 100 or 200 mg/kg (150, 300 or 600 mg/m^2)
Species	CD-1 mice
Number	4 subgroup of 4 mice per dose group (dosed at different times to compensate diurnal variation)
Schedule	single dose, IV tail vein, 1 mL/min, 10 mL/kg
Formulation	Isotonic saline
Active Control	Chlorpromazine HCl
Methods	Benwick Electronics activity platforms
Results	

Group		Mean activity					
	Observation period (min	5-15	15-25	25-35	35-45	45-55	55-65
1	Vehicle	1044 ± 391	909 ± 444	937 ± 589	846 ± 946	845 ± 1116	570 ± 880
2	Dexrazoxane 50 mg/kg	1121 ± 387	973 ± 314	944 ± 387	490 ± 315	146 ± 79	97 ± 56
3	Dexrazoxane 100 mg/kg	1279 ± 540 7	1064 ± 424	912 ± 424	636 ± 644	540 ± 733	396 ± 705
4	Dexrazoxane 200 mg/kg	1045 ± 454	912 ± 445	782 ± 411	544 ± 643	203 ± 291	135 ± 154
5	Chlorpromazine 3 mg/kg	163 ± 157	56 ± 77	19 ± 22	5 ± 7	4 ± 7	4 ± 4

4) ICF-187 (Cardioxane). Evaluation of effect on various cardiovascular and respiratory parameters in the anesthetized dog.

Major findings

Dexrazoxane caused no statistically or biologically significant changes in cardiac or respiratory parameters at single progressive doses of 25, 50 or 100 mg/kg (500, 1000, or 2000 mg/m^2).

Study number	EUC4/91579, Volume 2
Conducting laboratory	[redacted] b(4)
Date of study initiation	April 23, 1991
GLP compliance	Yes
QA report	Yes
Drug	Cardioxane®, Chiron BV, Amsterdam, Batch Q221289, purity >98%
Methods	
Doses	0, 25, 50 or 100 mg/kg (0, 500, 1000 or 2000 mg/m^2) given at 1 hour intervals with monitoring
Species	male beagle dog
Number	2
Schedule	single dose, IV tail vein, 1 mL/min, 10 mL/kg

Formulation	Isotonic saline
Anesthesia	induced with sodium thiopentone and maintained by IV α-chloralose
Methods	EKG, catheters, flow probes, tracheal cannula
Results	no significant dose related changes in cardiac or respiratory parameters

5) ICF-187 (Cardioxane). Assessment of the effects on motor coordination using the rotarod test in mice.

Major findings

A dose of 50, 100 or 200 mg/kg (150, 300 or 600 mg/m^2) of dexrazoxane caused no significant changes in motor coordination in trained mice.

Study number	EUC3/91536, Volume 2
Conducting laboratory	[redacted] b(4)
Date of study initiation	April 16, 1991
GLP compliance	Yes
QA report	Yes
Drug	Cardioxane®, Chiron BV, Amsterdam, Batch Q221289, purity >98%
Methods	
Doses	0, 50, 100 or 200 mg/kg (150, 300 or 600 mg/m^2)
Species	female CD-1 mice
Number	10 per dose group
Schedule	single dose, IV tail vein, 1 mL/min, 10 mL/kg
Formulation	Isotonic saline
Active Control	Mephenesin (80 mg/kg IV)
Methods	Rotarod test, 4 to 40 revolutions per minute over a 5 minute period
Results	

		Mean ± SD performance time (seconds)	
	Observation period	Pre-dose	Post dose
1	Vehicle	168 ± 48	210 ± 60
2	Dexrazoxane 50 mg/kg	171 ± 45	224 ± 63
3	Dexrazoxane 100 mg/kg	166 ± 52	208 ± 41
4	Dexrazoxane 200 mg/kg	165 ± 47	230 ± 44
5	Mephenesine 80 mg/kg	167 ± 46	21 ± 17 *

p < 0.0001 investigators calculation

6) ICF-187 (Cardioxane). Assessment of the effects on intestinal motility using the charcoal propulsion test in the mouse

Major findings

A dose of 50, 100 or 200 mg/kg (150, 300 or 600 mg/m^2) of dexrazoxane caused no significant changes in intestinal motility in mice.

Study number	EUC3/91580, Volume 2
Conducting laboratory	[redacted] b(4)
Date of study initiation	April 18, 1991

GLP compliance	Yes
QA report	Yes
Drug	Cardioxane®, Chiron BV, Amsterdam, Batch Q221289, purity >98%
Methods	
Doses	0, 50, 100 or 200 mg/kg (150, 300 or 600 mg/m^2)
Species	male CD-1 mice
Number	10 per dose group
Schedule	single dose, IV tail vein, 1 mL/min, 10 mL/kg
Formulation	Isotonic saline
Charcoal	0.25 mg of a 5% w/v suspension of charcoal in distilled water immediately after the drug dose
Active Control	Atropine sulphate, 5 mg/kg IV
Methods	distance charcoal bolus travels through the GI in 30 minutes
Results	

		Group mean distance traveled by charcoal as a percentage of total GI length (% ± SD)	Percent change relative to control
1	Vehicle	51 ± 6	
2	Dexrazoxane 50 mg/kg	46 ± 6	10
3	Dexrazoxane 100 mg/kg	52 ± 7	-2
4	Dexrazoxane 200 mg/kg	47 ± 9	9
5	Atropine 5 mg/kg	27 ± 5	47 *

$p < 0.001$ investigators calculation

7) **ICF-187 (Cardioxane). Assessment of the effects on urine volume and urinary electrolytes excretion in the rat.**

Major findings

A dose of 100 mg/kg (600 mg/m^2) of dexrazoxane caused a statistically significant decrease in urine volume output at 4 and 5 hours post dose. Doses of 50 or 200 mg/kg (300 or 1200 mg/m^2) did not cause changes in urine output relative to control so the effect was not dose dependent and thus probably not biologically significant. None of the three doses caused change in urine electrolytes.

Study number	EUC3/91581, Volume 2
Conducting laboratory	[redacted] b(4)
Date of study initiation	April 16, 1991
GLP compliance	Yes
QA report	Yes
Drug	Cardioxane®, Chiron BV, Amsterdam, Batch Q221289, purity >98%
Methods	
Doses	0, 50, 100 or 200 mg/kg (0, 300, 600 or 1200 mg/m^2)
Species	male Wistar rats
Number	8 per dose group
Schedule	single dose, IV tail vein, 1 mL/min, 10 mL/kg
Formulation	Isotonic saline
Active Control	Frusemide, 5 mg/kg IV
Methods	Urinary volume with time and urinary electrolytes
Results	

Group		Group mean total (cumulative) urine output (±SD) at time post dose					
	Observation period (min	1h	2h	3h	4h	5h	24h
1	Vehicle	3.1 ± 1.5	4.4 ± 0.6	4.8 ± 0.7	5.6 ± 0.6	5.8 ± 0.6	14 ± 3
2	Dexrazoxane 50 mg/kg	2.4 ± 1.0	4.0 ± 0.5	4.5 ± 1.1	4.9 ± 1.0	4.9 ± 1.0	16 ± 3
3	Dexrazoxane 100 mg/kg	3.3 ± 0.7	4.1 ± 1.2	4.2 ± 1.1	4.2 ± 1.1*	4.5 ± 1.2*	14 ± 3
4	Dexrazoxane 200 mg/kg	2.9 ± 1.5	4.4 ± 1.1	4.4 ± 1.0	4.6 ± 1.3	4.7 ± 1.2	15 ± 2
5	Chlorpromazine 3 mg/kg	8.2 ± 1.2***	9.3 ± 1.6***	9.6 ± 1.4***	9.7 ± 1.5***	10.0 ± 1.6***	17 ± 3*

* $p < 0.05$ investigators calculation
*** $p < 0.001$ investigators calculations

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Pharmacodynamic drug interactions

No studies submitted.

Pharmacokinetics and Toxicokinetics

Pharmacokinetics and Toxicokinetics Summary

Dexrazoxane plasma concentration decreases in three distinct phases, a rapid distribution phase that last for only a few minutes, an elimination phase lasting to about four hours, and a longer terminal elimination phase. The addition of doxorubicin significantly increases the exposure of dexrazoxane in male, but not female, rats. The two drugs possibly compete for some elimination process. Rats eliminated most of a dose of radioactivity associated with dexrazoxane in the urine within the first 8 hours after dosing (about 80% of total radioactivity). Elimination is negligible after that. Only 7 to 8% is found in the feces. The rats eliminated no significant amount of radioactivity in expired air and after 96 hours, they retained only about 1% in the carcass. High concentrations in the kidneys are consistent with a drug excreted predominantly in the urine. High concentrations in the liver are consistent with metabolism. The concentration in brain and eye is well below the concentration in plasma. The concentration in fat is also well below the concentration in plasma; the compound is not lipophilic and is not distributing to that compartment. Dexrazoxane is metabolized primarily to the open ring tetra-acetate. For further information, see the reviews of NDA 20-212 (appended).

Pharmacokinetics and Toxicokinetics Review

1) The excretion, metabolism and plasma kinetics of Cardioxane and total radioactivity following intravenous administration of ^{14}C-cardioxane to rats at a dose level of 20 mg/kg.

Major findings

Dexrazoxane plasma concentration decreases in three distinct phases, a rapid distribution phase that last for only a few minutes, an elimination phase lasting to about four hours, and a longer terminal elimination phase. The addition of doxorubicin significantly increases the exposure of dexrazoxane in male, but not female, rats. The two drugs possibly compete for some elimination process. Rats eliminated most of a dose of radioactivity associated with dexrazoxane in the urine within the first 8 hours after dosing (about 80%). Elimination is negligible after that. Only 7 to 8% is found in the feces. The rats eliminated no significant amount of radioactivity in expired air and after 96 hours, they retained only about 1% in the carcass. High concentrations in the kidneys are consistent with a drug excreted predominantly in the urine. High concentrations in the liver are consistent with metabolism. The concentration in brain and eye is well below the

concentration in plasma. The concentration in fat is also well below the concentration in plasma; the compound is not lipophilic and is not distributing to that compartment. Dexrazoxane is metabolized primarily to the open ring tetra-acetate.

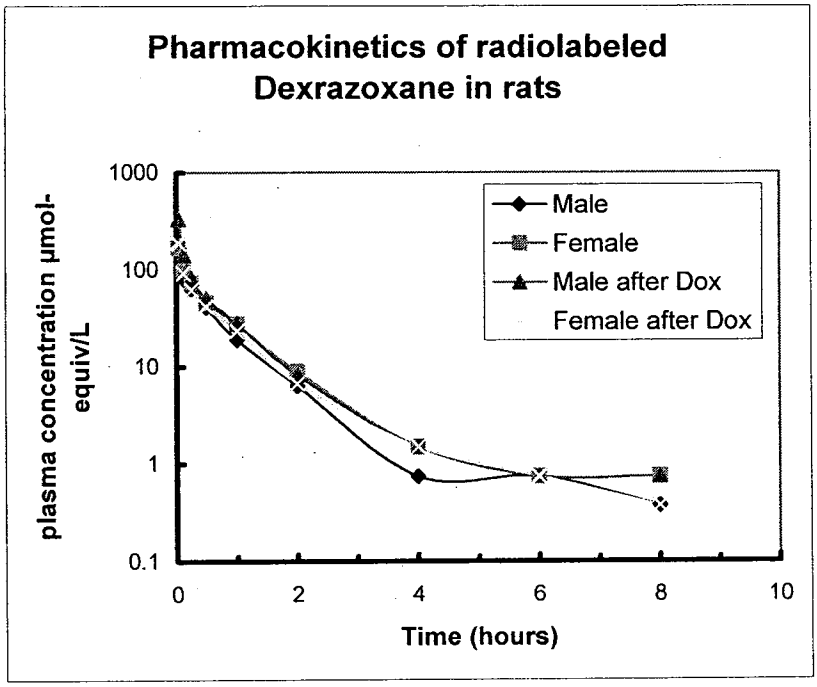
Study number	8248, Volume 3
Conducting laboratory	[redacted] b(4)
Date of study initiation	August 12, 2001
GLP compliance	Yes
QA report	Yes
Drug	Radio-labeled ^{14}C-dexrazoxane, batch CFQ.6444, 98.7% pure Specific activity 525 MBq/mmol, 14.2 mCi/mmol Non-radio-labeled dexrazoxane, batch Q221090
Doses	20 mg/kg, 120 mg/m^2
Species	male and female Sprague Dawley rats
Schedule	Single dose, IV tail vein in separate experiments (below)

Experiment	Descriptions	N
Phase 1	Plasma kinetics Dexrazoxane	4/sex
Phase 2	Plasma kinetics of Dexrazoxane after doxorubicin pretreatment (1 mg/kg 15 minutes before Dexrazoxane)	4/sex
Phase 3	Excretion/retention of total radioactivity	4/sex
Phase 4	Tissue distribution of total radioactivity	5/sex
Phase 5	Metabolic profiling	Animals from Phase 3 and 4
Phase 6	Provision of urine to the Sponsor	1 per sex

Formulation	Isotonic saline, 1.2 mg/mL
Methods	In phase 1 and 2 serial blood samples (0.2 m.) were drawn from the tail vein predose and at 2, 7, 15, and 30 minutes and 1, 2, 4, 6, 8 and 24 hr post dose, plasma was analyzed for total radioactivity by scintillation counting In phase 3 urine, feces, expired air, carcass and cage wash were collected and analyzed for total radioactivity by scintillation In phase 4 one male and one female was killed at 5 min. and 1, 6, 24 and 96 hours. Tissues were collected and analyzed for total radioactivity by scintillation. In phase 5, urine and feces from phase 3 animals and plasma from phase 4 animals were examined for metabolites by HPLC In phase 6, urine was non-radiolabeled dexrazoxane was collected and sent to [redacted] for analysis b(4) Statistical comparisons were done with Student's T-test

Plasma Pharmacokinetics (Phase 1 and 2)

The following graph (mine) shows that dexrazoxane plasma concentration decreases in three distinct phases, a rapid distribution phase that lasts for only a few minutes, an elimination phase lasting to about four hours, and a longer terminal elimination phase. In all cases, the concentration was below the limit of detection 24 hours after dosing.



The following table (mine) shows that male and female rats eliminate dexrazoxane similarly in the absence of Doxorubicin ($p = 0.13$), but the AUC in males is significantly higher than that in females in the presence of Doxorubicin ($p = 0.02$). The AUC in males in the presence of Doxorubicin is higher than that in males in the absence of Doxorubicin ($p = 0.014$). The investigators dismiss this difference and suggest that it is due to excess variation in the males receiving both drugs. Inspection of the individual data shows this not to be the case.

		Male	sd	Female	sd	Male after Dox	sd	Female after Dox	sd
AUC	µg base equiv*hr/ml	19.8	3.9	22.4	3.3	29.5	4	21.8	2.4
$t_{1/2}$	hr	~0.5		~0.7		~0.5		~0.7	

The following graph (mine) shows that the difference in AUC is due to higher concentrations in males receiving both drugs in the first 30 minutes. This suggests that the two drugs compete for some elimination process initially at high concentrations. I do not know if this is relevant to human elimination, I suspect it is not.

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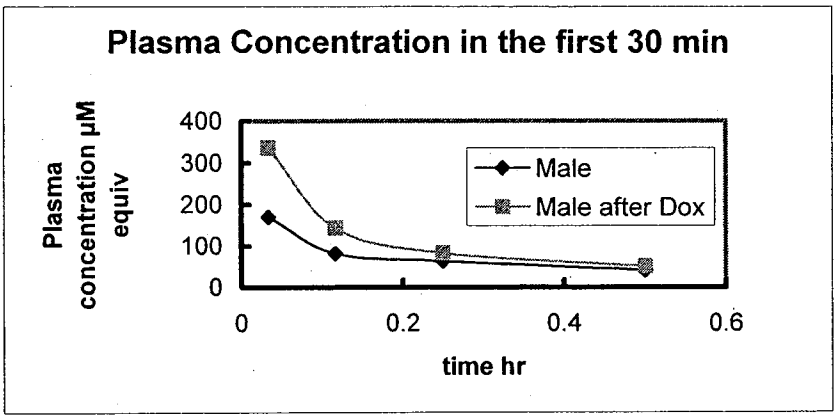

Excretion (Phase 3)

Consistent with the plasma pharmacokinetics, rats eliminated most of the radioactivity in the urine within the first 8 hours after dosing (80.6% males, 79.4% females). The following table (sponsor's) shows that elimination was negligible after that and that only 7 to 8% was recovered in the feces. The rats eliminated no significant amount of radioactivity in expired air and after 96 hours they retained only about 1% in the carcass.

Sex	Time	Mean % Dose		
		Urine	Faeces	Total*
♂	0-24	86.21	6.56	93.91
	0-96	86.99	7.58	97.68
♀	0-24	85.76	6.66	93.94
	0-96	86.72	7.94	97.93

* = Includes cage wash, gastro-intestinal tract and carcass

Tissue Distribution (Phase 4)

The following tables (sponsor's) show the concentration of total radioactivity in selected tissues in male and female rats. No statistics accompany these numbers because the investigators used only one rat per sex per time point. The concentration in bone marrow in females appears to be similar to the concentration in plasma but that in males is considerably higher. With an N=1 it is impossible to interpret this observation. High concentrations in the kidneys are consistent with a drug excreted predominantly in the urine. High concentrations in the liver are consistent with metabolism. The concentration in brain and eye is well below the concentration in plasma. The concentration in fat is also well below the concentration in plasma; the compound is not lipophilic and is not distributing to that compartment.

TABLE 7

Levels of Total Radioactivity in Organs, Tissues and Body Fluids of Male Rats Sacrificed at Intervals Following a Single Intravenous Administration of [^{14}C]-Cardioxane. Target Dose Level 20 mg base.kg^{-1}

Results expressed as µg base equiv.g^{-1} (ml^{-1})

Sample	Animal No./Time Point				
	29♂ (5 min)	28♂ (1 h)	25♂ (6 h)	26♂ (24 h)	27♂ (96 h)
Adrenals	21.78	7.29	0.43	0.12	0.07
Bone Marrow	53.96	5.97	0.36	0.40	0.01**
Bone Mineral	14.82	2.98	0.14	0.08	0.05*
Brain	1.16	0.41	0.07	0.02	0.01*
Eyes	6.77	2.28	0.27	0.05	0.04
Fat	7.43	1.78	0.14	0.05	0.02*
Heart	19.98	4.42	0.32	0.21	0.16
Kidney	80.30	22.76	3.07	0.43	0.05
Liver	50.39	46.44	14.26	1.27	0.04
Lung	25.78	4.98	0.33	0.16	0.05
Muscle	16.97	6.21	0.55	0.36	0.34
Pancreas	17.52	4.11	0.22	0.04	0.03
Pituitary	11.85	4.42	0.27	0.10*	0.04**
Skin	20.27	6.50	0.28	0.11	0.03
Spleen	23.41	4.27	0.37	0.20	0.09
Testes	4.93	2.09	0.45	0.14	0.08
Thymus	16.55	2.85	0.33	0.17	0.01*
Thyroid	26.11	5.41	0.06	0.09*	0.03**
Whole Blood	28.30	7.16	0.37	0.19	0.09
Plasma (ml^{-1})	32.46	10.07	0.25	0.03*	0.01**
Remaining Carcass	15.69	4.35	0.37	0.36	0.21
Stomach and Contents	4.84	1.42	0.28	0.06	0.06
Small Intestine and Contents	18.66	29.60	1.33	0.21	0.02
Large Intestine and Contents	7.92	4.47	30.63	3.54	0.35

* = Results calculated from data less than 30 d.p.m. above background
** = Results calculated from data less than 10 d.p.m. above background

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Levels of Total Radioactivity in Organs, Tissues and Body Fluids of Female Rats Sacrificed at Intervals Following a Single Intravenous Administration of [^{14}C]-Cardioxane. Target Dose Level 20 mg base.kg^{-1}

Results expressed as µg base equiv.g^{-1} (ml^{-1})

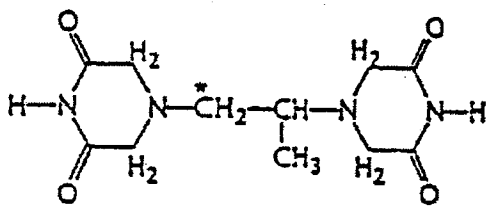
Sample	Animal No./Time Point				
	34♀ (5 min)	33♀ (1 h)	30♀ (6 h)	31♀ (24 h)	32♀ (96 h)
Adrenals	21.09	5.73	0.56	0.24	0.17
Bone Marrow	36.58	5.95	0.50	0.06*	0.00**
Bone Mineral	4.23	1.88	0.31	0.04*	0.03*
Brain	1.21	0.39	0.06	0.02	0.01*
Eyes	5.95	2.08	0.23	0.08	0.05
Fat	13.18	4.68	0.30	0.09	0.04
Heart	23.18	3.94	0.35	0.27	0.17
Kidney	100.08	13.41	2.32	0.50	0.08
Liver	52.00	42.19	13.86	1.68	0.06
Lung	27.11	4.26	0.38	0.20	0.06
Muscle	19.91	6.14	0.69	0.47	0.37
Pancreas	22.48	3.59	0.25	0.06	0.01*
Pituitary	16.47	4.94	0.43	0.42	0.00**
Skin	19.28	6.82	0.88	0.13	0.05*
Spleen	25.53	3.50	0.47	0.25	0.07
Ovaries	19.85	4.60	0.24	0.08*	0.01**
Thymus	17.40	4.09	0.35	0.20	0.02*
Thyroid	22.77	5.58	0.69	0.25	0.18
Whole Blood	26.70	6.09	0.39	0.23	0.13
Plasma (ml^{-1})	31.84	7.98	0.24	0.04	0.01**
Remaining Carcass	15.06	4.58	0.85	0.47	0.32
Stomach and Contents	5.22	1.63	0.48	0.23	0.09
Small Intestine and Contents	20.89	24.73	5.55	0.70	0.34
Large Intestine and Contents	8.23	5.01	40.25	6.09	1.31

* = Results calculated from data less than 30 d.p.m. above background
** = Results calculated from data less than 10 d.p.m. above background

The plasma/whole-blood ratio of radioactivity was ea 1:1 at 5 min and ea 1:1.5 at 6 h post dose for both male and female animals.

Metabolism (Phase 5)

The following chemical diagram shows the location of the radiolabeled carbon at the methylene bridge carbon not bound to a methyl group.



The following table (sponsor's) shows the results of the examination of plasma extract by HPLC.

The Profile of Metabolites in Plasma from Male and Female Rats, Following Intravenous Administration of [^{14}C]-Cardioxane, at a Dose Level of 20 mg base.kg^{-1}

Ion-Exchange HPLC System

Sample Type	Sex	Time Point	Extraction Efficiency	Column Recovery	Component 1		Component 3		Component 4	
					TR (min)	** %	TR (min)	%	TR (min)	%
Plasma	♂	5 min	NA	83	6	20	17	74	-	-
	♀	5 min	NA	88	5	20	16	72	-	-
	♂	1 h	NA	95	5	68	16	16	20	7
	♀	1 h	NA	77	5	50	16	23	21	17

NA = Not applicable, sample injected directly onto the column
% = % of radioactivity eluted from column
** = Results calculated as % radioactivity eluted

The investigators say that the retention time of component 3 was consistent with parent drug. The other two components increase in concentration after one hour. These two components did not migrate with retention times similar to two reference standards supplied to the investigators by the sponsor and labeled Decomposition Product A and B (not further defined). Component 1 migrated with a retention time similar to a reference standard supplied by the sponsor and labeled Decomposition Product C (not further defined). According to the sponsor, Decomposition Product C results from sequential decomposition Products A and B. The HPLC column used was a [redacted] a cation exchange column. Thus, the results suggest that Component 1 is an anion and that Component 4 has the same charge as the parent compound. b(4)

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Toxicology

Single Dose Toxicology Summary

A total dose of 600 mg/kg (1800 mg/m^2) given as two doses in a 24 hour period caused minimal toxicity in mice. A total dose of 600 mg/kg (3600 mg/m^2) given as two doses in a 24 hour period caused minimal toxicity in rats. Neither of these GLP range finding studies adequately demonstrated toxicity or provided information on the microscopic toxicity of dexrazoxane.

Single Dose Toxicology Review

1) Acute toxicity with Cardioxane by intravenous injection in the mouse.

Major findings

A total dose of 600 mg/kg (1800 mg/m^2) given as two doses in a 24 hour period caused minimal toxicity in mice.

Study number	018753, Volume 4
Conducting laboratory	[redacted] b(4)
Date of study initiation	August 2, 1989
GLP compliance	Yes
QA report	Yes
Drug	Cardioxane®, Chiron BV, Amsterdam, Lot Q070789, purity 100%
Methods	
Doses	600 mg/kg (1800 mg/m^2)
Species	CD-1 mice
Number	5 per sex per dose group
Schedule	two intravenous injections within 24 hours at a total dose of 600 mg/kg body weight (once at 400 mg/kg and once at 200 mg/kg) This was considered a maximum feasible dose
Formulation	Isotonic saline
Methods	clinical observation, mortality, gross necropsy
Results	

No animals died while on study. There were no clinical signs of toxicity during the 15-day observation period. The animals gained weight during this period but weight gain slowed in some animals. Two females had enlarged spleens.

2) Acute toxicity with Cardioxane by intravenous injection in the rat.

Major findings

A total dose of 600 mg/kg (3600 mg/m^2) given as two doses in a 24 hour period caused minimal toxicity in rats.

Study number	018742, Volume 4
Conducting laboratory	b(4)
Date of study initiation	August 2, 1989
GLP compliance	Yes
QA report	Yes
Drug	Cardioxane®, Chiron BV, Amsterdam, Lot Q070789, purity 100%
Methods	
Doses	600 mg/kg (3600 mg/m^2)
Species	Wistar rats
Number	5 per sex per dose group
Schedule	two intravenous injections within 24 hours at a total dose of 600 mg/kg body weight (once at 400 mg/kg and once at 200 mg/kg) This was considered a maximum feasible dose one male received only 400 mg/kg total dose
Formulation	Isotonic saline
Methods	clinical observations, mortality, gross necropsy
Results	

None of the animals died because of dosing. All of the animals gained weight during the 15-day observation period. Males showed signs of severe edema of the cervical region or the legs or both following the first dose on day 1. On day two only two males showed signs of edema of the cervical region. This was probably due to rough handling. One female was lethargic on day one. At necropsy, two males and four females had enlarged spleens.

Multiple Dose Toxicology Studies

Multiple Dose Toxicology Summary

Dexrazoxane daily for 28 days in rats at doses as high as 200 mg/kg (1200 mg/m^2) caused no mortality. The rats developed dose dependant hunched posture, pale appearance and rough coat. Dosing over this period caused significantly decreased weight relative to controls (~20). This dose caused significant myelosuppression and anemia. These serious decreases in blood counts were accompanied by decreased spleen size and decreased thymus size with microscopic signs of atrophy in these organs and lymph nodes. Testes were also atrophic. Kidney weight decreased significantly. Elevated AST suggested the beginning of damage to the liver. Lower doses caused a similar but less severe spectrum of toxicities.

Rabbits could not tolerate IV doses of 600 mg/m^2/d for more than 19 days.

Multiple Dose Toxicology Review

1) Subacute 28-day toxicity with Cardioxane by daily intravenous injection in the rat.

Major findings

Dexrazoxane daily for 28 days in rats at doses as high as 200 mg/kg (1200 mg/m^2) caused no mortality, but clinical signs demonstrated toxicity. The rats developed dose dependant hunched posture, pale appearance and rough coat. Dosing over this period caused a decrease in weight of as much as 28% in males and 16% in females relative to controls. Red and white cell parameters decreased as much as 73% (WBCs in males). These serious decreases in blood counts were accompanied by decreased spleen size and decreased thymus size with microscopic signs of atrophy in these organs and lymph nodes. Testes were also atrophic. Kidney weight decreased significantly. Elevated AST suggested the beginning of damage to the liver.

Study number	018764, Volume 4
Conducting laboratory	[redacted] b(4)
Date of study initiation	September 14, 1989
GLP compliance	Yes
QA report	Yes
Drug	Cardioxane®, Chiron BV, Amsterdam, Lot Q070789, purity 100%
Methods	
Doses	0, 10, 50 or 200 mg/kg (0, 60, 300 or 1200 mg/m^2)
Species	Sprague-Dawley rats
Number	5 per sex per dose group
Schedule	Daily IV doses for 28 days
Formulation	Isotonic saline
Methods	clinical observations, mortality, blood counts, clinical chemistry, ophthalmoscopic examination, gross necropsy and histopathology
Necropsy	Day 29. The battery of tissues examined microscopically was adequate in controls and HD and in animals that died or were killed before the end of study.
Results	

Parameter	Time of observation	Control	10 mg/kg	50 mg/kg	200 mg/kg
Mortality		2m, 1f not drug related	1f not drug related		
Clinical signs	twice daily			hunched posture, rough coat males	hunched posture, pale appearance, rough coat m&f, occasional incidence of diarrhoea, sedation and red discoloration of urine
Body Wt	daily				
	d 29 male		-10.4%	-14.2%	-28.4%
	d 29 female		-5.5%	-8.5%	-15.5%
Ophthalmoscopy	wk 4		NC	NC	NC
Hematology	d 29				
RBC male				-8.9%	-29.0%
HB male					-27.5%
Hct male					-30.2%
WBC male			-59.7%	-56.6%	-73.0%
Plts male			-13.3%	26.1%	50.2%
RBC female			-7.8%	-18.8%	-40.6%
HB female			-2.4%	-9.6%	-42.2%
Hct female				-9.1%	-43.2%
WBC female			-48.4%	-48.4%	-69.2%
Clinical Chemistry	d 29				
Creatinine male			-12.2%	-20.4%	-18.4%
AST male			-3.6%	-32.4%	31.1%
AST female				15.2%	36.3%
protein female			-4.5%	-9.1%	-18.2%
Organ Weight					
kidney male			-6.5%	-5.7%	-23.6%
Spleen male			-33.9%	-34.0%	-45.1%
Testes			-8.6%	-35.1%	-47.7%
kidney female			-7.5%	-4.3%	-15.5%
Spleen female			-21.1%	-24.8%	-31.3%
Gross Pathology	d29				
small testes				3/5	4/5
small thymus male				3/5	3/5
small thymus female				5/5	4/5
Histopathology	d29				
Thymic atrophy			9/10	10/10	10/10
Mes lymph node atrophy			10/10	10/10	10/10
Mandib lymph node atrphy			10/10	10/10	10/10
Splenic atrophy					10/10
Bone marrow atrophy					9/10
Testes atrophy				5/5	5/5

2) Subacute toxicity with Cardioxane following intravenous injection in the rat.

Major findings

Doses as high as 200 mg/kg/day (1200 mg/m^2/d) IV for 28 days caused no dose related mortality. This dose was associated with diminished weight (-20%) at the end of the dosing period relative to controls and with decreased food consumption. Dexrazoxane caused significant anemia and myelosuppression. Red cell parameters were decreased by nearly 50% in the high dose group and WBC was decreased by as much as 75%. This correlated with decreased organ weight in the spleen (~30%) and decreased size in the thymus. These toxicities were not completely recovered 42 days after the end of dosing. Increases in some liver enzymes and organ

weight changes suggested hepatotoxicity. Decreased organ weights suggested nephrotoxicity, particularly in females. Heart weights in males decreased significantly (21%) in males but this organ weight recovered by days 70 (42 days after the end of dosing). Dosing was also associated with atrophy in the thymus, lymph nodes, spleen, testes and in some animals, bone marrow.

A dose of 200 mg/kg/day (1200 mg/m^2/d) IV every 14 days for 10 weeks (5 total doses) caused no dose related mortality. This dose caused a spectrum of toxicity similar to but less severe than the daily for 28 days regimen. This regimen did cause significant toxicity in the testes and spleen.

Study number	027089, Volume 5
Conducting laboratory	[redacted] b(4)
Date of study initiation	February 5, 1990
GLP compliance	Yes
QA report	Yes
Drug	Cardioxane®, Chiron BV, Amsterdam, Lot Q241189, purity 100%
Methods	
Doses	Group 1, 0 mg/kg Group 2, 5 mg/kg (30 mg/m^2) Group 3, 50 mg/kg (300 mg/m^2) Group 4, 200 mg/kg (1200 mg/m^2) Group 5, 0 mg/kg Group 6, 200 mg/kg (1200 mg/m^2)
Species	Sprague-Dawley rats
Number	10 per sex per dose group in groups 1, 2, 3, and 4 5 per sex per dose group in groups 5 and 6
Schedule	Daily IV doses for 28 days for groups 1, 2, 3, and 4 once every 14 days for 10 weeks (5 doses total) for groups 5 and 6
Formulation	Isotonic saline
Methods	clinical observations, mortality, blood counts, clinical chemistry, gross necropsy and histopathology
Necropsy	Five animals per sex were necropsied on day 28 in groups 1, 2, 3 and 4 Five animals per sex were necropsied on day 70 in groups 1, 2, 3, and 4 after a 42 day recovery Five animals per sex were necropsied on day 70 in groups 5 and 6

Results

Two males in control groups 1 and 5 and one female in group 5 died in addition to those listed in the table below. The mortality seen in this study was probably not associated with the drug but with the handling during dosing. In the following table, values in bold were statistically significantly different from control (sponsor's calculation). The table shows that not all hematological parameters recovered to baseline 42 days after the end of dosing.

Parameter	Time of observation	5 mg/kg	50 mg/kg	200 mg/kg	200 mg/kg q14d
Mortality		2 m	1 f	1f	1f
Clinical signs	twice daily	ed discoloral	hunched posture, rough coat, pale appearance	hunched posture, pale appearance, rough coat m&f, occasional incidence of diarrhoea, sedation and red discoloration of urine. Swelling of limbs after start of Tx for a few days	None
Body Wt	daily				
male	d 29	-4.0%	-15.2%	-20.1%	
female	d 29	-6.8%	-15.0%	-21.8%	
male	d 71	-0.7%	-1.6%	-4.0%	3.4%
female	d 71	-12.0%	-17.5%	-25.6%	-8.8%
Food Consumption			decreased	decreased	
Hematology	d 29				
RBC male	d 29	-23.0%	-33.8%	-47.5%	
Hbg male	d 29	-12.2%	-18.9%	-42.2%	
Hct male	d 29	-9.5%	-14.3%	-45.2%	
MCV male	d 29	20.8%	30.2%	7.5%	
MCH male	d 29	16.7%	16.7%	8.3%	
WBC male	d 29	-64.0%	-74.0%	-77.3%	
RBC female	d 29	-18.5%	-28.2%	-48.0%	
Hbg female	d 29	-12.5%	-18.2%	-46.6%	
Hct female	d 29	-10.0%	-15.0%	-47.5%	
MCV female	d 29	10.7%	17.9%	1.8%	
MCH female	d 29	16.7%	16.7%	8.3%	
WBC female	d 29	-8.7%	-43.5%	-59.4%	
Plt female	d 29	61.3%	67.3%	135.4%	
RBC male	d 70	-8.0%	-9.8%	-11.5%	-3.8%
Hbg male	d 70	-4.2%	-5.2%	-2.1%	-5.3%
Hct male	d 70	-6.4%	-6.4%	-4.3%	-2.2%
MCV male	d 70	3.7%	3.7%	9.3%	0.0%
MCH male	d 70	9.1%	9.1%	9.1%	-8.3%
WBC male	d 70	20.7%	-10.3%	-21.6%	24.2%
RBC female		-3.4%	-1.4%	-0.3%	-4.0%
WBC female		5.1%	-17.9%	10.3%	-15.6%
Clinical Chemistry	d 29				
G-GT	d 29	0.0	0.0	53 nkat/L	0
AST male	d 29	23.7%	41.6%	122.6%	
glucose male	d 29	-15.5%	-11.3%	19.7%	
AST female	d 29	12.6%	17.4%	80.0%	
G-GT female	d 29			221.4%	
$PO4^{+++}$ female	d 29	9.9%	11.3%	20.2%	
Glucose female	d 29	-1.5%	7.6%	24.2%	
K^+	d 29	6.8%	9.1%	15.9%	
T-protein female	d 29	-4.5%	-11.9%	-7.5%	
Creatinine female	d 70				35.1%
Organ Weight					
Heart male	d 29	-12.5%	-17.8%	-20.7%	
Spleen male	d 29	-8.2%	-16.4%	-29.3%	
Testes	d 29	-10.0%	-38.3%	-49.0%	
Adrenals male	d 29	-14.3%	-20.0%	-34.3%	
Kidney male	d 29	5.5%	-2.7%	-12.3%	
kidney female	d 29			-6.7%	
Spleen female	d 29	5.6%	-21.0%	-30.8%	
Heart male	d 70	-1.4%	0.4%	1.1%	2.6%
Testes	d 70	-7.5%	-69.0%	-67.8%	-35.2%
Liver male	d 70	-9.0%	-1.9%	-7.5%	13.3%
Heart female	d 70	-9.1%	-11.3%	-13.7%	2.1%
Liver female	d 70	-26.8%	-30.5%	-19.6%	10.2%
Kidneys female	d 70	-13.6%	-15.2%	-16.7%	-5.2%
spleen female	d 70	11.6%	16.2%	6.7%	-18.3%
Gross Pathology	d29				
small testes	d29		1/5	4/5	
small testes	d 70		5/5	5/5	4/5
small thymus male	d29		2/5m 5/5f	4/5m 4/4f	
small thymus female	d 70		5/5	4/5	
Histopathology	d29				
Thymic atrophy		9/10	10/10	10/10	1f
Mes lymph node atrophy		3/5f	8/10	10/10	
Mandib lymph node atrphy		1/5f	5/5m 1/5f	9/10	
Splenic atrophy		1/5f	10/10	10/10	1m
Bone marrow atrophy			1/5m 2/5f	2/5m 5/5f	
Testes atrophy			5/5	5/5	5/5
Kidney pelvic dilation		2/2f	1/1f	1/1f	2/2f

3) Subacute 28-day toxicity with Cardioxane by daily intravenous injection in the rabbit.

Major findings

In the high dose group (2400 mg/m^2/d), four rabbits died between days 10 and 14 and the rest were moribund by day 15. The rabbits demise was associated with lethargy, ataxia, diarrhea and emaciation. Body weight began to decrease by day 8. There was gross and microscopic damage to the GI tract, thymus, spleen, lymph nodes, bone marrow and testes.

In the low dose group (600 mg/m^2/d), four rabbits died between days 13 and 22 and the other two were moribund by day 19. The rabbits showed toxicities similar to those seen in the high dose group. Rabbits do not tolerate these doses.

Study number	027067, Volume 9
Conducting laboratory	[redacted] b(4)
Date of study initiation	January 29, 1990
GLP compliance	Yes
QA report	Yes
Drug	Cardioxane®, Chiron BV, Amsterdam, Lot Q241189, purity 100%
Methods	
Doses	0, 50 or 200 mg/kg/d (0, 600 or 2400 mg/m^2/d)
Species	New Zealand white rabbits
Number	3 per sex per dose group
Schedule	Daily IV doses for 28 days
Formulation	Isotonic saline
Methods	clinical observations, mortality, blood counts, clinical chemistry, gross necropsy and histopathology
Necropsy	end of study and unscheduled death

Genotoxicity

See review of NDA 20212

Carcinogenicity

The sponsor submitted a study of the carcinogenicity of razoxane done by the NCI in 1978. The study report is available at:

http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr078.pdf

Razoxane is the racemic mixture of dexrazoxane, ICRF-187, and its R-enantiomer ICRF-186. It is referred to in the study report and elsewhere as ICRF-159. Razoxane has been studied to determine its potential as an anti-cancer drug and it has been used in the treatment of psoriasis. This study by the NCI concluded that the racemic mixture, razoxane, was carcinogenic in rats and mice. The following is the text of the abstract from that study as reported by the NTP. This information does not appear in the ZENICARD™ product label, but we have incorporated it into the product label for dexrazoxane because dexrazoxane is one component of a mixture shown to be carcinogenic.

1) TR-78: Bioassay of ICRF-159 for Possible Carcinogenicity

A bioassay of the experimental anticancer drug ICRF-159 for possible carcinogenicity was conducted by administering the compound by intraperitoneal injection to Sprague-Dawley rats and B6C3F_1 mice.

Groups of 35 rats and 35 mice were injected three times per week with ICRF-159 in buffered saline at one of the following doses, either 48 or 96 mg/kg body weight for the rats and either 40 or 80 mg/kg body weight for the mice. Both rats and mice were dosed for 52 weeks, then observed for 29-34 additional weeks. Untreated-control and vehicle-control groups each consisted of 10 rats and 15 mice of each sex; pooled-control groups consisted of the 10 vehicle controls of each sex of the rats combined with 30 vehicle controls of each sex of rats from similar bioassays of three other chemicals and the 15 vehicle controls of each sex of the mice combined with 30 vehicle controls of each sex of mice from similar bioassays of two other chemicals. All surviving rats were killed at 81-86 weeks; all surviving mice, at 86 weeks.

Mean body weights were depressed in rats and mice administered ICRF-159, and mortality was dose related among male and female rats and male mice. The high mortality among the male rats may have been associated with inflammatory lesions observed in the lungs, the liver, and the pleural and peritoneal cavities. Sufficient numbers of female rats and of both male and female mice were at risk for development of late-appearing tumors. In the male rats, time-adjusted analysis of the incidence of tumors was used for determining statistical significance.

In female rats, the incidence of uterine adenocarcinomas was higher in the low- and high-dose groups (P>0.001) than in the pooled controls (controls 0/38, low-dose 10/33, high-dose 11/32); the incidence was also dose related (P<0.001). In male rats, no tumors occurred in the dosed groups in a significantly increased incidence.

In female mice, the incidence of all hematopoietic neoplasms (histiocytic lymphomas, lymphocytic lymphomas, or lymphocytic leukemias), taken together, was higher in the low-dose group (P=0.038) and in the high-dose group (P=0.002) than in the pooled controls (controls 1/45, low-dose 5/31, high-dose 9/34); the incidence was also dose related (P=0.002). In addition, the incidence of these tumors in the high-dose group was higher (P=0.026) than that in the vehicle controls (0/15), and the incidence was dose related (P=0.021) using the vehicle controls. In male mice, lymphocytic neoplasms occurred only in two low-dose and two high-dose animals.

It is concluded that under the conditions of this bioassay, ICRF-159 was carcinogenic for female Sprague-Dawley rats, producing uterine adenocarcinomas, and was also carcinogenic for female $B6C3F_1$ mice, producing lymphomas.

Synonyms: (±)bis-4,4'-(1-methyl-1,2-ethanediyl)-2,6-piperazinedione

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Positive

Report Date: 1978

Reproductive Toxicology

See review of NDA 20212

APPEARS THIS WAY ON ORIGINAL

OVERALL SUMMARY AND ANALYSIS

Introduction

The US FDA has previously approved dexrazoxane for injection (ZINECARD®, NDA 20-212, May 26, 1995) for use in the prevention of the cardiomyopathy associated with doxorubicin cancer chemotherapy. The mechanism by which dexrazoxane exerts its cardio-protective activity is not well established. Dexrazoxane is a cyclic derivative of EDTA that, unlike EDTA, can cross cell membranes because it is uncharged. Some studies suggest that dexrazoxane hydrolyzes intracellularly to a tetra-acetate that chelates metallic cations such as iron. This chelation may interfere with the iron-mediated radical generation thought to be responsible, in part, for anthracycline-induced cardiomyopathy. TOTECT is the same chemical compound as Zinecard. The sponsor has proposed that this chemical is also prevents the formation of superficial but serious wounds that form after the extravasation of an anthracycline cancer chemotherapy drug such as daunorubicin or doxorubicin.

Toxicity

In short term toxicity studies, a total dose of dexrazoxane of up to 600 mg/kg (1800 mg/m^2) given as two doses in a 24 hour period caused minimal toxicity in mice. A total dose of up to 600 mg/kg (3600 mg/m^2) given as two doses in a 24 hour period caused minimal toxicity in rats. Neither of these GLP range-finding studies adequately demonstrated dose-limiting toxicities nor did they provide information on the microscopic toxicity of dexrazoxane.

In 28-day toxicology studies in rats, dexrazoxane at doses as high as 200 mg/kg (1200 mg/m^2) caused no mortality. The rats developed dose dependant hunched posture, pale appearance and rough coat. Dosing over this period caused significantly decreased weight relative to controls (~20%). This dose caused significant myelosuppression and anemia in males and females. A decrease in spleen and thymus size with microscopic signs of atrophy in these organs and lymph nodes accompanied these serious decreases in blood counts. Testes were also atrophic. Kidney weight decreased significantly. Elevated AST suggested the beginning of damage to the liver. Lower doses caused a similar but less severe spectrum of toxicities.

Rabbits could not tolerate IV doses of 600 $mg/m^2/d$ for more than 19 days. For more information on the toxicity of dexrazoxane, see the reviews of NDA 20-212 appended below.

Pharmacokinetics

In studies submitted to the ZINECARD™ NDA, a two-compartment open model with first-order elimination adequately described dexrazoxane pharmacokinetics in plasma in cancer patients. Dexrazoxane has been administered as a 15-minute infusion over a dose-range of 60 to 900 mg/m^2 with 60 mg/m^2 of doxorubicin, and at a fixed dose of 500 mg/m^2 with 50 mg/m^2 doxorubicin. Area under the concentration versus time curve increases linearly with dose within the range of 60 to 900 mg/m^2. The mean peak plasma concentration of dexrazoxane was 36.5 μg/mL (0.14 mM) at the end of the 15-minute infusion of 500 mg/m^2, which was given 15 to 30 minutes before a dose of doxorubicin (50 mg/m^2). The following table summarizes the pharmacokinetics of dexrazoxane in human cancer patients given both dexrazoxane and doxorubicin at a fixed ratio of 10 to 1. The values in parenthesis are the %CV.

Dose Doxorubicin (mg/m^2)	Dose dexrazoxane (mg/m^2)	N	Plasma Elimination Half-life (hr)	Plasma Clearance ($L/h/m^2$)	Renal Clearance ($L/h/m^2$)	Volume of Distribution (L/m^2)
50	500	10	2.5 (16%)	7.9 (18%)	3.4 (36%)	22 (22%)
60	600	5	2.1 (29%)	6.2 (31%)		22 (55%)

Initial distribution is rapid and is complete within 12 to 18 minutes. The estimated steady-state volume of distribution of dexrazoxane suggests its distribution primarily in the total body water (25 L/m^2). Qualitative metabolism studies with dexrazoxane have confirmed the presence of unchanged drug, a diacid-diamide cleavage product, and two monoacid-monoamide ring products in the urine of animals and man. In humans, forty-two percent of the 500 mg/m^2 dose of dexrazoxane was excreted in the urine. This is somewhat less than is seen in rats. *In vitro* studies have shown that dexrazoxane does not bind to plasma proteins.

In rats, dexrazoxane plasma concentration decreases in three distinct phases, a rapid distribution phase that last for only a few minutes, an elimination phase lasting to about four hours, and a longer terminal elimination phase. The initial rapid distribution phase seen in the human studies was probably obscured by the fact that the dose was given as a 15 minute infusion, not as a bolus as it was in rats. The addition of doxorubicin significantly increases the exposure of dexrazoxane in male, but not female, rats. The two drugs possibly compete for some elimination process. Rats eliminated most of a dose of radioactivity associated with dexrazoxane in the urine within the first 8 hours after dosing (about 80%). Elimination is negligible after that. Only 7 to 8% is found in the feces. The rats eliminated no significant amount of radioactivity in expired air and after 96 hours, they retained only about 1% in the carcass. High concentrations in the kidneys are consistent with a drug excreted predominantly in the urine. High concentrations in the liver are consistent with significant metabolism. The concentration in brain and eye is well below the concentration in plasma. The concentration in fat is also well below the concentration in plasma. Rats hydrolyze dexrazoxane primarily to the open ring tetra-acetate. For further information on the pharmacokinetics of dexrazoxane in animals, see the reviews of NDA 20-212 below.

Carcinogenicity and Mutagenicity

No long-term carcinogenicity studies have been done with dexrazoxane. The carcinogenic potential of pure dexrazoxane is unknown. Nevertheless, a study by the National Cancer Institute has reported that long term dosing with razoxane (Report PB 285 853, the racemic mixture of dexrazoxane, ICRF-187, and its enantiomer ICRF-186) is associated with the development of malignancies. In this bioassay, the investigators injected the rats IP with 0, 48 or 96 mg/kg (0, 288 or 576 mg/m^2) daily for 52 weeks. They injected the mice with 0, 40 or 80 mg/kg (0, 120 or 240 mg/m^2) on the same schedule. They determined that there was an increase in the incidence of uterine adenocarcinomas in rats. In female mice, the "incidence of all hematopoietic neoplasms (hystiocitic lymphomas, lymphocytic lymphomas, lymphocytic leukemias) taken together" increased with dose. The investigators concluded that razoxane was carcinogenic in female Sprague-Dawley rats and that it may have been carcinogenic in female B6C3F1 mice.

Dexrazoxane was not mutagenic in the Ames test but was found to be clastogenic to human lymphocytes *in vitro* and to mouse bone marrow erythrocytes *in vivo* (micronucleus test). See the reviews of NDA 20-212 below.

Reproductive Toxicity

No one has yet done standard reproductive toxicity studies of the possible adverse effects of dexrazoxane on the fertility of humans or experimental animals, male or female. Nevertheless, in long-term toxicity studies, dexrazoxane causes profound testicular atrophy in rodents when given daily at doses significantly lower than the proposed clinical dose. Dexrazoxane was toxic to pregnant rats at doses of 2 mg/kg (1/80 the human dose on a mg/m^2 basis) and embryotoxic and teratogenic at 8 mg/kg (about 1/20 the human dose on a mg/m^2 basis) when given daily during the period of organogenesis. Teratogenic effects in the rat included imperforate anus, microphthalmia, and anophthalmia. In offspring allowed to develop to maturity, fertility was impaired in the male and female rats exposed *in utero* during organogenesis at 8 mg/kg. In rabbits, doses of 5 mg/kg (about 1/16 the human dose on a mg/m^2 basis) daily during the period of organogenesis caused maternal toxicity. Doses of 20 mg/kg (1/4 the human dose on a mg/m^2 basis) were embryotoxic and teratogenic. Teratogenic effects in the rabbit included several skeletal malformations such as short tail, rib and thoracic malformations, and soft tissue variations including subcutaneous, eye and cardiac hemorrhagic areas, as well as agenesis of the gallbladder and of the intermediate lobe of the lung.

Efficacy in the Mouse Model

A single dose of an anthracycline such as doxorubicin or daunorubicin under the skin of a mouse consistently causes the formation of a wound in overlying skin and the underlying tissue over the course of four to five days. The initial injection is sufficiently painful that it necessitates anesthesia. The wound forms an eschar and slowly heals over the course of 20 to 40 days depending on its size. The body surface area of a mouse is about 1000 mm^2. A dose of 3 mg/kg (9 mg/m^2) of daunorubicin usually caused a wound with a surface area of about 110 mm^2 or less. Larger doses (375 mg/kg) in combination with an anthracycline caused significant mortality and morbidity. Thus, mice can sustain anthracycline wounds over about 10% of their body surface area. By measuring the size of the wounds each day and adding these sizes, the investigators in the various studies created a metric, area under the wound-area versus time, or wound AUC. They used this AUC metric as a measure of dexrazoxane efficacy, under the assumption that diminished AUC compared to controls demonstrates a treatment effect. They also compared the number of mice that formed wounds in the treatment group relative to controls (wound incidence) as an indicator of efficacy. The size of the wound increases with increasing anthracycline dose.

A single dose of 250 mg/kg (750 mg/m^2) dexrazoxane given IP immediately after a dose of 3 mg/kg daunorubicin SC decreased the wound area AUC from 1050 mm^2*day in controls to 433 mm^2*day in treated animals. These results were reproducible. Giving a dexrazoxane dose IP three hours after the daunorubicin dose ameliorated wound formation about as well as giving the dose immediately after the daunorubicin dose but efficacy diminished significantly if the dexrazoxane was given at a greater interval (6 hours or greater). Injecting the dexrazoxane directly into the wound site, the site of the daunorubicin dose, usually did not improve the results over those achieved with IP injection. IV administration of the dexrazoxane dose (250 mg/kg) actually resulted in larger AUC values than those obtained after IP injection of the same dose though the difference did not achieve statistical significance. A single dose of 62.5 mg/kg of dexrazoxane IP given immediately after 3 mg/kg of doxorubicin provided the same protection as 125 mg/kg and 250 mg/kg given at $t = 0$. Thus, these individual experiments did not establish a clear dose effect and they showed that multiple doses were no more effective than a single dose immediately after the toxic insult.

Doxorubicin is a less potent vesicant than daunorubicin, consistently forming smaller and fewer lesions at an equivalent dose on a mg/kg basis. Dexrazoxane was consistently more effective at preventing or ameliorating wound formation by doxorubicin when compared to daunorubicin. Some of the individual experiments did demonstrate that a larger dose of dexrazoxane was more effective than a smaller dose up to 250 mg/kg (750 mg/m^2). Mice treated with dexrazoxane (62.5 mg/kg q3hX3, total dose 187.5 mg/kg) after a dose of 3 mg/kg of doxorubicin rarely developed skin lesions, where as most untreated controls did. This regimen usually produced the best results for both doxorubicin and daunorubicin. An ice pack placed over the SC injection site did not improve the results.

Three doses of 62.5 mg/kg given at t=0, 3 and 6 hours of Zinecard and Cardioxane provided statistically equivalent protection against the formation of a skin wound after a single SC doses of daunorubicin or doxorubicin (3 mg/kg). The two commercial formulations of dexrazoxane are pharmacologically equivalent.

A dose of 0.05 mg/kg of Idarubicin SC caused little wound formation. A dose of 0.25 mg/kg caused wounds in about half the control mice treated with saline but only about 12% of mice treated with dexrazoxane (250 mg/kg) developed lesions after this dose. A dose of 0.75 mg/kg caused wounds in all mice treated with saline, but again dexrazoxane prevented wound formation in most mice. The mean wound AUC in mice treated with saline was about four times greater than it was in mice treated with dexrazoxane. Thus, dexrazoxane diminishes wound formation caused by idarubicin in mice though idarubicin is probably a more potent vesicant than daunorubicin on a mg/kg basis.

Epirubicin did not produce skin wounds as consistently as did daunorubicin or doxorubicin nor were the wounds as severe as measured by the AUC at an equivalent dose on a mg/kg basis. IP treatment with dexrazoxane had no effect on the formation of wounds caused by 3 mg/kg of epirubicin. But, a relatively high dose of 9 mg/kg of epirubicin consistently caused skin lesions. Treatment with single or repeat doses of dexrazoxane did not prevent the formation of wounds but the severity of the wounds decreased with dose and dose intensity as measured by wound AUC. Neither aclarubicin nor etoposide consistently caused skin wounds. Wounds formed in animals injected with mitoxantrone SC were clearly smaller in mice treated with dexrazoxane than in controls.

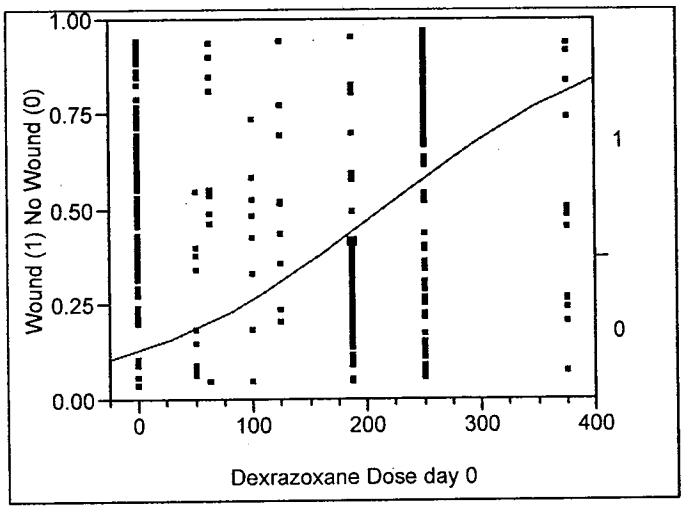
Dexrazoxane treatment had no effect on wound formation caused by SC injection of hydrogen peroxide. This suggests that dexrazoxane does not scavenge oxygen radicals. Systemic treatment with EDTA did not prevent wound formation by daunorubicin suggesting that dexrazoxane does not act by chelating the available iron. But this set of experiments may also mean that EDTA does not prevent wounds because of its high water solubility, or that release of iron ions may not be the ultimate cause of progressive anthracycline damage. α-Tocopherol, amifostine and N-acetylcysteine are well known radical scavengers. None of these drugs given IP had an effect on wound formation after a single SC dose of daunorubicin. These experiments all suggest that radical formation does not mediate anthracycline damage.

Merbarone is a topoisomerase 2-α inhibitor. Investigators determined that this drug given IP had no effect on wound formation after a single SC dose of daunorubicin. The experiment suggests that inhibition of topoisomerase 2-α may not be the mechanism of action of dexrazoxane or that the dose of merbarone may simply have been too low. It could also mean that merbarone and dexrazoxane may act at different sites on topoisomerase 2-α.

ADR-925 is the major double ring-opened metabolite of dexrazoxane. It has four acetate groups much like EDTA and is capable of similar tetrahedral chelation. This compound neither caused skin lesions when injected subcutaneously nor prevented wounds induced by a subcutaneous injection of daunorubicin. Thus, the metabolite is probably not responsible for prevention of daunorubicin skin damage.

The small sample size of the individual experiments limited the power of the statistical analysis. But, in this particular case, I consider that combining all the data for daunorubicin and doxorubicin from the individual experiments is reasonable. The experimental conditions were consistent, the drug used was the same, the mice were all of the same strain, the investigators were the same people, and they did the experiments over a short time. I analyzed the combined data for all the experiments with daunorubicin using JMP. A logistic regression analysis of the wound incidence versus dose demonstrated a clear dose response with an ED_{50} for completely preventing wound formation of about 200 mg/kg (dose that yields half 50% probability of wound formation. The following graph depicts this analysis. The table that follows shows a p value of < 0.0001 (Chi^2) for the regression, demonstrating a significant relationship.

Logistic Fit of Wound (1) No Wound (0) By Dexrazoxane Dose day 0



Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob > ChiSq
Difference	34.1	1	68.2	<.0001
Full	193.0			
Reduced	227.1			

RSquare (U)	0.1503
Observations (or Sum Wgts)	343

Converged by Gradient

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob > ChiSq
Intercept[0]	-1.9122	0.2436	61	<.0001
Dexrazoxane Dose day 0	0.0089	0.0012	54	<.0001

A similar analysis of the combined data for doxorubicin also yielded a significant relationship between dose and wound formation with a p value of < 0.0001 (not shown). In this case, the half-maximal probability of wound formation was at approximately 100 mg/kg. This is

consistent with the experiments that showed doxorubicin to be a less potent vesicant than daunorubicin. The data was too sparse to allow similar calculations for the other anthracyclines.

I also analyzed the Wound AUC versus Dose data in Microsoft Excel using the Solver module to fit the data to a standard hyperbolic response model by iterative regression. The data fit the following equation.

$W_m - W_{auc} = (\text{dexrazoxane dose}) * (W_m) / (\text{dexrazoxane dose} + k)$

Where W_m is the maximum wound size at a given dose of daunorubicin, W_{auc} is wound AUC, and k is a constant representing dose that causes the half-maximal response (ED_{50}). In this case maximum wound size was 1698 mm^2*day. $Wm-W_{auc}$ represents the decrease in the size of the wound brought about by dexrazoxane dosing. Here the dexrazoxane dose that causes the half–maximal response in Wound AUC is very low, k = 26 mg/kg. This means that the dose response curve may be very steep and that relatively low doses of dexrazoxane have a significant effect on wound formation. This could also explain why a clear dose effect was not evident in many experiments. Most of the doses used were high enough to be within the asymptotic portion of the dose response curve, the pseudo-linear region. Response was near maximal and did not vary significantly with increasing dose. This could explain why no wounds formed in many treated animals. But, the fit of the data to this equation was very poor. This is because so many of the values were near maximal, because there was so little data at low doses and because the formation of a wound is a threshold biological effect, that is, the response is not a continuous function. Nevertheless, despite its shortcomings the analysis does suggest a relationship between the dose of dexrazoxane and the decrease in wound AUC. One would expect such large variation in a biological metric with so many contributing parameters – dose, time, wound healing, clearance of the drugs and numerous others. I believe that a well designed experiment with sufficient animals and dose levels could demonstrate a dose effect in a parametric model, particularly if a damage metric that does not manifest with a threshold can be developed.

The sponsor proposes to give TOTECT™ clinically within six hours of anthracycline extravasation at a dose of 1000 mg/m^2 (maximum 2000 mg), with a second dose of 1000 mg/m^2 on day 2 and a third dose of 500 mg/m^2 on day three. The available evidence in mice does not support the efficacy of doses given on any day but the day of the initial insult. Indeed the most effective schedule in mice was IP injections of 62.5 mg/kg (187.5 mg/m^2) at time = 0, 3 and 6 hours (562.5 mg/m^2) after the subcutaneous injection of anthracycline. The next most effective schedule was a single IP injection of 250 mg/kg (750 mg/m^2) immediately after the anthracycline injection. In many cases, these two dosing regimens completely prevented the formation of an anthracycline-induced wound, particularly with doxorubicin. The sponsor has provided no evidence to justify administration of the drug two and three days after the extravasation.

The body surface area of a mouse is about 1000 mm^2. A dose of 3 mg/kg (9 mg/m^2) of daunorubicin in the absence of treatment with dexrazoxane caused maximal wounds of about 110 mm^2. Larger doses were lethal. Thus, mice could sustain wounds over about 10% of their body surface area. In induction therapy for acute myelogenous leukemia, the usual dose of daunorubicin is 60 mg/m^2. In the treatment of breast cancer, the dose of doxorubicin is also usually about 60 mg/m^2. So the doses used in the animal studies of this NDA to induce cutaneous wounds were about one sixth the total dose used clinically on a mg/m^2 basis. The animal studies cannot predict the efficacy of dexrazoxane in the situation where a large portion clinical dose is extravasated. The lack of information from any animal model other than the mouse hinders better extrapolation to the clinical situation.

Dexrazoxane probably works by binding to a site on DNA close to but distinct from the binding site of anthracyclines, thereby preventing the binding of the anthracycline and the resultant double-strand breaks associated with the inhibition of topoisomerase II (see S. Classen

et al. Proc Natl Acad Sci U S A. 2003 Sep 16;**100**(19):10629-34, not reviewed), but the sponsor has not established this mechanism. Thus, while I am confident in saying that dexrazoxane treatment works to prevent wound formation in mice after an anthracycline insult, I have insufficient evidence to confirm its efficacy in humans.

Chemistry Comment on the Formulation

Subject: TOTECT ™ Formulations Used in Clinical Trials

From Dr. Leon Epps

The proposed marketed pack for the TOTECT™ 500 mg powder and Solvent for Injection include: 10 vials of each 500 mg dexrazoxane Hydrochloride Salt "Zinecard" and 10 vials each 50 mL Sodium Lactate Injection component. One mL of the recommended reconstituted solution is used as prescribed.

Dexrazoxane [(+)-1,2-bis(3,5-dioxopiperazinyl)propane] is the S-enantiomer (called ICRF-187) of the racemic mixture of (+) and (-)- 1 ,2-bis(3 ,5-dioxopiperazinyl)propane, ICRF-159 (Razoxane). Due to ICRF-159 low solubility, it could not be formulated for parenteral use and was erratically absorbed when administered orally. Dexrazoxane is more soluble in water than ICRF-159, which enables parenteral administration of dexrazoxane.

In clinical practice, dexrazoxane is reported to have shown the desired efficacy when administered in a three day schedule with 1000 mg/rn^2 administered for the two first days and 500mg/m^2 the third day. The optimum formulation of 500 mg dexrazoxane/vial was selected. Due to instability of dexrazoxane in aqueous solutions it was not possible to prepare a solution for injection. A more readily soluble product containing the active ingredient was converted into its hydrochloride salt. The pharmaceutical development of dexrazoxane concentrated on the development of a formulation which would:

- be easily dissolved in water
- be stable after reconstitution for at least 4 hours.

The product Zinecard, marketed by Pfizer (formerly Pharmacia) for the indication of Prevention of cardiomyopathy associated with doxorubicin administration has been on the market in the USA since 1995.

The two clinical trials TTO1 and TTO2 performed to demonstrate the efficacy of dexrazoxane for accidental extravasations of antracycline used two different products. Cardioxane (Batch #TCO0K02-2) supplied by Chiron Corporation was used in the TTO1 trial ——— The Cardioxane (dexrazoxane hydrochloride salt) was reconstituted with 0.1 N HCl to a 2% solution. b(4)

Zinecard (Batches # ADR 059A, ADR 064A, ADR 067A, and ADR 069A) supplied by Pfizer (formerly Pharmacia) was used in the TTO2 trial. ——— b(4)

b(4)

[redacted] Both were lyophilized products of dexrazoxane containing only HCl as excipient but with slightly different pH values upon reconstitution due to process differences. [redacted] Zinecard was formulated to be reconstituted with a sodium lactate solution.

Information requests to the sponsor

1) Please submit tables of all the wound-area X day AUC (mm^2*day) values calculated for individual animals for all studies where that parameter was used to determine efficacy. We would prefer these tables in an easily readable electronic format such as SAS transfer files.

2) Please submit tables of all individual wound areas on given experimental days (mm^2 and day) for individual animals in an easily readable electronic format such as SAS transfer files.

The sponsor partially complied with these two requests

Further comments

1) On page 8 of study report SL246 the results sections says "The single high dose of dexrazoxane of 250 mg/kg given right after experimental **daunorubicin** extravasation..." The experiment details the effect after epirubicin extravasation. We assume this is a typographical error. You should clarify and amend this report.

2) Please confirm the calculated p values for the Student's t-tests done for Table 3 in study SL270 (page 9 of 14).

3) Most of the studies are unsigned. Please note that "draft" reports are not acceptable when filed as part of an NDA application. Please submit finalized, signed copies of all previously submitted but unsigned study reports.

4) The data file ***sl167auc*** identifies the test anthracycline as "DOX3" while the experiment describes the use of daunorubicin. Please confirm that the anthracycline used in this experiment was daunorubicin and correct the data file.

5) The data file sl214auc appears incomplete or incorrect. It describes the results for only 18 mice while the experiment specifies 75. Please provide the complete data set.

6) Your initial submission of the data sets for the experiments did not include files for experiments SL246, SL248, SL249 and SL270. Please submit these.

W. David McGuinn, Jr., Ph.D. D.A.B.T.

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/s/

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