

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:NDA 20-892

PHARMACOLOGY REVIEW(S)

Indication: Intravesical use in the treatment of patients with biopsy-proven carcinoma *in situ* of the urinary bladder refractory to BCG immunotherapy who are medically contraindicated for cystectomy or who refuse to undergo cystectomy.

Clinical Formulation: Cremophor EL and ethanol_{anhyd}, USP, 50/50 v/v
Diluent 0.9% NaCl injection USP

Route of Administration: intravesical instillation.

Clinical Protocols:

A9301 - Open-label study in patients who had not responded to ≥ 2 prior courses of intravesical therapy, including at least one course of BCG for CIS.

A9302 - Open-label study in patients who had not responded to ≥ 2 prior courses of intravesical therapy, including at least one course of BCG for CIS.

A9302 - Open-label study in patients who had not responded to 1 prior course of BCG for CIS, or had failed to complete 1 course of BCG therapy due to toxicity or contraindications.

Dose:	800 mg/75 mL,
Dwell time	60 to 120 min
Frequency:	Weekly
Total duration of drug exposure or cycles:	six weeks
Escalation doses:	None
Age of patient population:	≥ 18 years

Previous Reviews, Dates and Reviewer:

- 1) Dr. W. David McGuinn reviewed the NDA, June 23, 1998.
- 2) Dr. W. David McGuinn reviewed the new labeling on September 3, 1998.

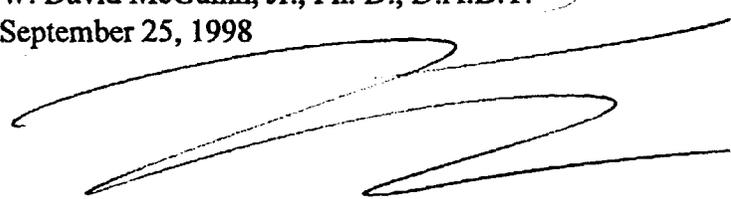
Labeling Review:

Recommendation:

The preclinical information supplied by the sponsor demonstrates that valrubicin is reasonably safe for the proposed indication.

/S/

W. David McGuinn, Jr., Ph. D., D.A.B.T.
September 25, 1998



Sept 25, 1998

cc: Original ~~IND~~ ~~NDA~~ 20-892
/HFD-150
/P Andrews
/A Staten
/WD McGuinn

Paul A. Andrews
9/25/98

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Age of patient population:	≥ 18 years

Previous Reviews, Dates and Reviewer:

Dr. W. David McGuinn reviewed the NDA, June 23, 1998.

Labeling Review:

Redacted 2

pages of trade

secret and/or

confidential

commercial

information

The following references are unnecessary.

REFERENCES

1. Krishan A, Israel M, Modest EJ, Frei E. Differences in cellular uptake and cytofluorescence of adriamycin and N-trifluoroacetyladiamycin-14-valerate. *Cancer Res* 1976;36:2108-10.
2. Krishan A, Dutt K, Israel M, Ganapathi R. Comparative effects of adriamycin and N-trifluoroacetyladiamycin-14-valerate on cell kinetics, chromosomal damage, and macromolecular synthesis in vitro. *Cancer Res* 1981;41:2745-50.
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 6. Israel M, Modest EJ, Frei E III. N-Trifluoroacetyl Adriamycin-14-valerate, an analog with greater experimental antitumor activity and less toxicity than adriamycin. *Cancer Res* 1975;35:1365-8.
 7. Vecchi A, Cairo M, Mantovani A, Sironi M, Spreafico F. Comparative antineoplastic activity of adriamycin and N-trifluoroacetyl Adriamycin-14-valerate. *Cancer Treatment Rep* 1978;62(1):111-6.
 8. Parker LM, Hirst M, Israel M. N-Trifluoroacetyl Adriamycin-14-valerate: additional mouse antitumor and toxicity studies. *Cancer Treatment Rep* 1978;62(1):119-27.
 9. Zirvi KA, Gilani SH, Hill GJ. Embryotoxic effects of doxorubicin and N-trifluoroacetyl Adriamycin-14-valerate (AD-32). *Teratology* 1985;31:247-252.

Recommendation:

The preclinical information supplied by the sponsor demonstrates that Valstar is reasonably safe for the proposed indication.

/S/

W. David McGuinn, Jr., Ph. D., D.A.B.T.
September 3, 1998

cc: Original IND
/HFD-150
/P Andrews
/A Staten
/WD McGuinn

Paul A. Andrews 9/3/98

JUN 23 1998

STATEN

DIVISION OF ONCOLOGY DRUG PRODUCTS, HFD-150
REVIEW AND EVALUATION OF
PHARMACOLOGY AND TOXICOLOGY DATA
Original Review No. 1, Filename 20892_01.doc

NDA No: 20-892 Serial No(s):, 000

Date of Submission: December 31, 1997
Letter Date: December 30, 1997
Date Review Completed: June 5, 1998

Information to be Conveyed to Sponsor: **No**

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

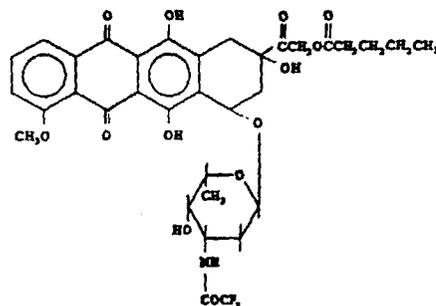
Title: Study of intravesical therapy with AD-32 in patients with carcinoma *in situ* (CIS) refractory to prior therapy with Bacillus Calmette-Guerin (BCG).

Sponsor: Anthra Pharmaceuticals, Inc.
Manufacturer: Anthra Pharmaceuticals, Inc.

Drug Name: Primary: AD-32
N-trifluoroacetyladiamycin-14-valerate

Chemical Name: Pentanoic acid, (2S-cis)-2-[1,2,3,4,6,11-hexahydro-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-4-[[2,3,6-trideoxy-3-[(trifluoroacetyl)amino]-α-L-lyxo-hexopyranosyl]oxy]-2-naphthaceny]-2-oxoethyl ester

Structure:
FW: 723.74
CAS: 56124-62-0
Molecular Formula: C₃₄H₃₆F₃NO₁₃



Related INDs & DMFs: IND
IND
IND
DMF
DMF
DMF

Class: anthracycline type cytotoxin
Indication: biopsy-proven carcinoma *in situ* of the urinary bladder who are refractory to BCG immunotherapy.

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Escalation doses:	None
Age of patient population:	≥ 18 years

Previous Reviews, Dates and Reviewer:

Preclinical studies reviewed in IND Reviewed by Doo Y. Lee Ham, Ph. D. 11/21/91.

I. Toxicology

- 1) single dose intravesical administration in rats
- 2) 3 intravesical doses in rat (two hour exposure, one week apart (summary only reviewed)
- 3) 6 intravesical doses in rat (two hour exposure, one week apart (summary only reviewed)
- 4) single dose i.p. administration in rats
- 5) 3 i.p. doses in rat (two hour exposure, one week apart.
- 6) Hemotoxicity
- 7) Urothelial contact toxicity with histopathology

II Genotoxicity

- 1) Ames Assay, 5 strains with and without S9

III Pharmacokinetics

- 1) PK in rats intravesical instillation

IV Pharmacology Studies

- 1) *in vivo* tumor activity
- 2) *in vitro* tumor activity
- 3) biochemical studies

Dr. Doo Y. Lee Ham reviewed preclinical studies from IND 11/21/91 held by Dana-Farber Cancer Institute. Dana-Farber Cancer Institute granted Anthra Pharmaceuticals the right to cross reference this information in IND

I Toxicology

- 1) i.v. weekly X 12 in rabbit

II Pharmacokinetics

- 1) i.v. in rat
- 2) i.v. in mouse
- 3) i.v. in monkey

Studies Submitted but not Reviewed:

- A.3 Report. Swaminathan, S. Cytotoxicity of AD 32 in human bladder cancer cell lines. 1997. Vol. 1.12, p 228
- B.1.3 Report AD32RATCHSTX1. A single dose toxicity study of AD 32 injected directly into the thoracic cavity of rats, 1996. Vol. 1.14, p 001
- B.1.4 Report AD32CHSTX1. A single dose toxicity study of AD 32 injected directly into the thoracic cavity of beagle dogs, 1995. Vol. 1.14, p 118.
- B.1.5 Report AD32PROSX1. A single dose toxicity study of AD 32 injected directly into the prostate of beagle dogs, 1995. Vol. 1.15, p 001
- C.3 Report. Sweatman TW and Israel M. Pharmacology of intraperitoneal AD 32 in the rat, 1991. Vol. 1.24, p 355
- C.5 Report. Sweatman TW. and Israel M. Validation report of the liquid chromatography assay for N-trifluoro-acetyl-diamycin-14-valerate (AD 32) and principal biotransformation products in human serum, 1997. Vol. 1.24, p 390
- C.6 Report. OLI-VRA595.1. Validation of a method for analysis of AD 32, AD 41 and AD 92 in human serum using following protein precipitation, 1997. Vol. 1.25, p 001
- C.7 Report OLI-VRA595.2. Cross-validation of a method for analysis of AD 32, AD 41 and AD 92 in human serum to dog plasma, 1997. Vol. 1.25, p 092
- C.8 Report OLI-RA595-9708-RAW-1. Analysis of AD 32, AD 41 and AD 92 in beagle dog plasma samples from Study 048-003, 1997. Vol. 1.25, p 238
- C.9 Report OLI-RA595-9708-RAW-2. Analysis of AD 32, AD 41 and AD 92 in beagle dog plasma samples from Study 3-E64, 1997. Vol. 1.25, p 270

The sponsor submitted three volumes of reprints from the scientific literature. These articles included studies of the pharmacology, biochemistry, cell biology and clinical science of AD 32. Some of this literature would have supported the labeling of AD 32, but since the NDA is not approvable, I have not reviewed any of these articles. I have appended a list of these articles to the end of this review.

Glossary:

AD 41	N-trifluoroacetyladrriamycin
AD 92	N-trifluoroacetyladrriamycinol
AD 151	7-deoxy-13-dihydroadrriamycinone
AD 112	7-deoxyadrriamycinone
Dox	Doxorubicin, adriamycin

Studies Reviewed in this IND:

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<i>B.5.1 Adriamycin cardiotoxicity. A comparison with adriamycin, AD 32, N-trifluoro-acetyladrriamycin-14-valerate, 1979. Report NSC 246131. Vol 1.23, p 273. This report is identical to study A.1. Vol. 1.12, p 86.</i>	9
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<i>B.2.2 Repeated exposure (6 dose) study in female albino rats with AD 32 administered by urinary bladder catheterization, 1991. Report WIL-178005. Vol. 1.17, p 001.</i>	14
<i>B.2.3 A repeated dose toxicity study of AD 32 administered intravesicularly to beagle dogs, 1997. Report 3-E64. SUBMITTED TO IND</i>	15
<i>B.2.4 Thunberg AL. Summary report on the analysis of dosing solutions from toxicology study 3-E64 (TSI Mason Laboratories): A repeated dose toxicity study of AD 32 administered intravesicularly to beagle dogs, 1997. Report OLI-RA247.06-9710-MJV-1, Vol. 1.19, p 462.</i>	17
<i>B.2.5 Report WIL-178003. Repeated exposure intraperitoneal toxicity study in female albino rats with AD 32,1991. SUBMITTED TO IND</i>	17
<i>B.2.6 Repeated dose toxicity study of AD-32 administered intraperitoneally to Sprague-Dawley rats, 1997. Report 048-004. Vol. 1.20, p 396.</i>	19
<i>B.2.7 Thunberg AL. Summary report on the analysis of dosing solutions from toxicology study 048-004 (Redfield Laboratories): Repeated dose toxicity study of AD 32 administered intraperitoneally to Sprague-Dawley rats, 1997. Report OLI-RA247.06-9710-MJV-2, Vol. 1.20, p 484</i>	20
<i>B.2.8. Repeat Dose toxicity study of AD-32 Administered intraperitoneally to beagle dogs, Study 048-003. 1997. Volume 21 page 1.</i>	20
<i>B.2.10. A multiple dose toxicity study of AD31 injected directly into the thoracic cavity of rats, Study AD32RATCHSTX4. 1997. Volume 21 page 191.</i>	21
<i>B.2.11 A multiple dose (X4) toxicity study of AD-32 injected directly into the thoracic cavity of beagle dogs, Study AD32CHSTX4. 1996. Volume 22 page 1.</i>	24

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Note: I have excerpted portions of this review directly from the sponsor's submission.

Pharmacology:

- C. 1. S. Garattini, 1977, Relationship of antineoplastic drug activity to *in vivo* effects, Report NIH-N01-CM23242. Vol 1.24, p a-2.

This is a large report with 10 separate studies. My review will be limited to section 7. I will not review the *in vitro* work in section 2. Section 7 and 8 deal primarily with Doxorubicin and Daunomycin.

Experiment 1a is a comparison of the efficacy of AD 32 with that of Dox. The author treated leukemia L1210 (implanted IP) bearing mice with 4 mg/kg Dox or 40 or 60 mg/kg of AD 32 IP daily for four days. In three of four experiments the author says that 90% of the mice were long term survivors (LST), in the fourth experiment 40%. In the text he defines long term survival as greater than 60 days, but in the accompanying data table he lists mean survival time (MST) for the AD 32 treated animals as 21 and 27 days or 2 to 3 fold increase over control. He lists LST as 9 of 10 and 9 of 10. The table seems inconsistent. Judging by MST the doses of AD 32 and Dox are approximately equipotent. Dissolving the Dox in 10% Tween 80 rather than saline decreases its efficacy by about 50%. Increasing the dose of Dox to 8 mg/kg causes 20% mortality. This is probably a well done experiment poorly reported.

In similar experiments, 1b and 1c, Dr. Garattini treated leukemia L1210 bearing mice with a single dose of AD 32 or Dox IV. Increasing the dose of AD 32 from 40 mg/kg to 120 mg/kg increased MST from 10 to 21 days. Dox was relatively ineffective at this schedule. A single dose of 10 mg/kg increased MST from the control value of 9 days to only 13 days.

Interestingly a single dose of AD 32 given as much as six days after tumor inoculation caused a 25% increase in MST. Past day three Dox was completely ineffective.

Next, in experiment 1d, Dr. Garattini implanted the same tumor line IV. A single IV dose of 60 mg/kg AD 32 increased MST from 10 days in controls to 19 days. Higher doses of AD 32 decreased MST probably due to toxicity. Again Dox was relatively ineffective.

Dox does not cross the BBB, so Dr. Garattini implanted the same leukemia cell line intracerebrally in mice to determine if AD 32 behaved differently. Even doses that caused some mortality did not increase MST for either compound. The positive control, BCNU, increased MST from 8 days in controls to 21 days at a dose of 20 mg/kg IP. These results imply that AD 32 does not cross the BBB in concentrations high enough to cause any anti-tumor effect.

In experiment 1f, Dr. Garattini confirmed that Moloney virus-induced LSTRA lymphoma in mice was relatively resistant to Dox. Nevertheless, AD 32 IV one day after tumor implantation caused a dose dependent increase in MST to two fold at 100 mg/kg. Higher doses decreased MST. Similarly doses of AD32 between 80 and 100 mg/kg caused a small (30 to 40%) increase in median survival time in mice implanted with 3LL (lung) carcinoma.

In section 3 of these experiments, Dr. Garattini determined that AD 32 IV was an even more potent immunosuppressant than Dox. He inoculated mice with sheep erythrocytes (SRBC) IP to induce an immune response. He quantitated this immune response by counting the number of specific antibody producing cells in the spleen (PFC/spleen). Immunosuppression increased exponentially with dose going from 135,381 PFC/spleen in controls to 75 PFC/spleen after a dose of 80 mg/kg AD 32. A dose of Dox that frequently causes mortality, 10 mg/kg, decreased the count to only 3,520 PFC/spleen. Doses of AD 32 as low as 10 mg/kg caused significant immuno suppression (38,502 PFC/spleen). The author calculated the ED₅₀ as 5 mg/kg AD 32. This is well below doses that cause significant antitumor effects. This immunosuppression was greatest when the drug was given one to two days after the antigen. When mice were injected with SRBC twice, ten days apart, AD 32 caused a significant but less dramatic decrease in the secondary immune response. Dr. Garattini calculated the ED₅₀ for suppression of the secondary immune response as 35 mg/kg. Like Dox, AD 32 also caused a significant decrease in the T-cell independent response.

In the fourth set of experiments Dr. Garattini again studied myelosuppression associated with AD 32 in mice. He injected mice with different doses then 24 hr later killed the mice and extracted the contents of the femoral shafts. He counted the cells with a Coulter counter then injected 3X10⁵ cells into bone marrow ablated mice (lethal irradiation). Eight days later he removed the spleens of these mice and determined the number of colony forming units (CFU). The following table shows that 20 mg/kg, a dose four times lower than that required for significant antitumor effect, caused profound myelosuppression. Again the dose response was exponential.

Drug dose mg/kg	CFU/Femur	SD	% control	Cells/femur X10-6	SD
0	2210	177	100	14.3	5.2
10	1561	182	71	10.3	3.4
20	919	102	42	8.1	2.6
40	746	52	34	5.3	1.1
60	240	23	11	4.4	1.2
80	123	8	5.6	3.8	1.4

The author then determined the kinetics of this myelosuppression. Using the technique described above, he determined myelosuppression on various days after dosing. The following

table shows that AD 32 caused an early nadir. It killed almost all stem cells by day 2. This is comparable to the early nadirs caused by Dox and daunorubicin at about 24 hr. The dose in this experiment was large, 120 mg/kg. AD 32 may cause such an early nadir by inducing apoptosis in stem cells. By day 11 the mice had recovered from the myelosuppression.

Day after Treatment	CFU/Femur	SD	% control	Cells/femur X 10 ⁴	SD
Control	2051	166	100	9.7	4.3
0.25	267	119	13	6.2	3.6
1	156	41	7.6	3.3	1.5
2	11	2	0.5	0.2	0.1
4	184	16	9	1.2	0.3
7	1448	40	71	6	1.2
11	1641	61	80	8.1	3.4

Next the author, incubated AD 32 with 9000Xg supernatant from the liver, kidney, brain, spleen, lung, and heart of male C57B1/6 mice. In duplicate experiments the incubation mixture also contained an NADPH regenerating system. At the end of the incubation he extracted the mixture to ether and determined AD 32 and its metabolites by ¹⁴C. With S9 from all organs but liver and brain only two compounds were found, AD 32 and the N-trifluoroacetyl adriamycin (AD 41) hydrolysis product. This hydrolysis product formed with and without the NADPH regenerating system suggesting that the hydrolysis was cytochrome P450 independent. This metabolite did not form in boiled controls, so the hydrolysis is enzymatic. Brain S9 did not hydrolyze AD 32. In liver an additional metabolite was found, M1, in the system that included NADPH. When the liver incubation mixture with NADPH was extracted to butanol, traces of two more polar metabolites, M2 and M3, were seen. A liver microsomal preparation (105,000Xg pellet) also formed M1. Thus the formation of this metabolite is probably P450 dependent. Formation of M2 and M3 may be P450 dependent or may be down stream P450 independent products of M1. When the investigator injected live mice with AD 32, the M1 metabolite was found in kidney and heart. M1 was not found in the blood. In other tissues the *in vivo* results were similar to the *in vitro* results.

In section 8, Garattini showed that at concentration less than 10⁻⁹ M, Dox had little effect on DNase I activity *in vitro*. At concentrations between 1.8X10⁻⁹ and 0.6X10⁻⁷ M, Dox increased DNase activity. This increase was probably a consequence of Dox interacting with the double helix in such a way as to make it more accessible to the DNase I. At concentrations above 10⁻⁶ M Dox inhibited DNase activity, as did Daunorubicin, Adriamycin acetate, and Adriamycin-14-octanoate (Adria-O). Daunorubicin caused inhibition at the lowest concentrations. This inhibition was competitive. AD 32 and N-trifluoroacetyl Adriamycin (AD 41, the major metabolite of AD 32) caused no inhibition of enzyme activity at concentrations as high as 10⁻⁵ M, suggesting that they do not interact directly with the double helix.

DNA added to a constant concentration of Dox quenched the characteristic fluorescence of this compound as a function of the ratio [DNA]/[Drug]. DNA also quenched the fluorescence of Daunorubicin, Adriamycin acetate, and Adriamycin-O. This suggests that the aromatic portion of these compounds interacts with the DNA, resulting in intersystem crossing. Added DNA did not quench the fluorescence of AD 32 and N-trifluoroacetyl Adriamycin suggesting that these compounds do not interact with the DNA. The results also suggest that a free glucosidic amino group on an anthracycline is necessary for DNA binding.

Section 9, 10 and 11 deal only with Dox.

Pharmacology Summary:

Four daily doses of 4 mg/kg of Dox and 40 to 60 mg/kg of AD 32 in tumor bearing mice are roughly equipotent against a range of tumors. At 4 mg/kg Dox is somewhat more toxic than equivalent doses of AD 32, thus AD 32 appears to have a better therapeutic index than Dox. This observation and the fact that AD 32 appears to be effective against some tumor implants resistant to Dox seems to have driven much of the research into the pharmacology of this compound.

AD 32 is not effective against intracerebral tumor implants, implying that it does not cross the BBB. *In vivo*, AD 32 is an even more potent immunosuppressant than Dox. This immunosuppression is probably the simple result of myelotoxicity. Like that of Dox, the myelotoxicity associated with AD 32 is very rapid; nadir occurs by day two of dosing.

The major metabolite of AD 32 is AD 41 in all tissues studied. Cytochrome P450 oxidizes AD 32 to more polar compounds only in very limited concentrations in the liver. Unlike Dox, AD 32 does not inhibit DNase I.

DNA added to AD 32 preparations, did not quench the fluorescence of AD 32. This suggests that the aromatic region of AD 32 does not interact directly with the DNA.

APPEARS THIS WAY
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Special Toxicology:

B.3.1 Eye irritation study of AD-32 in New Zealand white (NZW) rabbits, 1996. Study 048-001, Volume 1.23, p 2.

Animal	6 New Zealand white rabbits
Drug	AD-32, Lot 515-44-0002
Dose	0.1 ml in the right eye. Anthra supplied the vials of drug for this test. I assume it was the clinical formulation.
Procedure	Thirty seconds after application, both eyes of three of the rabbits were flushed thoroughly with distilled water. The eyes of the other 3 were not flushed.
Evaluation	1, 24, 48, 72, and 96 hr and 7 days after dosing.

This study was done by _____ signed the
GLP statement.

Results:

There were no clinical observations indicative of systemic toxicity. Treatment did not cause corneal opacity or iritis. Irritation was confined to the conjunctivae. The maximum average score (MAS) of irritation was 3.66 (maximum total score possible = 110), or 'mildly irritating'. Flushing the eyes for 30 seconds reduced the rating to 'practically non-irritating'.

B.3.2 Primary dermal irritation study of AD 32 in New Zealand white (NZW) rabbits, 1996. Study 048-002, Volume 1.23, p 30.

Animal	6 female New Zealand white rabbits
Drug	AD-32, Lot not specified
Dose	0.5 ml applied to test site for four hours. Anthra supplied the vials of drug for this test. I assume it was the clinical formulation.
Procedure	Rabbits were shaved 24 hr before dosing. Test site ~6.25 cm ² . Elizabethan collars. Dose applied via Hilltop Chamber wrapped with semi-occlusive dressing. After 4 hours the test area was rinsed with water.
Evaluation	30 min, , 24, 48, 72 hr. after patch removal.

This study was done by _____ signed the
GLP statement.

There were no clinical observations indicative of systemic toxicity. AD-32 caused no local skin irritation. The investigator classified it as a 'non-irritant'.

B.5.1 Adriamycin cardiotoxicity. A comparison with adriamycin, AD 32, N-trifluoroacetyladiamycin-14-valerate, 1979. Report NSC 246131. Vol 1.23, p 273. This report is identical to study A.1. Vol. 1.12, p 86.

Animal male and female NZW rabbits.
Drug AD 32 and Adriamycin
Dose Groups G1 saline control
 G2 Adriamycin, 2 mg/kg weekly for 12 weeks
 G3 AD 32, 11.27 mg/kg weekly for 12 weeks
 G4 Adriamycin, 2 mg/kg weekly 'until death or termination of the test'
 G5 AD 32, 11.27 mg/kg weekly 'until death or termination of the test'
N three animals per sex per dose group
Route IV marginal ear vein
Vehicle 0.9% saline
Schedule weekly
Observations
Body Wt Weekly
Clinical Chem before dosing and every three weeks
Hematology before dosing and every three weeks
ECG d-8, d-4, and every three weeks, 3 lead
Cardiac Cath before necropsy in G1, G3 and G5 surviving animals
Organ Wt at necropsy
Recovery Surviving animals in G2 and G3 were allowed to recover for 14 weeks
Necropsy 26 weeks or at unscheduled death, two controls were killed during the dosing period for comparison with dead animals.

In a pilot study the investigators gave six rabbits single IV dose of AD 32 at four dose levels. They did not specify these doses. They monitored mortality for 15 days and then calculated the following lethality parameters. They did not specify the method they used to calculate these parameters. They did not further describe the toxicity associated with this dosing but only used this study to determine a dose for subsequent studies

	mg/kg	lower 95% limit	Upper 95% limit
LD ₁₀	46	35	56
LD ₅₀	56	50	60
LD ₉₀	68	56	83

Results

Mortality No animals dosed with Adrimycin survived passed 12 weeks, one of these animals died form excessive bleeding during sampling wk 3.
 One control died from diarrhea
 G3 - 2 after wk 12, one after wk 3
 G5 - one after wk 9 (diarrhea, censored), one after wk 12, 1 after wk 24,
Clinical signs Adriamycin treated animals - transudate formation, subcutaneous edema, alopecia, and weight loss
Body Wt AD 32 treated animals decreased weight gain in animals treated 12 wk. (5%)
Clinical Chem G2 and G4 - major alterations in albumin, cholesterol, creatinine and CPK consistent with impending death.
 G3 and G5 - variations similar to controls.
Hematology G2 and G4 - decreased Hbg and Hct, 10 to 30 % just prior to death.
Organ Wt G2 and G4 - decreased testicular wt. (>60%)

	G3 and G5 - decreased testicular wt. (>50%), increased spleen wt. (to 50%) in some animals, increased lung wt. (to 40%) in some animals
ECG	Minor changes were seen in all dose groups including controls. One rabbit in G2 and one in G4 had a major conduction defect in wk 12. One rabbit in G5 had major heart block or ventricular premature beats at different times, wk 9.
Cardiac Cath	No significant differences among groups by ANOVA
Gross Pathology	G2 and G4 - cardiac edema, dilated right cardiac ventricles forming a rounded or globose apex with loss of tone. G3 and G5 - enlarged spleens with dilated sinusoids filled with erythrocytes and granulocytes at various stages of maturity.
Histopathology	G2 and G4 - Moderate to severe myocardial injury in all animals, including myocytolysis, fibrosis, or atrophy of the myocardial fibers. 4/12 bone marrow hypoplasia, 8/12 - renal lesions including glomerular vacuoles, dilated Bowman's spaces, tubular protein casts, cortical interstitial fibrosis 6/12 - moderate to severe liver lesions, including centrilobular necrosis, 4/6 - marked aspermia or testicular degeneration. G3 and G5 - moderate cytoplasmic edema and vacuolization in the hearts of four rabbits treated until death. spleen - filled with erythrocytes and granulocytes at various stages of maturity. 2/11 bone marrow hypoplasia, fatty changes in the livers of some animals

The changes in serum chemistry presaged the cardiac damage in week nine in the Dox treated rabbits, but these animals were very ill. Microscopic damage frequently correlated with gross damage for both drugs. The changes in the spleen associated with AD 32 suggest focal degenerative changes and were not associated with Dox. Neither did AD 32 cause the same spectrum of toxicity in the liver. The changes were relatively minor increases in fatty liver compared to the significant centrilobular necrosis caused by Dox. The changes in the liver caused by AD 32 at higher cumulative doses might be seen by a liver enzyme screen but the changes in the spleen and the heart may be occult.

This is a poorly organized and poorly reported study, but it provides perhaps the best toxicology data of any study in this submission. The authors conclude that AD 32 is less toxic than Dox. This is true on a molar basis. The average cumulative dose of Dox at 12 weeks was 60.7 mg (0.11 mmole) and that of AD32 was 492.1 mg (0.68 mmoles). A more interesting experiment would have been to study the two compounds at equitoxic doses, but this is difficult to determine without extensive range-finding. The data suggests that the spectrum of toxicities of AD 32 is significantly different from that of Dox, but it is still impossible to determine if higher cumulative dose AD 32 might not cause the same cardiac toxicities.

Special toxicology summary

AD 32 did not cause corneal opacity, iritis or any unusual ocular toxicity when instilled in the eyes of rabbits. It caused only mild irritation probably associated with the vehicle. Likewise, AD 32 caused no unusual local skin irritation. AD 32 does not appear to cause the

profound cumulative cardiac toxicity that Dox causes in rabbits. I have summarized the other toxicity results with the toxicology section.

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ON ORIGINAL

Toxicology:

Multi-dose studies:

B.2.1 Report WIL-178004. Repeated exposure (3 dose) study in female albino rats with AD 32 administered by urinary bladder catheterization, 1991. Vol. 1.16, p 001.

Animal	Female Crl:CD-BR rats
Drug	AD 32, Lot CJ78-8
Doses	untreated control, saline control, vehicle control, 78, 162, and 324 mg/m ² (13, 27, and 54 mg/kg, given as 8.5, 17, 34 mg/ml), 8 rats per dose group.
Vehicle	20% solution of NCI Diluent 12 (Lot BV-88-214) in 0.9% Sodium Chloride
Dose volume	0.4 ml/rat
Schedule	q7dX3 (days 0, 7, and 14)
Route	Urinary bladder catheterization
Exposure	2 hours
Anesthesia	IP injection of sodium pentobarbital 45 to 50 mg/kg with prophylactic antibiotic (Gentamicin Sulfate, 24 hr prior to the procedure)

Observations:

Clinical Obs	2 hours after recovery from anesthesia and twice daily
Body Wt.	Days -7, -1 and thrice weekly
Food Cons	daily beginning d-7
Water Cons	daily beginning d-7
Clin Chem	d6, d13, d21 (necropsy)
Hematology	d6, d13, d21 (necropsy)
Urinalysis	before necropsy
Necropsy	day 21 and at unscheduled death
Histopathology	Urinary bladder, urethra and gross lesions.

did this study for Anthra at its
signed the GLP statement.

Results:

Mortality	one LD and one HD animal on d14 after the third dose. One untreated control following recovery from anesthesia
Clinical Obs.	Urogenital staining and some trauma associated with dosing
Body Wt.	No test article related effects
Food Cons	No test article related effects
Water Cons	No test article related effects
Clin Chem.	No toxicologically significant differences
Hematology	No toxicologically significant differences
Urinalysis	No toxicologically significant differences
Gross Path	HD animal found dead, hemorrhage, reddened renal cortico-medullary junctions, orange discoloration of the urinary bladder, adhesions left and median lobes of the liver LD animal found dead, orange discoloration of the urinary bladder,

Control found dead, dark red intestinal contents, reddened renal cortico-medullary junctions, swollen liver lobes.

Surviving animals, including controls, had various adhesions possibly associated with the anesthesia.

Histopathology HD animal found dead, autolysis of the renal and intestinal tissue

LD animal found dead, urethral congestion

Control found dead, hyperemia of the renal cortico-medullary junctions.

Various lesions correlating with damage associated with anesthesia. Some damage to the to the urinary bladder and urethra. No dose related damage.

The distribution of the deaths among different dose groups and the gross signs on necropsy suggest that the animals that died suffered trauma associated with anesthesia. These animals all had at least six IP injections with Gentamicin or phenobarbital over a two week period. This treatment is sufficiently harsh to account for much of the damage to the peritoneum. The limited damage to the urinary tract was not dose dependent and was probably related to the catheterization procedure. At these doses AD 32 instillation into the urinary bladder of rats appears to cause damage only because of the procedure itself. The high dose, 324 mg/m², is comparable to but somewhat less than the clinical dose on a mg/m² basis (800 mg or ~ 470 mg/m² in humans), but bladder volume does not necessarily fit allometric scaling. The anesthesia will significantly induce CYP 2B1 and 2B2 in these animals.

B.2.2 Repeated exposure (6 dose) study in female albino rats with AD 32 administered by urinary bladder catheterization, 1991. Report WIL-178005. Vol. 1.17, p 001.

Animals	Female rats, 10 per dose group
Drug	AD 32, Lot CJ78-8, 94.0% purity
Vehicle	20% NCI Diluent 12 in sterile physiological saline
Dose	G1 - untreated controls G2 - saline controls G3 - vehicle control G4 - AD 32, 12 mg/kg given as 8.5 mg/ml G5 - AD 32, 24 mg/kg given as 17 mg/ml G6 - AD 32, 50 mg/kg given as 34 mg/ml
Dose volume	0.4 ml/rat
Schedule	single dose on days 0, 7, 14, 21, 28, and 35
Exposure	2 hr.
Pretreatment	Prophylactic antibiotic, Gentamicin 4.5 mg/kg IP, 24 hr. before dosing and after the procedure
Anesthesia	Sodium pentobarbital, ~50 mg/kg
Route	urinary bladder catheterization
Observations	
Clinical Signs	twice daily
Body Wt.	D-7, -1, and thrice weekly during treatment
Food Cons.	Daily from d-7 through the study
Water Cons.	Daily from d-7 through the study
Clin. Chem.	D6, d13, d20, d27, d34, and d42
Hematology	D6, d13, d20, d27, d34, and d42

Urinalysis Before necropsy
 Necropsy Day 42 or at unscheduled death
 Histopathology Urinary bladder, urethra and gross lesions.

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 signed the GLP statement. The investigators chose to treat only
 female rats because male rats are much more difficult to catheterize.

Results

Mortality 1 G1, 1 G2 and 1 G5 all during or immediately after the procedure, attributed to anesthesia.
 Clinical Signs Labored breathing, dried red material around the eyes, red urination secondary to anesthesia or the procedure
 Orange urogenital staining or urination associated with AD 32 dosing
 Body Wt. No dose related effects
 Food Cons. No dose related effects
 Water Cons. No dose related effects
 Clin. Chem. No dose related effects
 Hematology No significant dose related effects
 Urinalysis No dose related effects
 Necropsy swollen liver lobes and adhesions probably associated with IP anesthesia and prophylactic antibiotic treatment in all dose groups.
 Histopathology Microscopic damage consistent with the trauma of the procedure.
 No microscopic lesions associated with dosing.

Gross and microscopic findings suggest that the three deaths were caused by trauma associated with the catheterization procedure or anesthesia. The investigators concluded that 300 mg/m² (given as 50 mg/kg) was a NOEL. While I cannot agree that is a no effect level for the procedure, it does appear to be a no effect level for the drug. This dose is slightly less than the proposed clinical dose of approximately 470 mg/m² in patients.

B.2.3 A repeated dose toxicity study of AD 32 administered intravesicularly to beagle dogs, 1997. Report 3-E64. SUBMITTED TO IND

Animal Male beagle dogs
 Drug AD 32, Lot BVL 515-44-0003, purity 104.6% by label
 Doses G1 Control - 0.9% Saline
 G2 Control -10 ml of NCI Diluent 12 in 27.5 ml 0.9% Sodium Chloride
 G3 100 mg AD 32
 G4 200 mg AD 32
 G5 400 mg AD 32
 N 3 dogs per group except in HD 6 dogs
 Vehicle 10 ml of NCI Diluent 12 (50% Cremophor EL/50% ethanol) in 27.5 ml 0.9% Sodium Chloride
 Dose volume 37.5 ml
 Schedule once weekly for six weeks
 Route Urinary bladder via Foley urinary catheterization
 Exposure 2 hours

Anesthesia Atropine 0.02 mg/kg SC10 to 30 min before anesthesia
Brevital - 5 to 7 mg/kg IV loading dose maintained to effect

Observations:

Clinical Obs 2 hours after recovery from anesthesia and twice daily
Body Wt. Days 1 before dosing and weekly
Physical Exam before dosing and before necropsy
Temperature D-10, d-3, before each treatment, the day after each treatment and weekly during recovery
Ophthal. Exam before the first dose and before necropsy
Food Cons daily reported as daily average for each week
Water Cons daily beginning
ECG twice before first dosing, weekly weeks 1 through 6, and before necropsy
Clin Chem twice before first dosing, before each treatment, d2, wk8, 10 and 12.
Hematology twice before first dosing, before each treatment, d2, wk8, 10 and 12.
Urinalysis before each treatment and in wk 10
Necropsy day 43 G1 and 3 G5 animals
day 78 G2, G3, G4 and 3 G5 animals
Histopathology See table for tissues examined in G1 and G5 animals killed on d43
No tissues were examined for G2 and G3 animals
urinary bladder, testes and prostate gland from G4 and G5 killed on d78

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signed the GLP statement.

Results:

Mortality none
Clinical blood in the urine, vomiting, soft stool, penal discharge, discolored feces in all five groups
Body Wt. No toxicologically significant differences.
Food Cons No toxicologically significant differences.
Water Cons Water consumption varied during the study among all groups including controls
ECG No alterations attributable to AD 32 dosing
Body Temp AD 32 dosing had no effect on rectal body temperature.
Clin Chem No toxicologically significant differences
Coagulation Activated partial thromboplastin time decreased in all treated groups compared to saline controls on days 8 and 15.
Hematology No toxicologically significant differences
Urinalysis No toxicologically significant differences.
Organ Wt. No statistically significant differences.
G5 d43, 2/3 decreased prostate and testes wt
G5 d78, 1/3 increased testes wt
Gross Path. 3/3 G5 dogs at d43 had multiple red foci, 1 to 6 mm diameter, on mucosal surface of the urinary bladder, accompanied by diffuse tan discoloration and slightly raised surface.
Histopathology G5 d43 - 2/3, submucosal edema, hemorrhage, fibroplasia with mucosal hyperplasia of the urinary bladder. 3/3 neovascularization and pigment laden macrophages in the bladder.

2/3 mild to moderate atrophy of the prostate with inflammation. Diffuse decrease in acinar size, epithelial changes.
2/3 testicular degeneration. 1/3 marked germ cell depletion, Spermatid giant cells, karyomegaly.

G5 d78 - 3/3 neovascularization, 2/3 submucosal pigment, urinary bladder
2/3 decreased germ cells in the testes.

G4 d78, 1/3 neovascularization, 1/3 submucosal hemorrhage urinary bladder

G1 d43 - 2/3 inflammation of the prostate.

The clinical signs were associated with the dosing procedure and not related to the direct toxicity of AD 32. Some of the test compound leaked from the catheter after dosing on several occasions. This loss was significant only three times, but two of these times were with G5 dogs. Though there were no toxicologically significant differences in serum chemistry, there was considerable variation. None of these changes were dose dependent. These changes were possibly related to the dosing procedure but they did not occur after every dose. The procedure caused no kidney damage so changes in this organ were probably not responsible for the alterations in serum chemistry.

The toxicological significance of the decreased APTT is questionable. It could be due to the ethanol in the vehicle.

Unfortunately, the investigators did not examine tissues from the vehicle control animals microscopically. Other studies have shown that the NCI Diluent 12 causes significant local toxicity. Because of this omission it is impossible to know if the damage to the urinary bladder in G4 and G5 is due to AD 32 or to the vehicle. The atrophy of the prostate was seen in one G1 (control) and G5 dogs. This may be a spurious finding. The damage to the testes was possibly drug related. The high dose in this experiment was equivalent to the proposed clinical dose on a mg/m² basis.

B.2.4 Thunberg AL. Summary report on the analysis of dosing solutions from toxicology study 3-E64 A repeated dose toxicity study of AD 32 administered intravesicularly to beagle dogs, 1997. Report OLI-RA247.06-9710-MJV-1, Vol. 1.19, p 462.

This is an analysis of the dosing solutions from the previous study. All of the solutions were within 6% of the nominal concentration. These small deviations did not affect the outcome of the experiment.

B.2.5 Report WIL-178003. Repeated exposure intraperitoneal toxicity study in female albino rats with AD 32, 1991. SUBMITTED TO IND

Animal	Female Crl:CD-BR rats, 8 per dose group
Drug	AD 32, Lot CJ78-8
Doses	G1 - Dianeal G2 - Vehicle control G3 - AD 32, 156 mg/m ² (given as 0.85 mg/ml in 8 ml or 26 mg/kg) G4 - AD 32, 306 mg/m ² (given as 1.7 mg/ml in 8 ml or 51 mg/kg) G5 - AD 32, 636 mg/m ² (given as 3.4 mg/ml in 8 ml or 106 mg/kg)

Vehicle 2% solution of NCI Diluent 12 (Lot BV-88-214) Dianeal, Dianeal is PD-1 peritoneal dialysis solution with 1.5% dextrose
Dose volume 8 ml/rat
Schedule q14dX3 (days 0, 14, and 28)
Route IP
Anesthesia Metofane just prior to dosing

Observations:

Clinical Obs 2 hours after recovery from anesthesia and twice daily
Body Wt. Days -7, -1 and thrice weekly
Food Cons daily beginning d-7
Water Cons daily beginning d-7
Clin Chem d14, d27 before dosing and d35 (necropsy)
Hematology d14, d27 before dosing and d35 (necropsy)
Urinalysis before necropsy
Necropsy day 35
Histopathology Gross lesions.

did this study for Anthra at its
signed the GLP statement.

Results:

Mortality none
Clinical Obs. No AD 32 related effects
Body Wt. mean body wt in G5 decreased beginning day 17, ~10% < control. Did not recover by d35. Other dosed groups were less than controls but did not reach significance. The Dianeal controls weighed more than the vehicle control but the difference did not reach significance.
Food Cons G5 consistently less than G1. Occasional decreases in other dosed groups.
Water Cons No AD 32 related effects
Clin Chem. Mean total protein and its components albumin and globulin were decreased in G5 10 to 15% on d35. These parameters were somewhat lower in the other dosed groups but the difference was not significant. The toxicological significance of this decrease is unclear. Other variations were not toxicologically significant
Hematology d35 mean WBC decreased by 33% in G5 due to reduction in segmented neutrophils, lymphocytes and eosinophils.
Urinalysis No toxicologically significant differences
Gross Path No AD 32 related lesions.

Unfortunately the investigators did not choose to study AD 32 toxicity at higher doses. This is perhaps the best study available for AD 32 systemic toxicity and the doses are so low that the it is uninformative. Nevertheless, it does suggest that the dose limiting toxicity of AD 32 is myelosuppression.

B.2.6 Repeated dose toxicity study of AD-32 administered intraperitoneally to Sprague-Dawley rats, 1997. Report 048-004. Vol. 1.20, p 396.

Animal male and Female CrI:CD Sprague Dawley rats
Drug AD 32, Lot 515-44-0003
Doses G1 - Vehicle control "diluent" and 0.9% saline
G2 - AD 32, 240 mg/m² (given as 40 mg/kg)
G3 - AD 32, 480 mg/m² (given 80 mg/kg)
G4 - AD 32, 600 mg/m² (given 100 mg/kg)
Vehicle "AD-32 diluent" supplied by Anthra (Lot 583-44-0001) and 0.9" saline, proportions not specified.
Dose volume 15 ml/kg
N 3 males and 3 females per dose group
Schedule q21dX3 (days 1, 22, and 43)
Route IP

Observations:

Clinical Obs 1, 4 and 6 hr after dosing and twice daily
Physical Exam before the study and before necropsy
Body Wt. daily on weekdays
Food Cons weekly
Ophth. Exam before the study and before necropsy
Necropsy day 64
Histopathology See Table

did this study for Anthra at its
signed the GLP statement.

Results:

Mortality none
Clinical Obs. Injection site damage with bleeding
Swelling or firmness in the R and L inguinal areas in some G3 and G4 rats.
Bilateral hind limb impairment in most AD 32 treated animals.
Bilateral hind limb impairment in most AD 32 treated animals (curled toes, starting d17 reversible only in G2).
Orange urine after dosing.
Body Wt. Slight decreases associated with AD 32 dosing, more pronounced in males
Food Cons Slightly decreased in G4 males.
Ophth. Exam No treatment related changes
Gross Path No AD 32 related lesions.
Histopathology Edema and degeneration of the sciatic nerve - minimal to mild in G2 females, increasingly pronounced in both sexes at higher doses.
Proteinosis of the renal cortical tubules - minimal to mild in males of G3 and G4
Degeneration of the seminiferous tubules - mild to moderate G3 and G4

The swelling in G3 and G4 rats was probably due to local irritation associated with AD 32. The neuropathy seen both clinically and microscopically is significant. I have not found this toxicity in other studies. According to the authors of this study doxorubicin causes this toxicity at doses of 5 or 10 mg/kg. Jortner and Cho (1980, *Cancer Treatment Reports* 64(2-3):257-261 and 1977, *J Neuropath Exptl. Neurotox* 36:907-915) have described this toxicity as a Wallerian degeneration secondary to necrosis of the sensory neurons in the sciatic and other nerves. These authors postulated that the toxic effects are most pronounced in the lumbosacral dorsal root ganglia of rats because the blood neural barrier is absent in this area. I do not know why this toxicity did not manifest in the other IP study in rats.

Again AD 32 caused damage to the testes at relatively low doses. Myelosuppression was probably present but the investigators did not look for this toxicity.

B.2.7 Thunberg AL. Summary report on the analysis of dosing solutions from toxicology study 048-004 : Repeated dose toxicity study of AD 32 administered intraperitoneally to Sprague-Dawley rats, 1997. Report OLI-RA247.06-9710-MJV-2, Vol. 1.20, p 484

This is an analysis of the dosing solutions from the previous study. The mid and high dose solutions were within 5% of the nominal concentration. The low dose solutions were about 26% lower than the nominal concentration specified (2.0 mg/ml instead of 2.7 mg/ml). These dosing solutions were from the first dose period only so the sponsor does not know if the two subsequent doses were low. The low dose in the previous experiment may have been closer to 30 mg/kg than 40 mg/kg. No toxicity was seen in the low dose group.

B.2.8. Repeat Dose toxicity study of AD-32 Administered intraperitoneally to beagle dogs, Study 048-003. 1997. Volume 21 page 1.

Animals	male and female beagle dogs
Drug	AD-32, Lot 515-44-003
Doses	100, 200, and 400 mg/m ² (given as 5, 10, and 20 mg/kg) one per sex per dose in the toxicity portion of the study one per sex at the low and high dose for the Pharmacokinetics portion.
Dose volume	15 ml/kg
Vehicle	0.9% saline
Route	IP via Catheter
Schedule	single dose ever 21 days for three doses,
Observations	
Clinical Obs.	twice daily
Body Wt.	before dosing and weekly
Food Cons.	daily
Physical	before dosing and before necropsy
Ophthal. Exam	before dosing and before necropsy
Hematology	before dosing, 24 hours post injection, weekly
Clin. Chem	before dosing, 24 hours post injection, weekly
Necropsy	Day 64 for the toxicity portion of the study Day 2 for the pharmacokinetics portion,
Histopathology	Bone marrow, large and small intestine, kidneys, liver, lung, ovaries, testes, and gross lesions.

Pharmacokinetics on high and low dose dogs, Sample times, pre-injection, 1, 3, 5, 7, 12, and 24 hours.

This study was done by
GLP statement.

signed the

Results.

Mortality One LD male became entangled in the water line, strangled on day 48

Clinical Signs Diarrhea one MD dog, vomiting one HD dog.
Purple discoloration around injection site of some animals. Focal swelling of the abdomen, swelling around the eye 1 to 6 hours after injection. Related to injection not dose. These reactions were more numerous after third injection.

Body weight All surviving animals gained weight during the study

Food Cons No apparent treatment related effects

Hematology WBC was elevated 24 hr after the first dose in 6 animals, and in 3 after the second dose. This was probably an inflammatory response to the injection. These changes were reversible within 6 days.

Clinical Chem. No toxicologically significant changes

Gross Pathology No toxicologically significant changes

Histopathology degeneration of seminiferous tubules in MD and HD
Some degeneration in the liver at all doses

The high dose in this study approximates the total clinical dose on a mg/m² basis. This study shows that repeated doses (q21 d) causes little toxicity and no death. Much of the toxicity was associated with the injection. There were some signs of liver toxicity and a transient increase in AST in one HD female. Some liver toxicity with a drug of this class by this route would not be unusual. Notably the pathologist did not document any cardiac toxicity.

B.2.10. A multiple dose toxicity study of AD31 injected directly into the thoracic cavity of rats, Study AD32RATCHSTX4. 1997. Volume 21 page 191.

Animals	male and female CD rats				
Drug	AD-32, Lot 515-09-0002				
Doses					
Group	route	AD32 mg/kg	AD32 mg/m ²	# males	# females
Control				5	5
Saline	IT			10	10
Vehicle	IT			10	10
AD32	IT	100	600	10	10
AD32	IT	50	300	10	10
AD32	IT	25	150	10	10
AD32	IP	100	600	5	5
AD32	IP	50	300	5	5
AD32	IP	25	150	5	5

Vehicle NCI Diluent 12 (the author did not further specify the composition)

Dose volume 2.5 ml/kg for 100 mg/kg, 1.25 ml/kg for 50 mg/kg, 0.67 ml/kg for 25 mg/kg

Route intrathoracic IT under anesthesia, and IP

Schedule q21X3, a fourth planned dose was not given because of excess toxicity
 Observations
 Clinical Obs. twice daily
 Body Wt. five times weekly
 Hematology at necropsy
 Clin. Chem at necropsy
 Necropsy three weeks after last dose (day 63)
 Histopathology See table

This study was done by

signed the GLP statement.

Results

Mortality

Group	route	AD32 mg/m2	# males	# females
Control			0/5	0/5
Saline (SC)	IT		0/10	0/10
Vehicle (VC)	IT		3/10	1/10
AD32	IT	600	10/10	10/10
AD32	IT	300	10/10	4/10
AD32	IT	150	2/10	0/10
AD32	IP	600	5/5	3/5
AD32	IP	300	0/5	0/5
AD32	IP	150	0/5	0/5

Most HD IT males died immediately after the first injection. This appeared to be due to pleural effusion. Most HD IT females died immediately after the second dose. Most MD IT males died immediately after the second dose. Most MD females died immediately after the third dose. Death immediately after injection was usually preceded by respiratory distress. The description of these deaths is extensive but ultimately irrelevant. The dose limiting toxicities appear to be local, not systemic when AD-32 is given IT. Nevertheless, the toxicity does appear to be cumulative.

The volume of vehicle necessary to dissolve the HD was lethal to 4 of 20 rats. For adult rats this volume was approximately 0.5 ml. This is a lot of any thing to put in a rats thoracic cavity, nevertheless, no saline controls died. The rats in the vehicle group that died appeared to suffer effusion and pulmonary compromise. As one would expect, these signs were also seen in the HD and MD animals.

Rats that died after IP injection showed signs of general toxicity, roughened fur, kyphosis and abdominal distention.

Clinical Observation. Numerous signs and symptoms in the rats that died. Sparse in the LD IP and LD and MD IP groups.

Body Weight

The following table summarizes the results for males. The weighs of females followed the same pattern but the decreases were less severe. In all IT AD-32 groups weight decreased immediately after dosing. It then began to recover in the LD and MD groups.

Body Weight Decrease

	day 0	day 40	% of control	day 63	% of control
IT injection					
untreated controls	235	405	100%	467	100%
vehicle controls	236	374	92%	425	91%
LD males	240	380	94%	400	86%
MD males	238	239	59%		
HD males	238				
IP Injection					
LD males	239	413	102%	462	99%
MD males	240	417	103%	452	97%
HD males	237				

Hematology	Changes in Red Cell parameters consistent with dehydration in LD and MD IT groups, and vehicle controls IT group. Decrease in WBC (lymphopenia) in LD (~60% of controls) and MD (~40% of controls) IT groups.
Clinical Chem.	Some relatively small but statistically significant changes. None of these changes suggest dose dependent organ toxicity. Most changes seem associated with IT dosing and not the drug.
Gross Pathology	<p>VC -- 7/16 had small to moderate thoracic adhesions</p> <p>MD IT -- (all females) 4/6 thoracic adhesions, 5/6 pericarditis, 5/6 fibrinous pleuritis, 2/6 rounded liver lobes, 2/6 splenic atrophy, 1/6 thoracic effusion</p> <p>LD IT-- 13/18 thoracic adhesions, 9/18 pericarditis, 3/18 fibrinous pleuritis, 8/18 rounded lung lobes, 11/18 thoracic effusion.</p> <p>HD IP -- 2/2 dilated intestines, 1/2 splenic atrophy adhesions or ascites, 2/2 rounded liver edges</p> <p>MD IP -- 5/5 females no gross lesions, 4/5 m adhesions, 4/5 m ascites, 3/5 m rounded liver edges, 1/5 subcapsular kidney hemorrhage. 1/5 enlarged cecum.</p> <p>LD IP -- 7/10 no gross lesions, 3/5 m had drug induced toxicity including peritoneal adhesions, bloody ascites, swollen kidneys, rounded liver edges</p>
Histopathology	<p>VC -- 4/16 connective tissue on diaphragm, 1/16 lung surface trauma and hemorrhage, 6/16 adhesions or fibrosis, , 2/16 focal pneumonia, 1/16 surface atelectasis, 1/16 diaphragm with macrophages and new vessels</p> <p>MD IT -- 6/6 plural fibrosis, 1/6 pericardial fibrosis, 2/6 focal pneumonia, 2/6 fibrin deposition, 1/6 hemorrhage.</p> <p>LD IT -- 12/18 pleural fibrosis, 12/18 pericardial fibrosis, 4/18 focal pneumonia.</p> <p>HD IP -- 1/2 surface fibrosis of the diaphragm.</p> <p>MD IP -- 3/5 m fibrosis, 1/5 bilateral hydronephrosis and mild focal nephritis.</p> <p>LD IP -- 2/5 m moderate hydronephrosis</p>

The prolonged lymphopenia, three weeks after dosing is somewhat unusual for anthracyclines. It suggests that the drug may be cleared slowly from the thoracic cavity. After IT injection, some of the toxicity was caused by the vehicle, but AD 32 clearly exacerbates this toxicity. The damage was widespread but there was a great deal of individual variation at all doses. This study suggests that clinical studies of AD 32 given by the IT route would not be safe, particularly with this vehicle.

B.2.11 A multiple dose (X4) toxicity study of AD-32 injected directly into the thoracic cavity of beagle dogs, Study AD32CHSTX4. 1996. Volume 22 page 1.

Animals	two male and two female beagle dogs per drug dose group, one male and one female in the vehicle control group.
Drug	AD-32, Lot not specified
Doses	400 mg/m ² (given as 20 mg/kg)
Dose volume	50 ml
Vehicle	NCI Diluent 12 and saline, the author does not specify how much of each
Route	IT via cannula, the dogs were anesthetized.
Schedule	single dose ever 21 days for three doses (protocol originally specified 4 doses)
Observations	
Clinical Obs.	twice daily
Body Wt.	10 and 3 d before treatment, 1 and 3 hr after treatment, daily
Body Temp	10 and 3 d before treatment, 1 and 3 hr after treatment, daily
Food Cons.	10 and 3 d before treatment, 1 and 3 hr after treatment, daily
Ophthal. Exam	before dosing and before necropsy
ECG	Days -10, -3, 1, 4, 11, 18, 25, 32, 39, 46, 53, 60, 67
Hematology	Days -10, -3, 1, 4, 7, 11, 14, 18, 22, 25, 28, 32, 39, 43, 46, 49, 53, 56, 60, 63, 67, 70
Clin. Chem	Days -10, -3, 1, 4, 7, 11, 14, 18, 22, 25, 28, 32, 39, 43, 46, 49, 53, 56, 60, 63, 67, 70
Urinalysis	males only, days -3, 7, 14, 22, 28, 35, 43, 49, 56, 63, 70.
Necropsy	Day 71 for the toxicity portion of the study
Histopathology	tissues not listed but the author says they examined 50 tissues

This study was done by

signed the GLP statement

Results	
Mortality	one dosed male died on d23 after the second dose. One dosed female died on d50 after the third dose.
Clinical signs	severe transient anorexia after dosing. Numerous other signs particularly after dosing. These included lethargy, vomiting, loose stool, no stool, aggressive behavior, labored breathing, pain, dehydration.
Body Temp	Transient increases after dosing suggestive of an inflammatory response
Body Weight	Decreased 5 to 8% in dogs that survived all 3 doses.
Ophth	No toxicologically significant changes
ECG	No toxicologically significant changes
Hematology	All dosed animals had a transient increase in WBC (to 2.3 fold average above normal) after dosing. This increase was primarily due to neutrophilia suggesting an inflammatory response. By the fourth day after dosing the WBC dropped back below normal then slowly recovered. Fibrinogen also increased and then fell immediately after dosing, again consistent with an inflammatory response. Platelets tended to increase with time.

Clin Chem Increases in Alk Phos after dosing but no consistent dose related changes that would predict a specific organ toxicity.
Gross Path Thoracic effusions, fibrin depositions and adhesions
Histopathology Consistent with the damage seen grossly, fibrinous pleuritis.

The vehicle did not cause significant toxicity in this study. Evidently, it was diluted with sufficient saline to prevent the damage seen in study B.2.10. The dosed dogs were very ill throughout the study. The investigator says that the most severe physical symptom was rapid and difficult breathing. The dogs suffered pain when the investigators handled their thorax. They were so severely ill that the investigators opted to forgo the last dose. The primary toxicity appears to have been a severe intrathoracic inflammatory response. This response caused profound thoracic effusions. These effusions had a specific gravity greater than 1.017 and a total protein greater than 3.0 g/dl indicating that this effusion was an exudate. Glucose concentrations in the exudate were high implying that it was sterile. There is no evidence that IT AD-32 caused any systemic toxicity. All lesions appear to be local. Considering the amount of pathology found on day 71 (29 days after the last dose) a fourth dose probably would have killed the remaining dogs. Treatment with AD-32 by the IT route is probably too toxic to be a practical therapy.

Toxicology Summary

Three or six doses of AD 32 instilled in the urinary bladder of rats to 324 mg/m², seven days apart, caused only limited toxicity. The trauma and damage in the peritoneum, bladder and urethra were probably caused by the procedure. Six intravesical doses of AD 32 in the urinary bladder of male dogs to 400 mg caused neovasularization and the accumulation of pigment laden macrophages in the bladder epithelium. Treatment also caused atrophy and inflammation of the prostate, and testicular degeneration. Some of this damage remained after 35 days of recovery. Unfortunately the experiment did not include sufficient information about the vehicle controls, so I cannot distinguish AD 32 toxicity from vehicle toxicity (NCI Diluent 12).

Three IP doses to 636 mg/m² given 14 days apart to rats caused no mortality and only a 33% decrease in WBC. The doses in this study were among the highest systemic doses given to rats or dogs. The study suggests that myelosuppression is dose limiting, but the high dose was relatively low. Three IP doses to 600 mg/m² given 21 days apart to rats also caused no mortality. Nevertheless, this longer schedule did cause edema and degeneration of the sciatic nerve that increased in intensity with dose. This neuropathy is possibly associated with a diminished blood-neural-barrier in the lumbosacral area of rats. The significance of this toxicity to humans is questionable. In this study, IP dosing caused damage to the testes even at 480 mg/m².

In beagle dogs, three IP doses to 400 mg/m² given 21 days apart caused only mild degeneration in the seminiferous tubules and some degeneration in the liver. These toxicities are not unusual for an anthracycline. The dose approximates the proposed human dose which is relatively low.

Repeated intrathoracic dosing in rats to 600 mg/m² caused the death of 20 of 20 animals. Half this dose was lethal to 14 of 20 animals. In this experiment, vehicle at the volume given to high dose animals caused the death of four of 20 animals. Death appeared to be caused by pulmonary effusion. In surviving animals dosing caused prolonged lymphopenia, adhesions, pericarditis, fibrinous pleuritis and gross changes in the liver and spleen. Three intrathoracic

doses of 400 mg/m² given 21 days apart killed two of four dogs. Surviving animals suffered weight loss, an inflammatory response after dosing. Local toxicities included thoracic effusions, fibrin deposition and adhesions. Treatment with AD 32 by this route is probably too toxic to be a practical therapy.

Long term IV dosing with AD 32 appears to cause a different spectrum of toxicities than Dox. A total dose of 60.7 mg (0.11 mmoles) of Dox over 12 weeks caused cardiac edema, ventricular dilation, myocytolysis, fibrosis and atrophy in rabbits. This cumulative dose of Dox also caused direct damage to the glomerulus in the kidneys, centrilobular necrosis in the liver and aspermia and testicular degeneration. A dose cumulative dose 492.1 mg of AD 32 (0.68 mmoles) over the same period caused only moderate cardiac edema and vacuolization in rabbits. In this study AD 32 also caused enlargement of the spleen, bone marrow hypoplasia and fatty changes in the liver. The splenic enlargement was associated with sinusoids filled with erythrocytes and granulocytes at various stages of maturity.

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