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APPROVAL PACKAGE FOR:

**APPLICATION NUMBER
NDA 50-778**

Pharmacology Review(s)

Division of Oncology Drug Products, HFD-150
Review and Evaluation of Pharmacology and Toxicology Data
 Original Review

Keywords: Epirubicin, breast cancer, anthracycline

NDA: 21-010 and 50-778

Serial #: 000

Type: NDA

CDR date: 12/15/98

Information to be conveyed to sponsor: Yes(), No(x)

Reviewer: Doo Y. Lee Ham, Ph. D.

Date Review Completed: June 14, 1999

Applicant: Pharmacia & Upjohn
 Kalamazoo, MI 49001

Drug:

Generic Name: Epirubicin hydrochloride injection (4'-epi-doxorubicin; 4-epi-adriamycin hydrochloride)

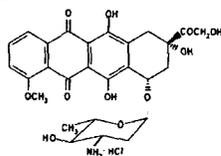
Trade Name: Ellence

Code Name: IMI 28, EPI

Chemical Name: (8S, 10S)-10-[(3-amino-2, 3, 6-trideoxy- α -L-arabinohexopyranosyl)-oxy]-8-glycoloyl-7, 8, 9, 10-tetrahydro-6, 8, 11-trihydroxy-1-methoxy-5, 12-naphthacenedione hydrochloride

CAS Register Number: 56390-09-1

Structure:



Molecular Formula: $C_{27}H_{29}NO_{11} \cdot HCl$

Molecular Weight: 579.99

Related IND & NDA: IND _____ and _____

Class: Anthracycline cytotoxic agent

Indication: Epirubicin is indicated for the treatment of the patients with advanced breast cancer. The recommended clinical dose is 100 mg/m² to 120 mg/m².

Formulation:

The complete formulation of the freeze dried dosage form (10 and 50 mg) is detailed in Table 9. This is the only formulation that has been used clinically.

Table 9: Formulation of Epirubicin Hydrochloride freeze dried

Strength	Formulation No.	Ingredient	Quantity per vial	Function	Reference to Standard
10 mg	F1 7701/1F1	Epirubicin Hydrochloride	10 mg	Active Pharmaceutical Ingredient	In-house
		Lactose	50 mg	Bulking agent	NF
50 mg	F1 7701/1F2	Epirubicin Hydrochloride	50 mg	Active Pharmaceutical Ingredient	In-house
		Lactose	250 mg	Bulking agent	NF

Route of Administration: Intravenous

Previous Reviews, Dates and Reviewers:

NDA — (Epirubicin HCl for Injection) 8/13/85 JSun

Background Data:

Epirubicin (4'-epi-doxorubicin, IMI 28) is a doxorubicin derivative which was synthesized in an effort to increase the therapeutic index of the parent compound.

Doxorubicin HCl is the most widely used cancer drug as a single agent or in combination with other anti-neoplastic agents to treat for both hematologic and non-hematologic malignancies since its approval 1970s.

NDA — for epirubicin was submitted by Farmitalia in 1984 for the treatment of advanced breast cancer. Due to insufficient data in the submission, this NDA was not approved. Farmitalia transferred the right for Epirubicin to Pharmacia & Upjohn.

NDA 21-010 for epirubicin was re-submitted for the same indication by Pharmacia & Upjohn in 1998. Pharmacia & Upjohn references NDA — for NDA 21-010 with regard to all non-clinical pharmacology, ADME and toxicology data. Besides reproductive toxicity studies, additional published data for pharmacokinetics, mutagenic and carcinogenicity studies have been submitted.

Studies Reviewed Previous Submission:

- I. Pharmacology
 - A. Mechanism of action
 - B. In vitro cytotoxicity
- 1) Safety Pharmacology:
 - A. Antitumor activity
- 2) General Pharmacology:
 - A. Effects on cardiovascular function in the normotensive rat
 - B. Effects on cardiovascular and respiratory functions in the anesthetized dog
 - C. Effects on diuresis in the rat
 - D. Effects on gastric emptying in the rat
 - E. Effects on the endocrine system in the rat
- II. Pharmacokinetics:
 - A. Pharmacokinetics in the mouse
 - B. Pharmacokinetics in the rat
- III. Toxicology:
 1. Single dose toxicity studies:
 - A. Acute iv toxicity study of IMI 28 vs doxorubicin HCl in mice
 - B. Acute iv toxicity study of FCE 21773 (13-OH-epirubicin, epirubicinol) in mice
 - C. Acute oral toxicity of IMI 28 in mice and rats

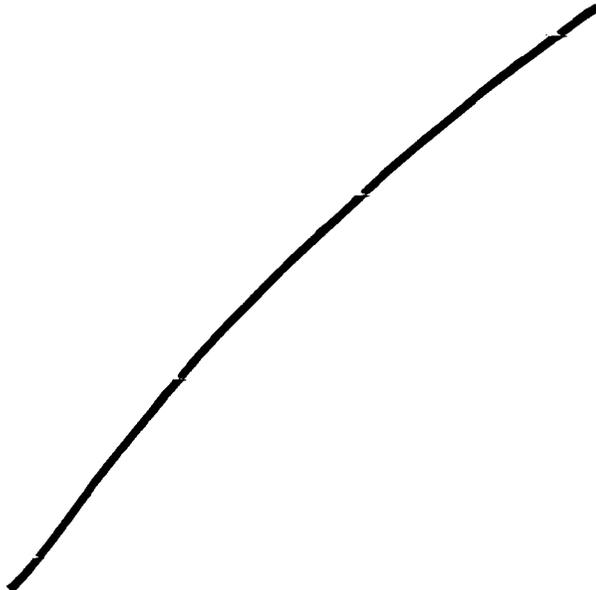
- D. Acute ip toxicity study of IMI 28 in mice and rats
- E. Acute iv toxicity study of IMI 28 in rat
- F. Acute iv toxicity study of IMI 28 in dog
- 2. Repeat dose toxicity studies:
 - A. 13-week iv toxicity study of IMI 28 in rats
 - B. 6-week iv toxicity study in rabbits vs doxorubicin
 - C. 6-week iv toxicity study in dogs vs doxorubicin
 - D. 13-week iv toxicity study in dogs
- IV. Special Toxicity Studies:
 - A. Hematotoxicity: dog
 - B. Immunotoxicity: mouse, guinea pig
 - C. Cardiotoxicity: mouse, rat
 - D. Local tolerance: rat
- V. Reproductive Studies:
 - A. Teratogenic study in rats
 - B. Teratogenic study in rabbits
- VI. Mutagenicity Studies:
 - A. Ames assay
 - B. In vitro gene mutation test on V79 Chinese hamster cell line
 - C. Chromosome aberrations
 - D. Micronucleus assay
- VII. Carcinogenic/Oncogenic Studies:
 - A. Carcinogenicity study (sc) in rats vs doxorubicin

Studies Reviewed with This Submission:

- I. Pharmacology:
 - A. Mechanism of action
 - B. In vitro cytotoxicity A summary table
 - C. Antitumor activity A summary table
 - D. Pharmacology studies A summary table
- II. Pharmacokinetics:
 - A. Comparative metabolism and pharmacokinetics of doxorubicin and 4'-epidoxorubicin in plasma, heart and tumor of tumor-bearing mice
 - B. Pharmacokinetics in normal and hepatectomized rats
 - C. Enterohepatic circulation and plasma protein binding of the radiolabeled compound in rats
 - D. Metabolism and excretion in rats vs Doxorubicin
 - E. Pharmacokinetics in rabbits
 - F. Pharmacokinetics after intrahepato-arterial and intravenous injections in rabbits
 - G. Pharmacokinetics in the dog
 - H. Pharmacokinetics in dogs vs various anthracyclines
 - I. Metabolism in animals: Absence of glucuronidation
 - J. Plasma protein binding of radiolabeled compound in man, mice, rats, rabbits and dogs
- III. Toxicology:
 - A. Acute iv toxicity study of PFS vs the lyophilized commercial formulation in mice
 - B. Acute iv toxicity study of PFS vs its artificially degraded formulation in mice
 - C. Acute iv toxicity study of PFS vs the lyophilized commercial formulation in rats
- IV. Special Toxicity Studies:
 - A. Intravenous hematotoxicity study in rats vs doxorubicin

- B. Intravenous bone marrow toxicity study in dogs vs doxorubicin
 - C. Intravenous immunotoxicity study vs doxorubicin in mice
 - D. Intravenous antigenicity study vs doxorubicin in guinea pigs-
 - E. Intravenous cardiotoxicity study vs doxorubicin in rats
 - F. Intravenous cardiotoxicity study vs doxorubicin in dogs
 - G. Local tolerability study in rat with it (intradermal) injection of epirubicin RTU
- V. Reproductive Studies:
- A. Segment I fertility study in rats (Tech Report (TR) IMI 28/433i & IMI28/435i)
 - B. Segment II teratogenic study in rats and rabbits (Report 9850197)
 - C. Segment III peri- and postnatal toxicity study in rat (Tech Report IMI 28/434i)
 - D. Segment III intravenous peri-postnatal toxicity study in rats (TR IMI 28/436i)
- VI. Genotoxicity Study: A summary table
- VII. Carcinogenicity Study:
- A. Carcinogenesis of the compound 4'-epi-adriamycin (IM 28) in inbred Wistar Lewis rats
 - B. Review of carcinogenicity studies; Intravenous carcinogenicity study in rats vs doxorubicin

Studies Not Reviewed:



* Portions of this review were excerpted directly from the sponsor's submission

I. Pharmacology:

A. Mechanism of Action:

Epirubicin (4'-epi-doxorubicin; 4'-epi-adriamycin; IMI 28; EPI) is the epimer of doxorubicin, with inversion of the 4'-hydroxyl group on the sugar moiety. The mechanism of action of epirubicin is not fully understood. However, many studies indicate that epirubicin, like doxorubicin (parent drug), forms a complex with DNA by intercalating into DNA strands, thus inhibiting DNA replication and transcription, and further inducing DNA fragmentation with inhibition of repair.

Recent studies by Bachur et al (1992) demonstrated that epirubicin and other anthracyclines inhibit DNA helicase activity, thus preventing the enzymatic separation of double-stranded DNA, and interfering with replication and transcription. Consequently, the observed inhibition of DNA and RNA polymerase activity by anthracyclines may result from an incomplete separation of DNA strands, which inhibits binding of these enzymes to DNA .

Anthracyclines also appear to affect regulations of gene expression and the integrity and activity of cellular membrane. Anthracyclines are reported to inhibit transmembrane dehydrogenase which has an important role in the control of tumor cell growth. Microsomal enzymes such as NADPH and cytochrome P450 reductase can reduce anthracyclines to semiquinone free radicals which appear to be implicated in damaging DNA, cell membrane lipids and mitochondria (Plosker, GL et al, 1993, *Drugs* 45:788-).

Helicase inhibition by anthracycline anticancer agents. Bachur, N.R., et al., *Mol. Pharmacol.* 41:993-998, 1992

These authors examined anthracycline antibiotic effects on SV40 large T antigen helicase activity, using a duplex DNA helicase substrate of ³²P-labeled 17-mer annealed to complementary M13mp19(+) circular single-stranded DNA. The T antigen helicase activity was potently inhibited by the anthracycline antibiotics. The T antigen helicase IC₅₀ values for the anthracycline antibiotics were as follows: nogalamycin, 2 x 10⁻⁷; daunorubicin, 4 x 10⁻⁷ M; doxorubicin, 4 x 10⁻⁷; idarubicin, 1.8 x 10⁻⁶; 4'-epidoxorubicin, 2 x 10⁻⁶; aclacinomycin, 4 x 10⁻⁶ M; and menogaril, 6 x 10⁻⁶. Partially purified helicases from HeLa cells and murine mammary carcinoma FM3A cells also were potently inhibited by these anthracyclines. This manuscript also included previously published data (DiMarco, A. et al., *Cancer Res.*, 36:1962-1966, 1976) on the effect of epirubicin on DNA melting temperature. The 12.5°C shift indicates that epirubicin intercalates into DNA similarly to daunorubicin and doxorubicin.

B. In Vitro Cytotoxicity: A Summary table

The in vitro cytotoxicity of epirubicin was investigated using HeLa human cervical carcinoma (colony efficiency test), human tumor specimens (stem cell assay) and murine embryo fibroblasts (MEF) infected or not with Moloney's murine sarcoma virus (MSV-M)(growth inhibition test).

A Summary of in Vitro Cytotoxicity Studies -

Test system	Compound	Conc. (ug/ml)	Exposure time						Results
			2 hrs		8 hrs		24 hrs		
			NC%	IC50 (ug/ml)	NC%	IC50 (ug/ml)	NC%	IC50 (ug/ml)	
HeLa cells	Epirubicin	0.125	78		59		60	In colony inhibition test, the cytotoxicity of epirubicin was time-dependent and the compound was less cytotoxic than doxorubicin	
		0.25	66	>5.0	54	0.35	40		
		0.5	60		47		35		
	Doxorubicin	0.025	102		83		42		
		0.05	78		72		36		
		0.1	65	0.33	62	0.19	18		
	0.25	55		35		6			
	0.5	40		14		0			
Murine embryo fibroblasts	Epirubicin	Conc. (ug/ml)	MSV-M			MEF		The activity of the two compounds on MEF and MSV-M-transformed MEF was approx. the same	
		0.0031	No. foci%	IC50(ug/ml)	No.foci%	IC50(ug/ml)			
		0.0062	90		62				
		0.0125	70	0.01	55	0.008			
	0.025	0		40					
	Doxorubicin	0.0062	46		61				
0.0125		24	0.0005	42	0.010				
0.025		0		37					
Human tumors	Tumor type	Conc.	Epirubicin		Doxorubicin	Average % survival	The cytotoxicity of epirubicin on the various types of human tumor was of the same order of magnitude as doxorubicin		
	Breast	0.05	7		6	4			
	Breast	0.01	9		16	15			
	Ovarian	0.01	80		67	68			
	Ovarian	0.01	50		55	46			
	Ovarian	0.01	28		31	29			
	Lung	0.01	60		57	54			
	Peritoneal	0.01	93		92	57			
Average% Survival		47		46					

NC= Number of colonies

B. Antitumor Activity Summary:

The antitumor activity of epirubicin was evaluated in comparison with doxorubicin on a panel of experimental tumor models including animal and human sources.

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A Summary of Antitumor Activities of Epirubicin

	Type of Tests	Test System	Dose/Schedule	Results
A	Activity against ip-implanted P388 and L1210 leukemias	BDF ₁ or CDF ₁ mice inoculated ip 1×10^6 leukemia cells	Epirubicin or doxorubicin ip at various doses on days 1 and/or 1-5, 1-9 or 1, 5, & 9	When given the optimal dose of 10 mg/kg on days 1,5, and 9, epirubicin was more active than doxorubicin against early leukemia with maximum T/C% values of >402 and >325, respectively
B	Activity against ip-implanted P388 leukemias resistant to doxorubicin	BDF ₁ mice inoculated ip P388 leukemia cells	Epirubicin ip at various doses on days 1, 5, & 9	Epirubicin was inactive against P388/DX leukemia
C	Activity against ip-implanted 180 sarcoma	Swiss mice inoculated 5×10^6 cells	Epirubicin or doxorubicin ip at 0.5-10 mg/kg	At optimal doses, T/C% values were 244 for epirubicin and 225 for doxorubicin, thus no differences exist between the two compounds
D	Activity against iv-implanted Gross leukemia	C3H/He mice inoculated iv 2×10^6 cells	Two forms of epirubicin PFS dissolved in saline or 5% glucose iv at various doses on days 1, 1-3, 1-5, or 1,5, & 9 Epirubicin or doxorubicin at various doses bid x 3 days	No differences in activity were seen between epirubicin PFS in saline or glucose Epirubicin and doxorubicin showed an essentially equal antitumor activity against disseminated Gross leukemias, independent of treatment schedules.
E	Activity against ip or sc implanted B16 melanoma	BDF1 mice inoculated sc or ip tumor	Epirubicin or doxorubicin iv at various doses on days 1-5, 1, 5, and 9	Epirubicin at optimal dose of 6 mg/kg/day resulted ~75% inhibition vs 87% observed in mice given 4 mg/kg/day doxorubicin. Epirubicin on days 1-5 was less active than doxorubicin against sc-implanted B16 melanoma

F	Activity against sc-implanted early and advanced mammary ca.	Mice 2×10^6 cells	Epirubicin or doxorubicin at various iv doses	When epirubicin or doxorubicin were given in two cycles of 6 doses each, starting from day 1, the survival time was increased but all animals had tumors. Epirubicin was slightly more active than doxorubicin against advanced mammary tumors
G	Activity against im-implanted Lewis lung carcinoma	C57BL/6 and BDF1 mice inoculated im 4×10^5 or 1×10^6 cells	Epirubicin or doxorubicin given sc to C57BL/6 mice on days 1-9	Epirubicin (3.0 mkd) and doxorubicin (2.5 mkd) sc given to C57BL/6 mice on days 1-9 slightly increased the survival time. T/C% values were 153 and 140. In BDF1 mice treated iv on days 1-4, survival time significantly increased with both compounds.
H	Activity against tumor metastases	BDF1 or BALB/c mice with pulmonary metastases by im-implanted MS-2 sarcoma and Lewis lung carcinoma	Epirubicin or doxorubicin given iv Q3D for 4 or 6 cycles on days 12-15 after tumor implantation	Epirubicin (5 & 10 mkd) increased the survival time of Lewis lung ca. Doxorubicin proved to be less effective than epirubicin. Similar effects were seen in the MS-2 system in which epirubicin was slightly more active.
I	Activity against human tumor transplant in athymic mice	21 different human tumors from patients	Epirubicin or doxorubicin iv weekly for 3-4 weeks	Epirubicin showed a spectrum of antitumor activity similar to that of doxorubicin.
J	Activity against other tumors	Rat Yoshida, AH-13, AH-66, colon 26	Epirubicin or doxorubicin iv on days 1-5 to Donryu rats	Both compounds showed the same activity against Yoshida, AH-13, AH-66, and colon 26.

D. Pharmacology Studies: A Summary table

A Summary of General pharmacology effects of epirubicin

Types of Tests	Species	Route	Doses, mg/kg	Results
Cardiovascular activity	Rat	IV	7, 10, 14 for epirubicin 4, 8, 10 for doxorubicin (as reference drug)	Both compounds at the tested doses had very little effect on mean blood pressure and heart rate
Cardiovascular/respiratory functions	Dog	IV	2 for epirubicin	No effect on systemic arterial pressure or heart rate and respiratory functions
Diuresis	Rat	IV	3, 6, 12 for epirubicin	No effect on renal excretion of water and electrolytes
Gastric emptying	Rat	IV	3, 6, 12	Epirubicin significantly slowed gastric emptying only at 12 mg/kg
Endocrine system	Rat	IV	0.4, 1.0, 2.5	Epirubicin had effects on the endocrine system from 1.0 m/kg as antianabolic, antiestrogenic, antigonadotrophic, and antithyroid activities

VIII. Pharmacokinetics:

- A. Comparative metabolism and pharmacokinetics of doxorubicin and 4'-epidoxorubicin in plasma, heart and tumor of tumor-bearing mice (Cancer Chemother Pharmacol 26:9-12, 1990):
(vol. 1.24, p. 026)

Method:

Pharmacokinetics and metabolism of 4'-epidoxorubicin, a stereoisomer of doxorubicin, was compared with doxorubicin in tumor-bearing mice. Following a single i.v. injection (10 mg/kg) of epirubicin or doxorubicin to female BALB/c mice bearing colon 26 tumors, plasma, heart and tumor samples were taken at 0, 5, 15 and 30 min and 1, 2, 4, 8, 24 and 48 hrs. Plasma and tissue samples were analyzed by HPLC with fluorescence detection.

Results:

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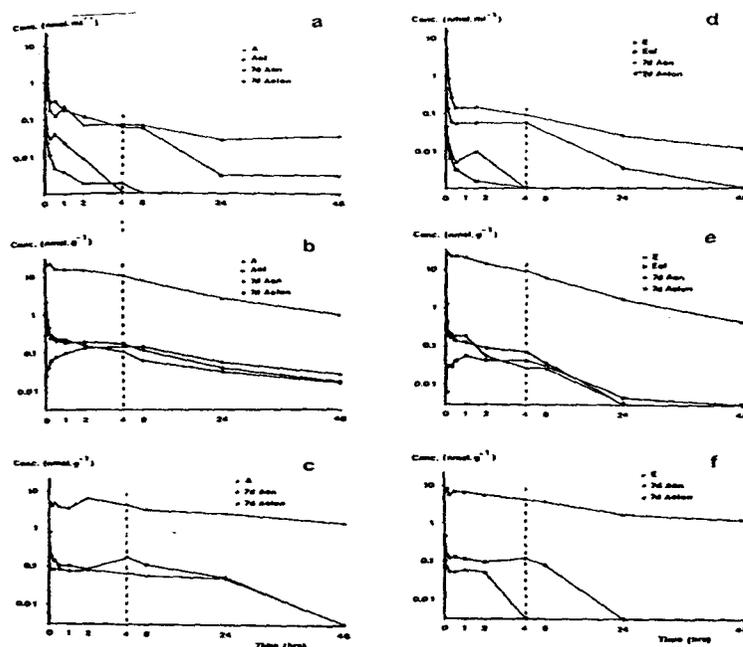


Fig. 1. Concentration-time curves for doxorubicin (A) and epidoxorubicin (E), including their metabolites, in a, d plasma, b, e heart and c, f tumor

Concentration-time curves for doxorubicin and epidoxorubicin including their metabolites, as determined in plasma, heart and tumor tissues, are shown in Fig. 1. The parent drugs and the two 7-deoxy-aglycones were present in all three compartments, whereas doxorubicinol and epirubicinol could only be detected in the plasma and heart. Half-lives (15.6 and 49.5 hr in plasma, 10.6 and 13.2 hr in heart, 24.7 and 32.1 hr in tumor for epirubicin and doxorubicin, respectively), and AUCs of doxorubicin and its metabolites were higher than the corresponding values for epirubicin and its metabolites in all three compartments as in Table 1.

Table 1. AUC values (0–48 h) for doxorubicin, epidoxorubicin and their metabolites in plasma, heart and tumor

Compound	Plasma (nmol·min·ml ⁻¹)	Heart (nmol·min·g ⁻¹)	Tumor (nmol·min·g ⁻¹)
A	204.9	14,064	8,697
Aol	1.7	228	0
7dAon	4.1	152	135
7dAolon	83.3	218	204
E	179.0	12,787	7,868
Eol	0.5	46	0
7dEon	1.6	49	11
7dEolon	53.9	66	99

A, doxorubicin; E, epidoxorubicin

B. Pharmacokinetics in normal and hepatectomized rats (Cancer Chemother Pharmacol 26:444-448, 1990):
(vol. 1.25, p. 057)

Method:

The study was to assess the influence of partial hepatectomy on the pharmacokinetics of 4'-epidoxorubicin (Epi-A, epirubicin) in rats. Male rats (BUF/MOL SPF) were given a single i.v. dose of 5 mg/kg Epi-A 10 min prior to a 2/3 hepatic resection or sham operation (controls). Epirubicin levels in liver tissue and plasma were determined using HPLC method with spectrofluorometric detection.

Results:

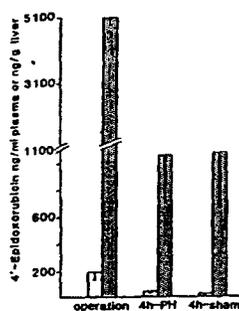


Fig. 1. Bar chart demonstrating Epi-A levels in plasma (□) and liver tissue (■) at the time of operation and at 4 h postsurgery. Mean values \pm SD are given. PH, partial hepatectomy.

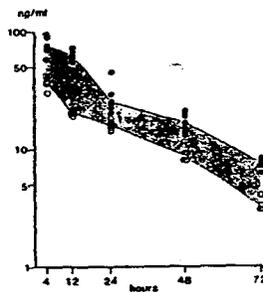


Fig. 2. Plasma concentration vs time curves for Epi-A in partially hepatectomized (●—●) and sham-operated (○---○) rats. The curves are drawn between mean values; points represent measurements in individual animals. Shaded area: the difference in AUC between the partially hepatectomized rats and the sham-operated animals.

The relationship between Epi-A levels in plasma and liver at 4 hr postdose is shown in Figure 1. A marked uptake of Epi-A in liver tissue was found at 10 min after injection. No other alterations in blood chemistry were observed in hepatectomized rats compared to the controls. The partially hepatectomized rats showed a 2-fold increase in plasma AUC between 4 and 72 hr as compared with the sham-operated controls as shown in Figure 2. The terminal half-life from 24 to 72 hr was not significantly changed by the partial hepatectomy (29 and 21 h in hepatectomized and sham-operated animals, respectively).

The results indicate that the liver plays an important role in the clearance of Epi-A.

- C. Enteric circulation and plasma protein binding of the radiolabeled compound in rats (Tech Report IMI-28/811i): (vol. 1.25, p. 038)

Method:

Enterohepatic circulation and plasma protein binding of ^{14}C -epirubicin were studied in rats. For enteric circulation, six bile duct-cannulated rats (318 g) were given 1 mg/kg ^{14}C -epirubicin intravenously and kept in  cage. Bile was collected at 1, 2, 4, 6, 12, 24 and 48 h post-dosing. Urine and feces were collected in the intervals 0-6, 6-12, 12-34 and 4-48 hr post-dosing. The 0-12 h bile of the above animals was pooled and given intraduodenally to another 6 bile duct-cannulated rats (two rats died during the experiments and were not analyzed at the dose of 45 ug-eq/kg. In recipient animals, bile was collected at the same schedule as the donor animals and the excretion balance was evaluated in urine and feces over the same time intervals as above. In plasma protein binding, 12 rats (4 rats/group, 303 g) were divided into 3 groups and given i.v. ^{14}C -epirubicin. Radioactivity in the samples was measured using liquid scintillation counting.

Results:

After i.v. dose of ^{14}C -epirubicin, cumulative biliary, urinary and fecal excretions of radioactivity are shown as in Tables 5E7, 8 and 9.

Table 5.E.7. Excretory Balance of Radioactivity in Rats Receiving IV 1 mg/kg of ^{14}C -Epirubicin (mean \pm SEM, n=6)

Time (h)	Excretion (% of the dose)		
	Bile	Feces	Urine
0-1	13.22 \pm 0.89		
0-2	18.13 \pm 0.99		
0-4	22.93 \pm 1.14		
0-6	25.25 \pm 1.14	1.18 \pm 0.26	7.31 \pm 0.25
0-8	28.39 \pm 1.21		
0-12	30.19 \pm 1.26	1.77 \pm 0.30	8.56 \pm 0.25
0-24	38.44 \pm 1.11	3.14 \pm 0.27	12.00 \pm 0.32
0-48	47.72 \pm 1.07	4.44 \pm 0.32	14.74 \pm 0.31

About 30% of dosed radioactivity were excreted in bile within 12 hrs post-dose, and about 38% and 48% were excreted with 24 and 48 hrs, respectively. Urinary excretion within the same time points were 9, 12 and 15 %, respectively. The major excretion route of ^{14}C -epirubicin was bile in rats. The present study showed that the pattern of excretion of epirubicin was similar to that of adriamycin.

Table 5.E.8. Excretory Balance of Radioactivity in Rats Receiving 0.45 $\mu\text{g}/\text{kg}$ of Radioactivity Following Intraduodenal Administration of the Bile Collected from the animals treated IV with 1 mg/kg of ^{14}C -Epirubicin (mean \pm SEM, n=4)

Time	Excretion (% of the dose)		
	Bile	Feces	Urine
0-1	0.06 \pm 0.01		
0-2	0.27 \pm 0.08		
0-4	2.01 \pm 0.95		
0-6	5.17 \pm 2.59		
0-8	7.05 \pm 2.85	Non detectable	0.54 \pm 0.06
0-24	14.72 \pm 1.42	26 \pm 9.22	2.14 \pm 0.24
0-48	17.32 \pm 2.14	48.78 \pm 3.06	3.19 \pm 0.14

At least 20% of the radioactivity appeared to be reabsorbed following intraduodenal administration. In addition, plasma protein binding was evaluated in vitro and in vivo using the radiolabeled compound and ultrafiltration.

Table 5.E.9. Protein Binding in Rat Plasma after IV Administration or *In vitro*

Sample	Concentration ($\mu\text{g}/\text{mL}$)	Protein binding (%)
<i>In vivo</i>		
30 min	0.164	80.7
6 h	0.077	85.6
24 h	0.048	88.2
<i>In vitro</i>		
0.05	0.056	81.7
0.2	0.223	81.0
1.0	1.081	82.2

In vivo, the protein binding of epirubicin to plasma protein showed a tendency to increase with time, while the in vitro measurements were constant around 82%. Since total radioactivity was measured (that is epirubicin and all metabolites present in plasma), this effect could be due to a strong affinity of epirubicin metabolites for plasma proteins.

D. Comparative metabolism and elimination of Adriamycin and 4'-epiadriamycin in rats (Cancer Chemother. & Pharmacol 19:201-206, 1987) (vol. 1.25, p. 063)

Method:

A comparative study was conducted to find a possible pharmacological basis for differences in toxicity between adriamycin (ADR) and 4'-epiadriamycin (epi-ADR) in rats. Female SD rats were given identical bolus or 24 hr infusion doses (10 mg/kg) of the two drugs. Blood samples were taken up to 24 hr after bolus from 5 animals/sampling time; biliary excretion (after bolus or 24 hr infusion) and mass balance (after bolus) was evaluated up to 48-60 hr. ADR and epi-ADR and metabolites were analyzed using HPLC with fluorescence detection.

Results:

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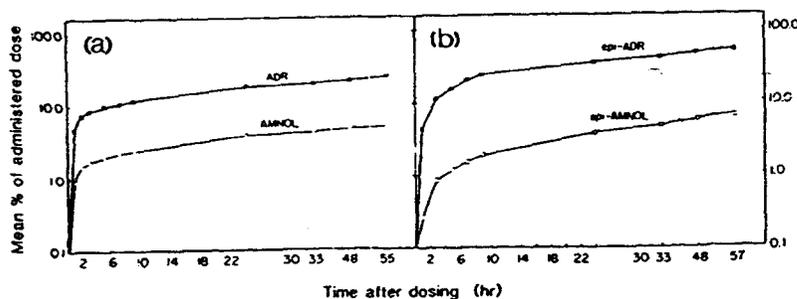


Fig. 1a, b. Mean cumulative biliary elimination of parent drug and metabolite in Sprague-Dawley rats following an i. v. bolus dose of 10 mg/kg of a ADR or b epi-ADR. $n=6$ for each group

As in Figure 1, no differences were seen in plasma drug levels of two agents at 4, 7, and 24 hr. However, differences in rate and extent of drug elimination were clearly noted. Following bolus and 24 hr infusion, the parent drugs were eliminated largely in the bile, with recovery of epi-ADR (50% (infusion) and 40% (bolus) in 54 h and 55 h, respectively) exceeding that of ADR (31% (infusion) and 23% (bolus) at the corresponding time). Urinary excretion of a bolus dose was 4.8% epi-ADR vs 3.3% for ADR in 60 h. For each drug the only significant metabolite seen was the corresponding 13-carbinol derivative, adriamycinol (AMNOL) or 4-epi-adriamycinol (epi-AMNOL). The 13 carbinol metabolite represented a larger part of the recovered dose for ADR 15% (infusion) and 18% (bolus) in bile, 0.3% in urine than epi-ADR 7% (infusion) and 11% (bolus) in bile, 0.04% in urine (Table 3).

Table 3. Cumulative elimination of anthracycline in rat urine following an i.v. bolus dose (10 mg/kg) of ADR or epi-ADR

Time (h)	ADR	epi-ADR	AMNOL	epi-AMNOL
0-6	2.08 ± 1.26	2.43 ± 0.29	0.22 ± 0.13	0.024 ± 0.047
0-24	2.77 ± 1.27	3.90 ± 0.25	0.26 ± 0.13	0.030 ± 0.045
0-60	3.33 ± 1.46	4.83 ± 0.34	0.30 ± 0.15	0.044 ± 0.043

Figures represent percentage of dose (mean ± SD, $n=6$ in each group)

The study suggests that the reported lower toxicity of epi-ADR compared to ADR for the heart may result from a more extensive elimination of parent drug in bile and urine, as well as diminished metabolic conversion to its 13-carbinol metabolite by aldo-keto reductase enzymes.

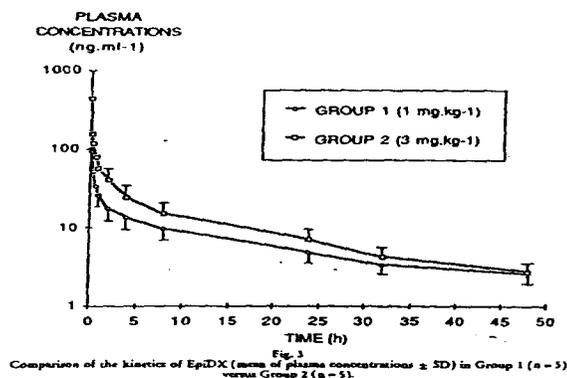
E Pharmacokinetics in rabbits (Acta Therapeutica 17:213-227, 1991): (vol. 1.25, p. 070)

Method:

The pharmacokinetics of epirubicin was studied following i.v. doses of 1 mg/kg ($n=5$) and 3 mg/mg ($n=5$) in the male and female NZW rabbits. Plasma samples were obtained up to 48 hrs and plasma concentrations of epirubicin and metabolites were determined by HPLC with fluorometric detection.

Results:

As in Figure 3 below, epirubicin concentrations declined triexponentially with the terminal half-lives ($t_{1/2\gamma} = 22.8 \pm 6.85$ h for 1 mg/kg and 21.3 ± 5.5 h for 3 mg/kg). Epirubicin was no longer detectable after 48 hrs.



A statistically significant difference ($p < 0.05$) between groups 1 and 2 was observed for the AUC and the initial volume of distribution (Vd). The results of Epirubicin pharmacokinetic parameters are summarized in Table 5.

Table 5. Pharmacokinetic Parameters Obtained after IV Administration of Epirubicin in Rabbits at the Dose Levels of 1 and 3 mg/kg.

Compound	Parameter	1 mg/kg dose	3 mg/kg dose
Epirubicin	$t_{1/2\alpha}$ (h)	0.16 \pm 0.12	0.71 \pm 0.39
	$t_{1/2\beta}$ (h)	3.41 \pm 2.81	7.39 \pm 3.93
	$t_{1/2\gamma}$ (h)	22.85 \pm 6.85	21.28 \pm 5.51
	AUC (ng·h/mL)	420 \pm 160	620 \pm 90
	CL (l/h)	11.76 \pm 7.11	19.35 \pm 3.16
	V_d (L)	21.84 \pm 15.32	81.72 \pm 28.37
Epirubicinol	$t_{1/2}$ (h)	367.89 \pm 179.09	587.16 \pm 144.43
	$t_{1/2}$ (h)	0.63 \pm 0.28	1.66 \pm 1.80
	$t_{1/2}$ (h)	13.97 \pm 2.61	33.94 \pm 17.64
Epirubicin-glucuronide	AUC(0-24 h) (ng·h/mL)	69.46 \pm 11.64	204.86 \pm 148.26
	$t_{1/2}$ (h)	0.15 \pm 0.02	
Epirubicinol-glucuronide	$t_{1/2}$ (h)	1.02 \pm 0.20	0.49 \pm 0.20
	$t_{1/2}$ (h)	7.64 \pm 2.58	15.83 \pm 7.13
	$t_{1/2}$ (h)	0.21 \pm 0.12	0.39 \pm 0.12
	AUC(0-2 h) (ng·h/mL)	1.47 \pm 0.66	4.01 \pm 1.52

After rapid i.v. administration of epirubicin to rabbits, in addition to unchanged drug, several metabolites were detected in plasma. Epirubicin was rapidly metabolized to epirubicinol (chief metabolite identified as the C-13 reduced metabolite), and unidentified metabolite, an aglycone derivative. Two additional metabolites, the glucuronic acid derivatives and epirubicinol, were also detected in plasma soon after injection and were no longer detectable after 2 hrs. As in humans, the metabolism of epirubicin in the rabbit is characterized by the existence of glucurono-conjugates, which are unique in the anthracycline series. The rabbit appears as the only animal species to produce glucurono-conjugates of epirubicin and epirubicinol, since no glucuronide conjugates could be detected in mouse, rat, hamster or guinea pig (Maessen et al).

F. Pharmacokinetics after intrahepato-arterial and intravenous injections in rabbit (Meth Fin Exp Clin Pharmacol 14:655-9, 1992): (vol. 1.25, p.086)

Method:

This study is to compare the pharmacokinetics of a formulated emulsion to epirubicin solution after intrahepatic (i.h.) and intravenous (i.v.) administration in rabbits. Ten NZW rabbit (5/sex/group) was given either i.h. injection of epirubicin emulsion or i.h. and i.v. epirubicin aqueous solution. The drug dose used was 1 mg/kg. Blood samples were taken at 0, 2.5, 5, 10, 15, 30, 60, 120 up to 360 minutes post-dose. Plasma concentrations of epirubicin were measured by HPLC with fluorimetric method.

Results:

Table 1 summarizes the i.v. pharmacokinetic parameters of epirubicin by the two formulations. The $AUC_{(0-\infty)}$ is higher while clearance is slower, the apparent volume of distribution smaller and elimination half-life is longer after an i.v. solution.

TABLE 1. Mean (SEM) kinetic parameters of epirubicin after intravenous (i.v.) injection of emulsion and plain solution

	i.v. Emulsion (n = 10)	i.v. Solution (n = 10)	P<
$AUC_{(0-\infty)}^0$ (ng·ml ⁻¹ ·min)	8054.7 (2849.3)	10846.1 (2763.3)	0.20
Cl (ml·min ⁻¹)	1561.9 (450.2)	812.8 (217.6)	0.10
V_d (ml·kg ⁻¹)	48783.2 (11378.8)	30908 (8396.5)	0.20
MRT (min)	50.1 (9.6)	75.8 (39.7)	0.50
$T_{1/2}$ (min)	37.7 (6.4)	63.4 (29.2)	0.20

Table 2 summarizes the i.h. pharmacokinetic parameters. Intrahepatic injection of emulsion has lower AUC, but clearance is faster with similar volume of distribution. Intrahepatic emulsion has shorter half-life. Bioavailability after i.h. injection is less than 50%, indicating a high first-pass clearance by the liver.

TABLE 2. Mean (SEM) kinetic parameters of epirubicin after i.h. emulsion, i.h. solution and i.v. solution

	i.h. emulsion (n = 10)	i.h. solution (n = 10)	i.v. solution (n = 10)	P_a	P_b	P_c
$AUC_{(0-\infty)}^0$ (ng·ml ⁻¹ ·min)	2185 (574.6)	3931.2 (680.7)	10846.1 (2763.2)	<0.05*	>0.20	<0.005*
Cl (ml·min ⁻¹)	3778 (1096.9)	2701.8 (850.3)	812.8 (217.6)	>0.20	<0.02*	<0.01*
V_d (ml·kg ⁻¹)	136673.8 (28342.9)	128653.7 (31603.7)	30908 (8396.5)	<0.05*	<0.05*	<0.002*
MRT (min)	66.6 (19.6)	83.1 (29.7)	75.8 (39.7)	>0.50	>0.50	>0.50
$T_{1/2}$ (min)	52.1 (15.9)	68.4 (20.9)	63.4 (29.2)	>0.50	>0.50	>0.50
Bioavailability	0.2	0.36	1.0			

P_a , P_b and P_c are P (t test) between i.h. emulsion and i.h. solution, i.h. solution and i.v. solution and i.h. emulsion and i.v. solution, respectively. *Denotes significance.

It is important to note that clearance after intrahepatic injection whether emulsion or solution, is much increased compared to intravenous injection.

G Pharmacokinetics in the dog (Tech Report IMI-28/813i): (vol. 1.25, p. 092)

Method:

This study was to determine the toxicokinetics of ADR-529 (dexrazoxane) and high doses of epirubicin given concomitantly in a 4-week i.v. tolerance study in dogs. Epirubicin was given at 0.25, 0.5, or 1 mg/kg and ADR-529 at a 20:1 dose ratio (ADR-529 at 5, 10, or 20 mg/kg) approximately 25 min prior to epirubicin once weekly for 4 weeks. Plasma samples were taken at predose, 2, 4, 6, and 8 hr for ADR-529 and at predose, 8, 24, 32, and 48 hrs for epirubicin after the fourth treatment. ADR-529 and epirubicin were determined using with

Results:

Following ADR-529 administration, the AUC_{2-8} for 10 mg/kg and 20 mg/kg dose groups were $2,711 \pm 279$ and $5,573 \pm 1,175$ ng.hr/mL, respectively, and showed a 2-fold increase with doubling of the dose (Figure 1). This indicated that systemic drug exposure to ADR-529 increases linearly with the dose over this narrow dose range.

FIGURE 1: Mean Plasma Concentrations of ADR-529 in Beagle Dogs (n=4) Following Administration of 10 or 20 mg/kg ADR-529 (with 0.5 or 1.0 mg/kg Epirubicin, respectively)

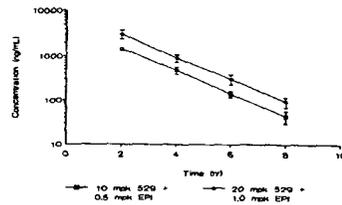


FIGURE 2: Mean Plasma Concentrations of Epirubicin in Beagle Dogs (n=4) Following Administration of 1.0 mg/kg Epirubicin

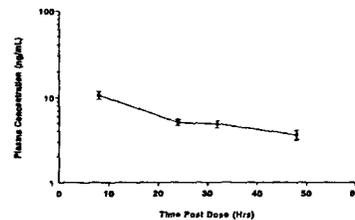


Figure 2 shows the mean plasma concentrations vs. time profile for epirubicin. Data post 20 hr was assumed to be in the post-distribution phase. Elimination and systemic exposure estimates for epirubicin are summarized in Table 5. The AUC_{8-48} ranged from 214.4 to 245.8 (mean= 233.2) ng.hr/mL and showed a less than 10% variability. The estimated of $t_{1/2 \lambda}$ ranged from 35 to 77 hr, and were similar to those previously reported for epirubicin.

Table 5
Toxicokinetic Disposition Estimates for EPI (Group 4)
Following Administration of a 1.0 mg/kg I.V. Epirubicin

Dog Number	C_p^* (ng/mL)	AUC_{8-48} (ng hr/mL)	λ_z (hr ⁻¹)	$t_{1/2 \lambda}$ (hr)
18090				
18089				
18102				
18099				
Mean	10.66	233.2	0.0147	51.1
S.D.	1.10	15.0	0.0044	18.0
%RSD	10.3	6.4	29.8	35.3

* Plasma Concentration at 8 hours post dose.

Mean terminal half-lives for ADR-529 were 1.8 and 1.2 hr for the two epirubicin dose groups, respectively.

H Pharmacokinetics of anthracyclines in dogs: evidence for structure-related body distribution and reduction to hydroxy metabolites (Pharmaceutical Res 1:33-8, 1984): (vol. 1.25, p.128)

Method:

The study was conducted to compare the pharmacokinetic parameters of the five anthracyclines [adriamycin (ADM, doxorubicin), 4'-epi-adriamycin (4'-epi-ADM, epirubicin), daunorubicin (DAR), carminomycin (CAM), and 4-demethoxy-daunorubicin (4-dem-DAR)], and the formation of their reduced C13 hydroxy metabolites in dogs. Two beagle dogs each were given randomly as a single i.v. bolus injection with an interval of 4 weeks of ADM (1.27 mg/kg), 4'-epi-ADM (1.27 mg/kg), DAR (1.2 mg/kg), CAM (1.0 mg/kg), or 4-dem-DAR (1.0 mg/kg). An additional dog was given an i.v. bolus injection of doxorubicin and doxorubicinol at 1.27 mg/kg. The pharmacokinetic parameters and 13-dihydro metabolites were determined in plasma and urine using HPLC with fluorescence detection.

Results:

The pharmacokinetic parameters of various anthracyclines are shown in Table 1. After administration of doxorubicinol, the terminal half-life of this compound was much shorter than that observed after doxorubicin administration, suggesting formation rate limitation for the disposition of the 13-dihydro derivative of doxorubicin.

Table 1. Pharmacokinetic Parameters of the Anthracyclines in Dog after i.v. Bolus Injection^{a,b}

Drug	dog	A	B	C	$t_{1/2\alpha}$	$t_{1/2\beta}$	$t_{1/2\gamma}$	V_d	V_r	Cl_p	Cl_m	Cl_m	AUC _p	AUC _m	AUC _m /AUC _p
		ng/ml	ng/ml	ng/ml	min	h	h	l	l	ml/min	ml/min	ml/min	µg·h/l	µg·h/l	
		mean ±SD													
ADM	604	531	10.9	6.4	5.4	1.12	21.5	19.1	798	607	55 ± 20	15 ± 3	288	94	0.33
ADM	617	789	28.8	11.2	5.9	0.82	30.7	10.4	641	426	47 ± 20	19 ± 6	333	107	0.32
4'-epi-ADM	604	125	9.2	4.0	7.1	2.11	40.0	71.9	1698	589	59 ± 31	12 ± 8	280	151	0.47
4'-epi-ADM	617	100	15.3	3.7	8.0	1.80	35.7	84.1	1584	662	50 ± 25	26 ± 10	252	154	0.61
DAR	604	198	6.0	1.5	5.3	1.13	24.2	51.0	2537	2031	134 ± 17	59 ± 11	86	476	5.53
DAR	617	74	3.8	1.7	5.8	1.29	19.3	107.2	2679	2163	62 ± 30	67 ± 35	66	280	4.27
CAM	604	92	16.7	3.9	20.3	1.13	12.1	98.5	782	1358	12 ± 2	7 ± 1	129	698	5.42
CAM	617	47	20.5	2.3	20.5	1.33	15.8	122.4	829	1233	9 ± 5	9 ± 5	115	898	7.81
4-dem-DAR	604	330	14.9	4.7	6.0	2.10	18.9	34.5	540	711	6 ± 3	6 ± 3	305	1893	6.22
4-dem-DAR	617	249	45.1	5.7	7.1	1.65	20.4	33.4	517	601	4 ± 2	8 ± 3	319	1286	5.59

^aThe parameters were corrected for the dosage used, which was set to 1 mg/kg.

^bFor explanation of the symbols used, see text.

The structural variation at the C₁₄ position (-H versus -OH) results in large difference in the plasma concentration of the hydroxy metabolite. When the ratios of the AUC_m of the metabolite and the AUC_p of the parent compound (AUC_m/AUC_p) for ADM and DAR are compared, a rise from 0.33 for ADM to 5.53 for DAR was observed. For CAM and 4-dem-DAR, which have different substituents in R₂ (-OCH₃, -OH, -H) in addition to the proton in R₁ (-OH, -H) at the C₄ position, the AUC_m/AUC_p ratios are approximately the same, ie, 5.53, 5.42 and 6.22, respectively. The presence of a C₁₄ hydroxy group (adriamycin and 4'-epi-adriamycin) drastically reduces appearance of the C₁₃-dihydro metabolites in plasma. Substitution of the C₄H with C₄-OH and C₄-OCH₃ in this rank order, decreases the AUC of the parent compound and their C₁₃-dihydro metabolites.

I. Metabolism of epidoxorubicin (epirubicin) in animals: Absence of glucuronidation
(Cancer Chemother Pharmacol 20:85-7, 1987): (vol. 1.25, p. 136)

Method:

Metabolism of epirubicin was studied in plasma of five different animal species and man at 2 hr after intravenous (or intraperitoneal) administration of 4 mg/kg. Plasma samples were analyzed by HPLC method.

Results:

Concentrations of epirubicin and its metabolites in plasma 2 hr after administration of epirubicin are summarized in Table 1. None of the animals showed significant glucuronidation of epirubicin, only glucuronides (low concentrations) could be detected in the rabbit. No animal model is suitable to study the effect of glucuronidation on the cardiotoxicity in humans.

Table 1. Concentrations of epidoxorubicin (E) and its metabolites in plasma 2 h after administration of 4 mg E/kg ($10^{-7}M$)

Animal	Route of administration	Eol-Glu	E-Glu	Aolon	Eol	7d-Aolon	7d-Aon	E
Mouse	i.p.	0	0	0	0.2	20	0	49
Rat	i.v.	0	0	0.1	0.4	4.2	0	56
Hamster	i.p.	0	0	0.1	3.3	21	1.3	83
Guinea pig	i.p.	0	0	0	0.2	1.7	0	39
Rabbit	i.v.	0.5	1.7	0.3	23	4.2	0	87
Man*	i.v.	90	460	1	15	25	14	46

* After a dose of 1.5 mg E/kg

Large differences in formation of epirubicinol and 7-deoxy-doxorubicinol aglycone were observed between the species. These phenomena may be relevant for interpretation of the interspecies differences with regard to anthracycline-induced toxicities.

J. Plasma protein binding of ^{14}C -epirubicin (IMI 28) in man, mice, rats, rabbits and dogs
(Tech Report #IMI-28/812i): (vol. 1.25, p.138)

Method:

The binding of epirubicin to plasma proteins of mice, rats, rabbits, dogs and man was measured *in vitro* by equilibrium dialysis using the ^{14}C -epirubicin (IMI 28). ^{14}C -epirubicin (103 uCi/mg) and non-radioactive IMI 28 were added in order to obtain plasma concentrations of 20, 100, 500 and 1000 ng/mL of plasma. The extent of ^{14}C -IMI 28 binding to plasma proteins was measured *in vitro* by equilibrium dialysis, using

Results:

At initial concentrations of 20, 100, 500 and 1000 ng/mL, the binding of ^{14}C -epirubicin to plasma proteins was 76-78% for man, 79-80% for the mouse, 79-83% for the rat, 77-78% for the rabbit and 80-81% for the dog. The percent of epirubicin bound to plasma proteins was practically the same in all animal species and in man.

Table 2 - Binding of ^{14}C -IMF28 to plasma proteins of man, mouse, rat, rabbit and dog.
Results are given as mean percentages \pm S.D. n = 3.

Initial Concentration ng/ml	Man	Mouse	Rat	Rabbit	Dog
20	78.09 \pm 0.09	79.89 \pm 0.08	82.76 \pm 0.30	78.46 \pm 0.22	80.32 \pm 0.45
100	77.35 \pm 0.08	79.98 \pm 0.49	81.27 \pm 0.52	78.17 \pm 1.02	81.22 \pm 0.77
500	76.78 \pm 1.31	80.35 \pm 0.63	78.67 \pm 0.65	78.40 \pm 0.27	80.68 \pm 0.44
1000	76.52 \pm 0.12	79.06 \pm 0.43	80.49 \pm 0.26	77.06 \pm 0.89	80.90 \pm 0.65

Summary of Pharmacokinetics:

Pharmacokinetics and metabolism of 4'-epidoxorubicin, a stereoisomer of doxorubicin, was compared with doxorubicin after a single i.v. injection of 10 mg/kg epirubicin or doxorubicin to female BALB/c mice bearing colon 26 tumors. The parent drugs were largely present in plasma, heart, and tumors during the 48 hr study. AUCs of doxorubicin and its metabolites were higher than the corresponding values for epirubicin and its metabolites in all three compartments. Half-lives of elimination of epirubicin were smaller than those of doxorubicin in all compartments.

The influence of partial hepatectomy on the pharmacokinetics of 4'-epidoxorubicin was studied following a single i.v. dose of 5 mg/kg given to hepatic resection in rats. The partially hepatectomized rats showed a 2-fold increase in AUC between 4 and 72 hr as compared with the sham-operated controls. The terminal half-life from 24 to 72 hr was not significantly changed by the partial hepatectomy.

Enterohepatic circulation and plasma protein binding were studied after i.v. injection of 1 mg/kg ^{14}C -epirubicin to bile-duct cannulated rats. The major excretion route of ^{14}C -epirubicin was in bile and the pattern of excretion was similar to that of doxorubicin in rats. In vivo the protein binding of epirubicin to plasma protein showed an increase with time, while the in vitro measurements were constant.

A comparative study was conducted to assess pharmacological basis for differences in toxicity between adriamycin and 4'-epiadriamycin. After bolus or 24 hr infusion doses of 10 mg/kg epirubicin were given to rats, no differences were seen in plasma drug levels but differences were noted in rate and extent of drug elimination. The parent drugs were eliminated largely in the bile, recovery of 50% (bolus) and 40% (infusion) of epi-adriamycin vs 31% (bolus) and 23% (infusion) of adriamycin. The only significant metabolite seen and the recovered dose was the 13-carbinol derivatives, adriamycinol or 4'-epiadriamycinol. The reported lower toxicity of epi-adriamycin compared to adriamycin for the heart may result from a more extensive elimination of parent drug in bile and urine and diminished metabolic conversion to its 13-carbinol metabolites.

Pharmacokinetics were determined following i.v. doses of 1 and 3 mg/kg epirubicin in rabbits. After rapid i.v. dose of epirubicin, epirubicin declined triexponentially with the terminal half-lives of 21-23 hrs. Epirubicin was rapidly metabolized to epirubicinol and unidentified metabolites due to glucuronic acid conjugation. The rabbit is the only animal species to produce glucuroconjugates of epirubicin.

Pharmacokinetics of a formulated emulsion and epirubicin solution were compared after intrahepatic and intravenous administration in rabbits. Intrahepatic injection of emulsion has lower AUC, but clearance is faster with high volume of distribution with a shorter half-life. Clearance after i.h. injection of emulsion or solution was greatly increased compared to intravenous injection.

A toxicokinetic study was undertaken in a 4 week i.v. tolerance study in dogs to determine the toxicological effects of ADR-529 (5, 10 or 20 mg/kg) and high doses of epirubicin (0.25, 0.5 or 1 mg/kg) given concomitantly in dogs. Systemic ADR-529 exposure increased

linearly with the two highest doses. The plasma disposition of ADR-529 is not modified by epirubicin at the doses tested in dogs.

Pharmacokinetic parameters of the five anthracyclines and the formation of reduced C₁₃ hydroxy metabolites were studied in dogs following 4 weekly single i.v. doses of each compound (1.27 mg/kg adriamycin and epirubicin, 1.2 mg/kg DAR, 1.0 mg/kg CAM and 4-dem-DAR). The structural variation at the C₁₄ positions (-H vs -OH) results in large difference in the plasma concentration of the hydroxy metabolite. The presence of a C₁₄ hydroxy group (adriamycin and 4'-epi-adriamycin) reduces appearance of the C₁₃-dihydrometabolites in plasma. Substitution of the C₄H with C₄-OH and C₄-OCH₃ decreases the AUC of the parent compound and their C₁₃-dihydrometabolites.

Metabolism of epirubicin was studied five different animal species and man after i.v. or i.p. dose of 4 mg/kg. Large differences in formation of epirubicinol (adriamycinol) and 7-deoxydoxorubicinol aglycone were observed in the plasma between the species. None of the animals showed significant glucuronidation of epirubicin except small amounts of glucuronides could be detected in the rabbit and in humans.

The binding of epirubicin to plasma proteins of mice, rats, rabbits, dogs and man was measured in vitro. At initial concentrations of 20, 100, 500 and 1000 mg/mL, the percent of epirubicin bound to plasma was practically the same in all species and in man.

III. TOXICOLOGY:

A. **Acute iv toxicity study of PFS vs the lyophilized commercial formulation in mice** (Technical Report #IMI 28/450i): (vol. 1.5)

An acute toxicity study was conducted to compare the i.v. toxicity of the PFS (Preservative Free Solution or RTU= ready to use) formulation with that of the lyophilized commercial formulation (LCF) in mice.

Animals: CrI:CD-1(ICR) BR male mice, 10/group; 20-26 g, 4 weeks of age
 Compounds: Epirubicin (EPI) RTU 2 mg/ml (Batch #6001LB) or Epirubicin LYO 50 mg (Batch # 5005) at 18.00, 20.70, 23.81, 27.38, or 31.48 mg/kg in 0.9% NaCl. Controls received the vehicle (saline)
 Treatment: Single iv doses of EPI RTU or EPI LCF were given at a volume of 20 ml/kg
 Observation: Animals were observed for daily for 28 days for death and clinical signs. Gross necropsy were done during and at the end of the test period
 GLP: Yes

Results:

A dose-related and majority of the mortality occurred between the 5th and the 12th day of observation with EPI RTU and with EPI LCF between 6th and the 11th day. Clinical signs included ruffled fur, lost of fur, hunched posture, wasting, sedation, hind-leg weakness, hypothermia, and weight loss. Some animal doses at ≥ 23.81 mg/kg exhibited diarrhea and abdominal swelling. Dose-related decreases in mean body weight gains were observed, especially at 27.38 mg/kg. At this dose level on day 4, body weight loss was 14% EPI RTU vs 13% EPI LCF and on day 8 the body weight loss was 29% EPI RTU vs 28% EPI LCF.

At necropsy, mice that died after treatment showed poor or cachectic conditions and gross alterations including reduction in size of the thymus and spleen (with pale color). In animals treated with mid-high doses, hyperemic changes (glandular region of stomach, intestine), and hydroperitoneum and hydrothorax (with EPI RTU) were observed in the lungs.

Dose-related marked reductions in size of thymus and testes, and kidney alterations were observed. A decrease in size of the spleen was seen in 2 animals treated with 23.81 mg/kg EPI RTU, whereas hydroperitoneum and hydrothorax was observed in one mouse treated with the same dose of EPI LCF. The LD₅₀ values were 22.78 and 23.70 for EPI RTU and EPI LCF, respectively. The LD₁₀ values were calculated to be 19.77 and 21.28 mg/kg, respectively.

Two epirubicin formulations induced similar dose-related acute toxic effects.

B. Acute iv toxicity study of PFS vs its artificially degraded formulation in mice
(Technical Report #IMI 28/448i): (vol. 1.5)

This study was conducted to compare acute i.v. toxicity of EPI RTU (ready to use) vs the same formulation artificially degraded in male mice (performed by Farmitalia Carlo Erba Research & Development.)

Animals: Crl:CD-1(ICR) male mice, 10/group; 20-26 g, 31 days old
Compounds: Epirubicin RTU 2 mg/ml (Batch #1585/AF) or Epirubicin ADF (artificially degraded formulation, Batch #6001-LC/A) at 0, 18.0, 20.5, 23.4, 26.7, 30.4 and 34.7 mg/kg in distilled water. Control received the vehicle (water).
Treatment: Single iv doses of EPI RTU or EPI ADF were given at a volume of 25 ml/kg necropsy was done during and at the end of the test period
GLP: Yes

Results:

Dose-related mortality occurred between the 6th and the 12th day of observation with EPI RTU starting at doses of 23.4 mg/kg and 6th and the 11th day with EPI ADF starting at doses of 18 mg/kg. Clinical signs included ruffled fur, clonic convulsions (a few seconds), marked dyspnea (1-2 min), hypoactivity or sedation, hypothermia and weight loss (dose-related). Dose-related, moderate to severe body weight changes (13-37% EPI RTU vs 15-44% EPI ADF) starting from days 3-8 and partial recovery (32% EPI RTU vs 42% EPI ADF) starting day 12 of observation were observed.

Necropsy findings from mice that died showed generally poor condition, marked reduction in thymus and spleen volume, and hyperemic changes (stomach, duodenum, jejunum). One mouse had congestion of the lung. Necropsy findings from mice killed at end of the study revealed similar gross alterations and one mouse each at 18 and 23.4 mg/kg had slight reduction in testis size. One mouse at 23.4 mg/kg had paler than normal liver.

RTU ADF induced qualitatively similar gross findings to that observed with EPI RTU. The LD₅₀ and LD₁₀ values (calculated on the 15th day of observation) were 23.80 and 21.03 mg/kg for EPI RTU and 21.84 and 17.91 mg/kg for EPI ADF, respectively.

C. Acute iv toxicity study of PFS vs the lyophilized commercial formulation in rats(Tech Report #IMI 28/451i): (vol. 1.6)

An acute toxicity study was performed to compare the i.v. toxicity of the RTU formulation with that of the lyophilized commercial formulation (LYO) in rats.

Animals: Crl:CD(SD) BR male rats, 10/group; 153-185 g, 6 weeks of age
Compounds: Epirubicin (EPI) RTU 2 mg/ml (batch #6001LB) or Epirubicin LYO 50 mg (batch #5005) at 0, 10.00, 12.00, 14.40, 17.20 or 20.74 mg/kg in 0.9% NaCl. Controls received the saline.
Treatment: Single i.v. doses of EPI RTU or EPI LYO were given at a volume of 12 ml/kg into caudal vein

Observation: Animals were observed for daily for 28 days for mortality and clinical signs.
Gross necropsy was done during and at the end of the test period

GLP: Yes

Results:

A dose-related mortality occurred starting from the dose of 12.00 mg/kg on days 6-13 with EPI RTU and days 9-14 with EPI LYO. Clinical signs included poor general condition, wasting, hypoactivity, muscle relaxation, dirty, ruffled thinned fur, diarrhea, dyspnea, and abdominal swelling. Body weight losses were moderate to severe (19% and 36% Epi RTU vs 17% and 33% EPI LYO on days 4 & 8, respectively).

Necropsy findings from rats that died showed severe reduction in size of thymus and spleen, prostate, seminal vesicles and testes. Dose-related alterations in glandular region of stomach (hyperemia, hemorrhage and erosion), intestine (hyperemia, enteritis-like signs), paler kidney, liver, pancreas, and adrenals, lungs (congestion, hemorrhage), and reddish areas were seen in apex of the heart. In animals sacrificed at the end of the study exhibited more or less similar findings without any dose-dependence for severity. Reduction in size of spleen, and kidney lesions (paler, juxta-medullary hyperemia, hydronephrosis and enlargement) were observed. Transparent/reddish fluid was found in the pericardium and in the abdominal cavity.

EPI RTU induced qualitatively similar gross findings that observed with EPI LYO. The LD₅₀ and LD₁₀ values (calculated) were 13.85 and 12.16 mg/kg for EPI RTU and 15.15 and 11.91 mg/kg for EPI LYO, respectively.

Summary of Toxicology:

Acute intravenous toxicity studies were conducted 1) to compare the epirubicin PFS (RTU) formulation with that of commercially available epirubicin lyophilized formulation in mice, 2) to compare the epirubicin RTU with that of epirubicin artificially degraded formulation in mice, and 3) to compare the epirubicin RTU with that of lyophilized commercial formulation in rats.

Dose dependent acute toxicities were observed including mortality, clinical signs and gross alterations. Acute toxic effects induced by EPI PFS (RTU) were qualitatively or quantitatively similar to those with EPI LCF, EPI ADF or EPI LYO.

IV. Special Toxicity Studies:

- A. Hematotoxicity of doxorubicin, epirubicin and idarubicin in male rats after single i.v. injection (Tech Report IMI 28/465i): (vol. 1.7, p. 040)

Method:

CrI-CD (SD) BR male rats (3-4/group, 214-318 g) received single i.v. doses of 3.00 and 6.00 mg/kg doxorubicin, 1.0, 3.00 and 6.00 mg/kg epirubicin, and 0.75, 1.50 and 3.00 mg/kg idarubicin in 0.9% saline via caudal vein. Blood samples were taken on days 8, 15, 29 and 57. Hematology parameters (RBC, Hb, Hct, WBC and platelets) were determined.

Results:

All three anthracyclines caused slight to marked dose-related decreases in leukocytes with the nadir on day 8 (on day 15 only for doxorubicin 3.00 mg/kg) which was followed by rapid recovery. Decreases were ~40% and 60% for doxorubicin 3.00 and 6.00 mg/kg; ~10%, 30% and 60% for epirubicin 1.50, 3.00 and 6.00 mg/kg; and ~35%, 70% and about 80% for idarubicin 0.75, 1.50 and 3.00 mg/kg, respectively. These changes were reversible by day 15. Platelet decreases were ~40% and 65% with doxorubicin 3.00 and 6.00 mg/kg; ~90% with

epirubicin 6.00 mg/kg; and ~50%, 95% and 100% with idarubicin 0.75, 1.50 and 3.00 mg/kg, respectively. Erythrocytes and Hb and Hct were only marginally affected.

All three anthracyclines induced a similar dose-related decrease in WBC with the nadir on day 8; epirubicin and doxorubicin at equal doses (3 and 6 mg/kg) were equitoxic on leukocytes. RBC and related parameters were only marginally affected, whereas slight to marked decreases in platelets were seen at both doses of doxorubicin and at the highest dose of epirubicin.

B. Bone marrow toxicity study after single intravenous administration of epirubicin hydrochloride in the male dog (Tech Report IMI 28/442i): (vol. 1.8)

Method:

Male beagle dogs (4/group, 6-9 months old) were given single i.v. doses of 0, 1 and 2 mg/kg IMI 28 (epirubicin) and 1 mg/kg of doxorubicin (Dox). Effect of the two anthracyclines on bone marrow toxicity was compared.

Results:

Epirubicin was lethal at 2 mg/kg (3/4 dogs died). In hematology, decreases in RBC, Hb, Hct, WBC and platelet were observed in animals treated with 1 mg/kg IMI 28 and 1 mg/kg Dox-treated group. The bone marrow tests showed depressed differentiation of myelocytes in all treated groups with epirubicin and doxorubicin but no abnormality in number of bone marrow cells were seen in any group.

The development of toxic changes was earlier and less severe with doxorubicin but the recovery was more rapid with epirubicin. No remarkable drug-induced changes were observed, however, one doxorubicin-treated dog showed dark-red change in the part of lung and in mucosa of small intestine. These findings suggested that these two anthracyclines are qualitatively comparable at equitoxic dose.

C. Intravenous immunotoxicity study of IMI 28 vs doxorubicin in mice (Tech Report IMI 28/302i): (vol. 1.21, p.117)

Method:

The action of epirubicin (IMI-28) on immune response was compared with adriamycin. Crl:CD-1(ICR)BR female mice (n=10) were used to test the antibody response to sheep red blood cells (SRBC). BDF1 male mice were used for the delayed hypersensitivity reaction. C3H/HeN and C57B1/6 female mice were used as skin graft donor and recipients, respectively. Single iv doses of 6 and 12 mg/kg epirubicin or 4 and 8 mg/kg doxorubicin were given before, concomitantly with or after immunization with SRBC. Daily i.v. doses of 3 mg/kg/day epirubicin or 2 mg/kg/day doxorubicin were given for three consecutive days before the antigen were used. Controls received saline. Humoral immunity was evaluated as the primary immune response to optimal doses of SRBC. The secondary response was assessed only on sera. Cell-mediated immunity was studied by two methods where delayed hypersensitivity to SRBC was induced and measured as the increase in thickness of the footpad in animals receiving 12 mg/kg of epirubicin or 8 mg/kg doxorubicin and rejection time of a skin allograft was evaluated in 50% of the animals. The doses of epirubicin and doxorubicin were 12 and 8 mg/kg, as single doses, and 5.25 and 3.5 mg/kg/day, as repeated doses, respectively.

Results:

Epirubicin exerted an immunosuppressive activity that was greater than that of doxorubicin on the humoral type immune response. The effect on the IgG was more pronounced with IMI-28 than Dox, irrespective of the treatment schedule used. Epirubicin (IMI-28), but not

doxorubicin, had a slight immunosuppressive effect on delayed hypersensitivity reactions. Similarly to doxorubicin, epirubicin had no effect on cutaneous transplant rejection, even after repeated doses.

D. Intravenous antigenicity study vs doxorubicin in guinea pigs (Tech Report IMI-28/212i):
(vol. 1.21, p. 136)

Method:

Female guinea pigs (Hartley strain, 10/group, 4 weeks old) were immunized with epirubicin doses of 0.1 and 1.0 mg/kg s.c. or 0.1 mg/kg i.p. Similar groups of animals were immunized s.c. with an emulsion containing Freund's complete adjuvant (FCA) plus 1.0 mg/kg epirubicin or 1.0 mg/kg doxorubicin, as positive control. The corresponding antigens, epirubicin and doxorubicin, were injected i.v. in each animal to induce passive systemic anaphylaxis.

Epirubicin was subsequently injected i.d. (intradermally) at the doses of 0.01, 0.1, or 1 ug/site in guinea pigs previously immunized by s.c. or i.p. injections of 0.1 mg/kg epirubicin to detect skin hypersensitivity reaction.

Results:

No passive systemic anaphylaxis or skin hypersensitivity reactions were induced by epirubicin or doxorubicin in guinea pigs. A delayed skin hypersensitivity reaction was observed when epirubicin was injected i.d. (intradermal) at doses of 0.1 and 1.0 mg/kg to guinea pigs previously immunized with an emulsion containing FCA plus epirubicin.

Guinea pigs previously immunized with an emulsion containing FCA plus 1.0 mg/kg doxorubicin were treated i.d. with doxorubicin and showed the delayed type skin hypersensitivity but not the immediate type of reaction.

No allergic reaction was observed with epirubicin only, so it was concluded that severe allergic reactions would unlikely develop during the clinical use of epirubicin.

E. Intravenous cardiotoxicity study vs doxorubicin after a single-dose regimen in rats (Tech Report IMI-28/431i):
(vol. 1.21, p.184)

Method:

Male Crl:CD (SD) rats (n=55) received either a single dose of 6 and 9 mg/kg epirubicin or 3 and 6 mg/kg doxorubicin. Eight animals/dose were killed after 2, 4, 8, 12, 22, 27 and 35 weeks post-dosing to determine the onset of cardiac and renal lesions. Mortality, clinical signs, and general behaviors were observed, and gross/histopathological lesions especially the myocardium and kidneys were examined.

Results:

Tab. 1 MORTALITY AND BODY WEIGHT (g)

Group	Compound	Dose (mg/kg)	Mortality/ 56 rats	Body weight							
				Pretest	Observation week						
				2nd	4th	8th	12th	22nd	27th	35th	
1	Saline	/	0	177	309	379	470	532	602	647	672
2	IMI 28	6	0	176	291	352	429	483	553	588	603
3	IMI 28	9	2	174	368	323	387	387	454	478	506
4	DE.HCL	3	1	175	291	357	436	495	563	613	631
5	DE.HCL	6	4*	176	266	313	366	386	442	484	537

DE.HCL = doxorubicin HCL

* - one rat incidentally killed by handling

Mortality and body weight changes are shown in Table 1. Two animals treated with 9 mg/kg epirubicin and 1 and 3 animals treated with 3 and 6 mg/kg doxorubicin died towards the 19th week. One rat treated with 6 mg/kg doxorubicin was incidentally killed by handling. Dose-related body weight gain decreased during the first 12 weeks in those given the higher dose (25% with 9 mg/kg IMI-28; 26% with 6 mg/kg dox). No recovery (25% with IMI-28; 22% with dox) was seen during the 35th week in the high dose groups.

At necropsy, abnormal incisors, diffuse cysts in the medulla and cortex of the kidneys were observed. Two drugs induced a qualitatively similar histological lesions in myocardium and kidneys. For cardiac lesions, micro- and macro-vacuolization of the cardiocytes, cell and/or interstitial edema, atrophy, necrosis and fibrosis in the left ventricle and interventricular septum, and more serious damages affected vast zones of the organs were observed. Kidney lesions consist of renal tubules (dilatation of the lumen, hypotrophy, hyaline degeneration, epithelial vacuolization and desquamation), glomeruli (vacuolization, hypertrophy and proliferation of the epithelial cells, fibrosis), and interstitium (infiltrates and fibrosis).

Histological examination showed that renal damage occurred before cardiac damage. The renal lesions were observed from the second week even when no myocardial alterations were present.

F. Intravenous cardiotoxicity study vs doxorubicin after a multiple-dose regimen in rats
(Tech Report 9550019) (vol. 1.22, p.001)

Method:

This study was to determine the potential cardiotoxicity of idarubicin in comparison with the two reference articles doxorubicin and epirubicin. Crl:CD (SD) BR male rats (36/group) were given 0.3 and 0.6 mg/kg/week Idarubicin (IMI-30) and 1.0 mg/kg/week for both reference drugs doxorubicin and epirubicin once a week for 7 consecutive weeks. Animals were observed daily (mortality), weekly (body weight, hematology) and terminal necropsy on week 36.

Results:

Mortality occurred from the week 13 in 12/36 rats given 0.6 mg/kg idarubicin, 9/36 and 5/36 rats given the reference articles from the weeks 17 and 19 for doxorubicin and epirubicin, respectively. Clinical signs included ruffled fur, pale mucosa and weight loss. Renal impairment and cardiotoxicity were identified as the probable cause of death in the animals treated with reference drugs. Dose-related decreases in body weight were observed during the treatment (~15%) and week 7 (~35%) with 0.3 mg/kg/week (mkw). A marked decrease in body weight gain starting from week 4 and reaching the maximum of ~75% were noted at 0.6 mkw at the end of treatment period. Animals given the reference articles doxorubicin and epirubicin showed a trend similar to that observed in animals given the highest dose of idarubicin.

In hematology, decreases in leukocytes (35-40%) at 0.3 mg/kg/wk weeks 4 and 8 and the 0.6 mg/kg/wk group decreases were 50 and 55% at weeks 4 and 8, respectively. Moderate decrease 45% in leukocytes was observed at 1 mg/kg/wk with the reference compounds.

Clinical chemistry included the changes that already known with anthracyclines such as late overt impairment of renal function (week 35) with severe hyperazotemia (due to increases in urea and creatinine), hyperphosphatemia and hyperkalemia. Marked increases in serum lipids (triglyceride, total cholesterol, phospholipid) were observed from week 8-22. All treated animals showed a progressive decrease in albumin due to persistent proteinuria. Main urinary change was persistent proteinuria with leukocyturia.

Drug-induced gross alterations included statistically significant increases in the kidney weight (kidney enlargement), marked decreases in size of the testes, small prostate, and pale

liver. Almost all treated with 0.6 mkw idarubicin, 1.0 mkw doxorubicin and epirubicin had kidney changes (enlargement, pitted surface and blanching of the parenchyma), small spleen and thymus, testes, and abnormally colored area in the heart.

Histopathological findings were dose-related and included drug-induced cardiotoxicity. Slight to marked multifocal vacuo-degeneration often associated with fibrosis and atrial thrombosis. The cardiotoxicity of epirubicin, expressed as mean total score (MTS), was compared statistically with that induced by doxorubicin. The statistical comparison between the three anthracyclines and significant differences ($p < 0.05$) in cardiotoxicity are summarized as in the table below.

WEEKS	CONTROLS	IDARUBICIN 0.3 mg/kg	IDARUBICIN 0.6 mg/kg	DOXORUBICIN 1.0 mg/kg	EPIRUBICIN 1.0 mg/kg
4	0	0	0.17	0.83*	0.33
8	0	0	1.83	4.00**	2.67*
12	0	0.33	2.67*	4.67**	3.33**
22	0	0.67	3.00*	5.00**	3.33**
27	0	0.83	-	4.00**	2.17**
35	0	1.33*	-	-	1.00

*: $p < 0.05$
 **: $p < 0.01$

Idarubicin induced a dose-related cardiotoxicity. At 0.3 mkw, minimal to slight cardiac lesions, time-related frequency and severity were observed from week 12 and reaching the maximum MTS (mean total score) of 1.33 at week 35. At 0.6 mkw, slight to moderate cardiac lesions were noted in all rats from week 8, reaching the maximum of 3.0 at the last week 22.

The cardiotoxicity (MTS 1.33) induced by idarubicin at 0.3 mkw was characterized by late onset week 35 with respect to the time of treatment. By contrast, the substantially equi-myelotoxic doses of doxorubicin and epirubicin (1.0 mkw) induced marked or moderate cardiotoxicity (MTS 5.0 and 3.33, respectively) with cardiac lesions already present at week 8. Cardiac lesions after treatment with idarubicin at 0.6 mkw reached the maximum MTS value of 3.0 at week 22.

G. Local tolerability study in rat with it (intradermal) injection of epirubicin RTU (Tech Report IMI-28/447i): (vol. 1.21, p.071)

Method:

After a single injection of Epirubicin RTU in saline or glucose solution and farmorubicin, local intradermal tolerability were compared in rats.

Male SD CrI:CD (SD) BR rats (10/group) were treated with a following scheme as in Table 2 below. A total volume of 0.1 ml was injected intradermally in the shaved area of dorso-scapular region. Animals were observed for behavior and general condition daily and the skin around the injection site was examined on days 1, 3, 5 and 7 days after treatment and then twice weekly for 6 weeks. Main local lesions (ulcers, eschar) and other lesions were recorded.

TABLE 2 - GROUP MEAN LESION AREA MEASUREMENT FOR EACH OBSERVATION PERIOD (mm²)

COMPOUND	DOSE mg/rat	CONC. mg/ml	VOLUME ml/rat	OBSERVATION DAY															
				2	4	6	8	12	15	19	22	26	29	33	36	40	43		
SALINE (0.9% NaCl)	-	-	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.5% GLUCOSE SOLUTION	-	-	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EPIDRUBICIN RTU IN SALINE	0.1	1	0.1	0	0	6.4	10.6	15.1	14.5	8.4	6.3	4.4	4.8	5.1	2.6	0.4	0	0	
EPIDRUBICIN RTU IN SALINE	0.2	2	0.1	0	0	14.5	33.3	36.1	33.2	20.1	12.8	7.6	7.4	7.8	4.1	2.5	1.2	0	
EPIDRUBICIN RTU IN 0.5% GLUC.SOL.	0.1	1	0.1	0	0	4.8	9.2	16.8	9.9	3.7	3.4	1.8	0.5	0.6	0	0	0	0	
EPIDRUBICIN RTU IN 0.5% GLUC.SOL.	0.2	2	0.1	0	0	8.0	13.1	25.0	25.5	15.3	7.5	3.7	1.2	4.1	1.0	0.2	0.1	0	
FARMORUBICIN	0.1	1	0.1	0	0	12.4	10.2	12.8	11.4	6.4	4.0	2.5	1.7	1.6	0.6	0	0	0	
FARMORUBICIN	0.2	2	0.1	0	1.6	19.4	24.2	28.7	27.5	20.9	16.3	11.6	10.2	10.6	5.4	3.4	2.0	0	

No mortality or no drug-induced clinical signs or behavioral changes were observed. No treatment-related lesions were observed in the control animals. Table 2 summarizes the mean lesions (mm²) for each treatment and for each observation period. Treatment with 1 and 2 mg/mL epirubicin RTU both in saline and glucose caused slight to moderate edema on days 4-8, slight to marked hyperemia on days 2-3, and ulcers and eschar on days 4-8. Complete healing occurred between 9-43. Rats treated with Farmorubicin showed slight to moderate edema and slight hyperemia 24 hrs after injection and hyperemia disappeared days 4-6, the edema days 6-15; ulcers and eschar appeared on days 2-15 and complete healing took place between days 19-43.

Epirubicin RTU in saline solution produced more severe lesions (~36.1 mm² on day 12) than 28.7 mm² on day 12 for farmorubicin and 25.5 mm² on day 15 for epirubicin in glucose solution.

Summary of Special Toxicity:

After i.v. administration of single doses of epirubicin and doxorubicin to male rats, the main hematology finding observed was a qualitatively similar dose-related decrease in WBC with the nadir on day 8. The two compounds were equitoxic for leukocytes. Slight to marked decreases in platelets were seen at 6.0 mg/kg for both compounds while RBC and related parameters were marginally affected by both compounds.

In a bone marrow toxicity study, a single i.v. dose of 2 mg/kg epirubicin was lethal to dogs. Animals given an equitoxic dose of 1 mg/kg for both compounds showed qualitatively similar effects of depressed differentiation of myelocytes in bone marrow in dogs.

The immunosuppressive activity of the two anthracyclines was evaluated using different i.v. doses (12 or 6 mg/kg IMI-28; 8 or 4 mg/kg Dox) and schedules (single and repeat doses) on the humoral immune response, delayed hypersensitivity reaction, and transplant rejection. Results indicated that both Dox and IMI-28 completely suppressed the humoral immune response, IgM and IgG. Like doxorubicin, epirubicin had no effect on cutaneous transplant rejection.

In intravenous antigenicity study, guinea pigs immunized with s.c or i.p. doses of epirubicin or doxorubicin and challenged with corresponding antigens i.v. did not experience passive systemic anaphylaxis. However, slight reactions of both immediate and delayed type skin hypersensitivity were seen when 0.1 and 1.0 mg/kg epirubicin or doxorubicin i.d. was given to guinea pigs previously immunized with an emulsion containing FCA plus epirubicin or doxorubicin.

After a single dose of 0, 6, or 9 mg/kg epirubicin or 3 and 6 mg/kg doxorubicin to male rats, cardiotoxic effects of two drugs were evaluated. Both compounds produced qualitatively similar dose-related toxic effects: higher mortality given doxorubicin, decreases in body weight gains, and inflammatory and/or degenerative changes in the kidney and heart.

In cardiotoxicity study after a multiple dose regimens, male rats received epirubicin and doxorubicin at equi-myelotoxic dose of 1 mg/kg i.v. weekly for seven weeks. Both compounds

induced renal impairments and cardiotoxicity with myocardial lesions at week 4. The myocardial damage was moderate for epirubicin (MTS 3.33) and severe for doxorubicin (MTS 5.0).

In local tolerability study, epirubicin RTU in saline solution produced more severe lesions (hyperemia, edema, ulcers, eschars) than farmorubicin and epirubicin in glucose solution.

V. Reproductive Toxicity Studies:

A preliminary test of reproductive study (dose-finding study, Tech Report IMI 28//433i) was performed by treating with epirubicin i.v. doses at 0.03, 0.1, 0.3 and 1.0 mg/kg to 6 weeks old males (n=6) for 9 weeks before mating, and 0.03, 0.1, 0.3, 1.0 and 2.0 mg/kg to 13 weeks old females (n=6) for 2 weeks before mating and to day 7 of gestation to mated females.

Animals were observed daily for clinical signs and mortality; body weight and food intake, estrous cycle, live/dead fetuses, mating and fertility.

Results:

No mortality but male at 1.0 mg/kg/day exhibited piloerection, ptosis, anemia, falling body temperature, soft stool after day 12-14 after the start of dosing. These symptoms increased in number and were observed in all animals after day 26. One male each was sacrificed on days 29, 31 & 32, 2 males on day 34 and one each on days 35 and 36 from deteriorated general conditions. Females treated with 2.0 mg/kg/day exhibited similar toxic signs plus salivation 8 days after the start of dosing. All 6 females died from deterioration of general conditions during the treatment period before mating. No changes in estrous cycle were noted in any group. Decreases in body weight gain and food intake were observed at doses ≥ 0.03 mkd for males and at doses ≥ 0.3 mkd for the both sexes.

Necropsy findings included decreased organ weights (testes, epididymides), atrophy (lymph nodes, testes, epididymides, prostate, seminal vesicles in males only; thymus, spleen, liver and pancreas for both sexes), and fluid in thoracic/abdominal cavity 11/12 males at 1.0 mg/kg, and 5/6 females at 2.0 mkd had hemorrhage of stomach.

No females were available for evaluation at 2.0 mkd dose. No pregnancy or 2/6 female had only trace of implantation and pregnancy was not evaluable at 1.0 mkd. No pregnancy in 6/6 was observed at 0.3 mkd at C-section.

In litter data, decreased body weight of F₁ fetuses at 0.03 and 0.10 mkd was observed. Only one of 93 fetuses had a malformation (omphalocele) at 0.03 mkd dose. At 0.10 mkd dose, increased embryoletality (17%) was seen.

A. Segment I fertility study in rats (Tech Report IMI 28/433i & IMI28/435i):

(vol. 1.15, p.153)

Species:	CrJ:CD (SD) — rats (24/sex/group, 6 weeks old males and 13 weeks old females)
Dose levels:	0, 0.01, 0.03, and 0.10 mg/kg/day, i.v. epirubicin (Lot 10050100) for males; 0, 0.01, 0.03, 0.10 and 0.30 mg/kg/day for females
Treatment:	Males were dosed for 9 weeks before mating and female rats for 2 weeks before mating and up to day 7 of pregnancy. A male treated for 9 weeks and a female treated for 2 weeks were mated. Females treated with 0.30 mkd were mated with untreated males. If the copulation did not occur within 2 weeks, the males were mated with untreated females and the females were mated with males with successful copulation in the same group
Observation:	General conditions (mortality, body weight), estrous cycle, number of corpora lutea, implantation, live/dead fetuses, and organ weights.

Results:

No mortality was observed but males given 0.03 and 0.10 mg/kg/day epirubicin showed dose-related decreases in body weight gain and food consumption. At the end of mating, autopsy showed slight atrophy (thymus, testes, epididymides), slightly decreased spermatogenesis (16/24) and decreased testes weights at the highest dose only.

Similar decreased body weight gain was observed in females given ≥ 0.01 mg/kg, but body weight was significantly depressed with 0.30 mg/kg during the gestation period. The cesarean section showed significant increase in embryo/fetal deaths in the groups given 0.10 mg/kg and at 0.30 mg/kg (20% and 76%, respectively). Significant decreases in body weight of live fetuses were observed in the group treated with 0.03 mg/kg. Only significant increased placenta weight was noted with 0.3 mg/kg. There were no remarkable change in general condition or significant differences in sex ratio of live fetuses and no external malformation.

There was no effect on either copulation or fertility, although slight atrophy of testis and hypospermatogenesis were observed in most of them with 0.10 mg/kg.

B. Segment II teratogenic study in rats and rabbits (Abbreviated Report 9850197):
(vol. 1.16, p.001)

Species: Crl:CD (SD)BR ————— pregnant female rats, n=5
 Dose levels: 0, 2, or 4 mg/kg/day, i.v. epirubicin PFS (Lot 8001LB)
 Treatment: pregnant females were dosed on days 9 and 10 of pregnancy
 Observation: Clinical signs daily; body weight/food consumption were recorded; dams were sacrificed on day 20, uterine contents were examined.

Results:**Maternal data:**

Only one rat at 4 mg/kg died on day 19. At this dose vaginal bleeding was observed in 3/5 rats (60%) on days 17 & 18. Dose-related effects on body weight and food consumption were seen at both doses. Body weights decreased after the first dose at 2 mg/kg. Mean body weight subsequently tended to recover until day 14 of pregnancy, but it was markedly depressed toward the end of pregnancy (about 25 grams and 50 grams less than that of control rats on days 18 and 20, respectively).

Drug-related macroscopic changes included small thymus, spleen, and kidney at both doses. Reduced gastric content and numerous pinpoint hemorrhagic ulcers in the gastric mucosa were observed at the high dose only.

Litter data:

The high dose of 4 mg/kg was 100% Embryoethal. At 2 mg/kg/day, embryoethality was 50% of implants (37/75). The post-implantation loss was about 50%, and the mean live litter size was reduced to about one-half of that controls. Reduced fetal body weight and placental weight were observed compared to the controls (Table 6).

TABLE 6: SUMMARY OF MEAN LITTER DATA FOR FEMALES KILLED ON DAY 20 OF PREGNANCY (p. 1)

Dose (mg/kg/day)		Corpora Lutea	Implan- tations	Embryofetal Deaths					Live Fetuses	Implantation Loss %		Litter Weight (g)	Mean Fetal Weight (g)	Mean Placental Weight (g)
				Resorptions		Dead Fetuses	Aborta	Total		Pre	Post			
				Early	Late									
0 (vehicles)	No.	39	39	39	39	39	39	39	39	39	39	39	39	39
	Mean	17.95	15.79	0.95	0.03	0	0	0.97	14.82	11.28	6.26	53.58	3.62	0.46
	SDEV	2.32	1.87	1.10	0.16	0	0	1.11	2.15	10.51	7.24	8.04	0.20	0.05
2	No.	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	17.40	15.00	0.60	6.60	0.20	0	7.40	7.60	12.64	49.97	23.56	3.13	0.34
	SDEV	2.51	1.58	0.89	3.21	0.45	0	2.97	3.51	13.17	21.49	10.29	0.19	0.07
4	No.	4	4	4	4	4	4	4	4	4	4	--	--	--
	Mean	16.75	12.25	7.75	4.50	0	0	12.25	0	25.16	100	--	--	--
	SDEV	1.71	3.30	4.11	6.14	0	0	3.30	0	24.92	0	--	--	--

(a) : Animals providing background control data for comparison of the scheduled variables

As in Table 7, all fetuses obtained from five litters at 2 mg/kg had malformations. External anomalies include anal atresia (23/38), short and or bent, curled or kinked tail (20/38), and abnormal genital tubercle (29/38). Visceral malformations affecting the digestive system (esophageal agenesis or trachesophageal fusion; agenesis, hypoplasia and atresia of stomach and intestine (38/38) and the urinary system (hydronephrosis, hydroureter; kidney, ureter and/or vesical bladder agenesis or hypoplasia) (36/38). A high incidence of cardiovascular malformations (anomalies of the heart, great vessels with their branches) occurred in 22/38 fetuses. Several other viscera were absent, underdeveloped or mal-developed (eg, trachea, spleen, pancreas, genital organs). Skeletal malformations included deformity of the long bones of the fore- and hind-limbs in 22/38, rib anomalies (ie, bent, thickened and misshapen ribs) in 21/38. Ossification of the spine was compromised (34/38). Spinal ossification sites revealed mostly irregularities of thoraco-lumbar centra, and sometimes sacro-caudal centra, indicating reduced and defective ossification.

Table 7: SUMMARY OF THE PRINCIPAL MALFORMATIONS OBSERVED IN LIVE FETUSES

EXTERNAL EXAMINATION	FETUSES No.	(LITTERS No.)
ANASARCA	4	(5)
SHORT/ KINKED/ BENT/ CURLED TAIL	20	(5)
ANAL ATRESIA	23	(5)
ABSENT/ REDUCED/ ENLARGED/ MISSHAPEN/ DISPLACED GENITAL TUBERCLE	29	(5)
FRESH VISCERAL EXAMINATION		
CARDIOVASCULAR SYSTEM:		
WIDE VARIETY OF SEVERE ABNORMALITIES INVOLVING HEART, GREAT VESSELS AND BRANCHES	22	(5)
DIGESTIVE SYSTEM:		
ESOPHAGUS: AGENESIS, TRACHESOPHAGEAL FUSION; STOMACH AND INTESTINE: ABSENT/ RUDIMENTARY/ HYPOPLASTIC/ IMPERFORATE	38	(5)
URINARY SYSTEM:		
KIDNEY, URETER AND/OR BLADDER: AGENESIS/ HYPOPLASIA; HYDRONEPHROSIS; HYDROURETER	36	(5)
OTHER VISCERA:		
TRACHEA: AGENESIS	10	(4)
LUNGS: HYPOPLASTIC	8	(3)
LIVER: SMALL/ MISSHAPEN	3	(3)
SPLEEN: AGENESIS/ RUDIMENTARY	27	(4)
PANCREAS: AGENESIS/ RUDIMENTARY	23	(3)
TESTES: DISPLACED	3	(2)
UTERUS: AGENESIS/ HYPOPLASIA/ ATRESIA	5	(4)
OVARIES: AGENESIS/ HYPOPLASIA	2	(2)
ADRENALS: ENLARGED/ SMALL	2	(2)
SKELETAL EXAMINATION		
FORE-HIND LIMBS:		
BENT/ SHORT/ THICKENED HUMERUS AND/OR FEMUR, ULNA, RADIUS, TIBIA, FIBULA	21	(5)
RIBS:		
BENT/ THICKENED/ MISSHAPEN	21	(5)
GIRDLES:		
BENT/ SHORT/ MISSHAPEN SCAPULA AND/OR CLAVICLE, ILEUM, ISCHIUM	18	(5)
SPINE:		
UNOSSIFIED/ BIPARTITE/ DUMBBELL/ INCOMPLETE THORACOLUMBAR CENTRA; MISSHAPEN SACROCAUDAL VERTEBRAE	34	(5)
TOTAL EXAMINED	38	(5)
TOTAL AFFECTED	38	(5)

Rabbits:

Species: NZW Hy/Cr pregnant rabbits, n=5
 Dose levels: 0, 1, or 3 mg/kg/day, i.v. epirubicin PFS (Lot 8015LC)
 Treatment: Pregnant females were dosed on days 10 to 12 of gestation
 Observation: Clinical signs daily; body weight/food consumption was recorded; dams were sacrificed on day 29, uterine contents were examined.

Results:**Maternal data:**

At 3 mg/kg, vaginal bleeding in 3/5 and 2 rabbits aborted on day 16 of pregnancy.

At 1 mg/kg, vaginal bleeding in 1/5 and 3 rabbits aborted on days 19, 21, or 25 of pregnancy. All rabbits 5/5 in the HD died on days 5 & 6 and 1/5 in the LD died on day 6 had vaginal bleeding. Three rabbits that aborted survived up to termination.

Dose-related effects on body weight and food consumption occurred at both doses but severe at 3 mg/kg/day (62% on day 16 and died on day 17). Reduction in body weight gain and food consumption was less severe at 1 mg/kg/day (57% on day 16 and 34% on day 29).

At necropsy, animals at 1 and 3 mg/kg/day showed poor nutritional status and had duodenal ulcer (1/5), enlarged heart (1/5) and pale liver in another.

Litter data:

100% post-implantation loss and no live fetuses were seen at 3 mkd. The single female rabbit at 1 mg/kg that reached term with pregnancy had 11 live fetuses with no embryonal deaths. External, visceral and skeletal examination of these 22 fetuses revealed no malformations.

C. Segment III peri- and postnatal toxicity study in rat (Tech Report IMI-28/434i):
 (vol. 1.16, p. 058)

Rats: Species: CrJ:CD (SD) ————— pregnant rats, n=8
 Dose levels: 0, 0.03, 0.1, 0.3, 1.0 or 2.0 mg/kg/day i.v. epirubicin (Lot 10050100)
 Treatment: Pregnant dams were given i.v. doses of epirubicin from day 17 of pregnancy to day 7 after delivery
 Observation: Dams-clinical signs, mortality, body weight/food consumption, delivery, # of implantation, dead/live fetuses
 Offsprings (birth to weaning)-mortality, fetal size and weight, fetal anomalies, sex, birth index, autopsy at day 7

Results:**Effects on dams:**

Doses of 1 and 2 mg/kg/day were toxic to dams. No mortality but blotted fur (around nose and mouth, lower abdomen), soft/black stool, piloerection and emaciation were observed. General conditions of these rats deteriorated and they showed loss of nest-building and nursing action. Both 1 and 2 mg/kg doses showed significantly depressed body weight gain during the perinatal period, and remarkably decreased body weights and decreased food intake after delivery.

Gross alterations at necropsy included atrophies of lymphoid organs (thymus, spleen) and hemorrhage of stomach. Other changes such as hypertrophy of submandibular lymph node, dark red lung, and atrophy and paleness of pancreas was also observed in dams given 1 and 2

mkd. Atrophy of thymus (6/8 rats at 0.3 mkd, 1/8 rat at 0.1 mkd) and spleen (1/8 at 0.3 mkd) was observed. No abnormal finding was seen at 0.03 mkd.

Conditions of delivery:

Gestation index was 100% in all treated including a control group. No significant changes in number of implantations, number of still-borns/live offsprings, and birth index were observed and no external anomaly was found.

Litter Data:

In development of offsprings, dose-related decreases in body weight gain were observed in both sexes with 0.03, 0.1 and 1 mg/kg and significant difference was detected continuously. The 2 mg/kg dose showed decrease in body weight from day 0 to day 4 after birth, while rats given 0.3 mkd had no changes in body weight or a similar growth to that in the control group.

Decreased body weight gain of F₁ offsprings at 1 mg/kg was considered to be due to insufficient nursing and decreased body weight and reduced viability index (days 0-4 after birth), at 2 mg/kg, were caused by intoxication of dams by the drug. All the newborns in this group died on days 4-7 after birth.

D. Segment III intravenous peri-postnatal toxicity study in rats (Tech Report IMI 28/436i):
(vol. 1.17, p.001)

Species: CrJ:CD (SD) pregnant rats, n=25
 Dose levels: 0, 0.05, 0.15, or 0.50 mg/kg/day i.v. epirubicin (Lot 10050100)
 Treatment: Pregnant dams were given i.v. doses epirubicin from day 17 of pregnancy to day 21 after delivery (during perinatal and lactation periods)
 Observation: Dams- mortality, clinical signs, body weight/food intake, delivery (dead/live fetuses, size/weight of fetus, anomalies, nursing ability and autopsy at weaning
 Offsprings (birth to weaning)-mortality, birth index, viability index, weaning index, developmental index, general development; (at weaning)-autopsy, spontaneous locomotor activity, functional tests
 Different tests were given to offsprings after weaning: At 5 weeks: open-field test, spontaneous locomotor activity; at 7 weeks: various reflexes and T-maze test; at 9 weeks: conditioned avoidance response; at 10 weeks: reproductive performance

Results:

Effects on Dams:

The 0.50 mg/kg dose was a toxic to dams. One dam given 0.05 mg/kg died from dystocia (incidental not drug-related) on day 23 of pregnancy. Dams given 0.50 mg/kg dose had significant decreases in body weight gains on days 17-21 of pregnancy and during lactation and decrease in food intake on day 21 of pregnancy and days 1-14 after delivery. Slightly reduced ability in nursing pups was noted. Necropsy findings at weaning included decreased organ weights (spleen, ovary and uterus) at 0.50 mkd but atrophy of thymus was noted at 0.50 mg/kg (16/24) and 0.15 mkd (1/23).

Litter data:

The viability index (day 4 after birth) and body weight gains of F₁ were depressed during the lactation period. These changes appear to result from the reduced nursing ability caused by intoxication of dams, for these changes were not observed after weaning. At autopsy, at weaning, F₁ showed decreased various organ weights in both sexes at 0.50 mkd. In males, right adrenal weight was decreased at doses \geq 0.15 mg/kg, and weights of heart, spleen and

kidney were significantly decreased at 0.5 mg/kg whereas in females, weights of brain, heart, liver, kidney, adrenal and ovary were decreased.

Functional tests:

Increased spontaneous locomotor activity was observed in male rats treated with 0.15 mg/kg and 0.50 mg/kg (on day 21 after birth). No abnormalities were found in various reflex activity (righting reflex, pupillary reflex, auricular reflex, corneal reflex, auditory test (preyer reflex) and visual test (cliff avoidance) either in the treated or in the controls.

Behavioral tests:

In water-filled multiple T-maze test (learning performance), the time required for animals to reach the goal was significantly decreased in the third trial on day 3 in males with 0.15 mg/kg and increased in the third trial on day 1 in females with 0.15 and 0.5 mg/kg. Acquisition of learning performance was confirmed in these rats.

In reproductive performance tests of F₁ (10 weeks old):

No significant difference was observed in copulation index, duration of mating period and fertility index between the treated and control animals. At C-section, no remarkable changes in number of corpora lutea, number of implantations, number of dead/resorbed fetuses, number of live fetuses and sex ratio were observed.

In live fetuses, significant increase of body weight was seen in both sexes with 0.05 mkd, females with 0.15 mkd and both sexes with 0.50 mkd. External anomalies were observed only in one animal with 0.15 mg/kg (exencephaly) and one animal each with 0.15 and 0.5 mg/kg (hematoma of hind legs).

Summary of Reproductive Studies:

In a preliminary dose-finding study, i.v. doses of 0.03, 0.1, 0.3 and 1.0 mg/kg were administered to male rats for 9 weeks before mating, and 0.03, 0.1, 0.3, 1.0 and 2.0 mg/kg to females for 2 weeks before mating and to day 7 of gestation. Epirubicin was lethal for male rats and females at doses of 1.0 and 2.0 mg/kg/day, respectively. At 0.3 mkd, body weight gain was depressed in both sexes, male rats showed atrophy of testes, seminal vesicles and/or epididymides, and no females were pregnant.

In a Segment I fertility study, epirubicin had no effect on fertility at i.v. doses of 0, 0.01, 0.03 and 0.10 mkd administered to male rats for 9 weeks before mating while females were given i.v. doses of 0.01, 0.03, 0.10 and 0.3 mkd for 2 weeks before mating and up to day 7 of gestation. No treatment related effects were seen on precoital periods, mating performance, or fertility. Males given 0.03 and 0.1 mkd epirubicin showed dose-related decreases in body weight gain (at 0.1 mkd only), and decreases in size/weight of the testes, epididymides, and reduced spermatogenesis. In females, decreases in body weight gain and increases in placental weights were seen at ≥ 0.3 mkd. Increased mortality and decreases in body weight of live fetuses were observed from dams given doses at ≥ 0.1 and ≥ 0.03 mkd, respectively.

The segment II teratogenic effects of epirubicin were studied in rats given i.v. doses of 0, 2 or 4 mg/kg/day on gestation days 9 and 10. Both doses showed dose-related maternal toxic effects such as mortality, decreases in body weight and food intake, and vaginal bleeding at 4 mkd. At 2 mg/kg, embryoletality affected 50% of implants, and all 38 fetuses from surviving dams showed specific malformations of the GI tract, urinary system, and a variety of cardiovascular malformations. Skeletal ossification was also severely affected in most fetuses. The most characteristic findings included deformity of the long bones of the fore- and hind limbs, girdles, and rib abnormalities. These findings were similar to those seen with doxorubicin and other anthracyclines.

The segment II teratogenic effects of epirubicin were determined in rabbits given i.v. doses of 0, 1 or 3 mg/kg/day on days 10 to 12 of gestation. Epirubicin at i.v. doses of 1 or 3 mg/kg/day induced abortion in 80 and 100% of animals. Dose-related toxic effects were decreases in body weight gain and food intake and vaginal bleeding. No teratogenic effects were observed by the examination of 22 fetuses.

In segment III peri- and postnatal toxicity study in rat, pregnant rats were dosed at 0, 0.03, 0.1, 0.3, 1.0 or 2.0 mg/kg/day epirubicin from day 17 of pregnancy to day 7 after delivery. Epirubicin i.v. doses of 1 and 2 mg/kg/day were lethal to dams and reduced ability to nurse. At 0.30 mg/kg/day, no remarkable changes were seen in dams or newborns.

In another segment III peri-postnatal toxicity study, pregnant rats were treated with i.v. doses of epirubicin at 0, 0.05, 0.15 or 0.50 mg/kg/day from day 17 of pregnancy to day 21 after delivery. At 0.5 mkd, epirubicin induced maternal toxicity characterized by significant decreases in body weight and food intake and, at autopsy, atrophy of thymus and decreased organ weight of spleen and uterus. In offsprings of this group decreased viability index and body weight gain were noted probably due to the reduced nursing ability of the dams. No remarkable changes in developmental, functional tests and reproductive performance were seen in both treated and control groups.

V. Genotoxicity Studies: A Summary table

Epirubicin was assayed in a battery of in vitro and in vivo genotoxicity assays. Epirubicin was tested in comparison with doxorubicin in most studies. The two compounds had similar genotoxic activity in all experimental systems in vitro and in vivo as in the following table.

A Summary for Genotoxicity studies

Type of Study	Test System	Concentrations/Doses	Metabolic Activation	Results
In vitro Gene mutation on <u>S. typhimurium</u> (Ames assay)	Salmonella strains: TA 98, TA 100, TA1535, TA1537, TA 1538	0, 1.25, 2.5, 5, 10, 20, 40, 80 ug/plate	-/+	Positive Mutagenic on 3 strains of <u>S. typhimurium</u> : TA 1538, TA 100 and especially TA 98
In vitro gene mutation test on the V79 chinese hamster cell line	V79 chinese hamster lung cells	EPI: 0, 0.12-1 ug/ml Doxo: 0.25-1 ug/ml	-	Positive/ clastogenic
Induction of chromosome aberrations in vitro	Human lymphocytes	0, 0.05, 0.1, 0.2, 0.4, 0.8 ug/ml	-/+	Positive/clastogenic Epirubicin induced both chromatid and chromosome aberrations, including interchanges
Induction of chromosome aberrations in mouse bone marrow	Mouse (Iva:NMRI), n=5/sex	0, 5, 10 or 20 mg/kg epirubicin vs doxorubicin -sampling at 24 h postdose	NA	Positive

VII. Carcinogenicity Studies:

A. Carcinogenesis of the compound 4'-epi-adriamycin (IMI 28) in inbred rats (Tech Report IMI 28/402i): (vol. 1.20, p.159)

Method:

Newborn inbred  rats (24-36 hrs after birth, weight (not provided) received s.c. doses of 0.75 and 1.0 mg/kg IMI 28 (Lot SR966/75) once daily for 4 consecutive days with a 5 days rest between the two courses (a total of 8 treatments). The animals were classified according to sex when newborns reached 50 days of age. Animals were observed twice a month

for 530 days for tumor appearance. When animals were in distress due to tumor burden, animals were sacrificed and necropsied. Tissue and any apparent visible lesions were fixed for histologic examination. The tumors found in the experimental animals were tabulated for comparison with the control animals.

Results:

The numbers and kinds of tumor were summarized as in Table 1. The incidence of tumors increased from 21% in the control (females) to 80-85% in the treated females whereas in males the incidence goes from absence of tumors in the controls to 66 and 81% in the treated groups for 1 and 0.75 mg/kg epirubicin, respectively. There appears to be more males 83% (10/12) than females 41% (7/17) affected by malignant tumors (Table 3). Overall tumor incidence are similar between the sexes.

Table 1
Carcinogenic activity of 4'-epi-adriamycin administered repeatedly s.d. to newborn rats (530 days after treatment).

TREATMENT	Dose mg/Kg	Animals with tumour/total	%	Sex	No. of Histol. preparations examined	Histological Classification
CONTROLS		4/19	21	f	3	1 adenocarcinoma 2 adenomas
		0/9	0	m	0	-
4'-epi-adriamycin	0.75	12/14	85	f	9	1 mammary adenocarcinoma 1 " " " " + 1 adenosis 1 fibrosarcoma 1 adenocarcinoma 3 fibroadenomas 1 fibroma
		9/11	81	m	9	1 giant cell sarcoma 2 sarcomas 1 leiomyosarcoma 1 leiomyosarcoma 1 fibroma 1 fibrosarcoma 1 ossifying fibroma ² 1 animal with 2 tumours ³
4'-epi-adriamycin	1.10	12/15	80	f	11	1 sarcoma 2 fibrosarcomas 1 mammary adenocarc. + rhabdosarcoma ¹ 1 Schwann-cell tumour - malignant 2 fibrosarcomas 1 fibroadenoma + fibroma + verruca 2 adenomas 4 sarcomas 2 fibrosarcomas
		10/15	66	m	10	1 osteogenic sarcoma and liposarcoma ¹ 1 rhabdosarcoma 1 ameloblastoma 1 fibroadenoma

- ¹ Tumours present in the same animal
² Uncertain classification
³ Neoplasms not verified histologically

As shown in Table 3, there are a higher number of malignant tumors with 1 mg/kg dose (17/26) than with the 0.75 mg/kg dose (8/18). Most of the tumors appear at about the 400th day (~13 months). The median tumor appearance was 8.3 to 15 months.

Mortality and non-neoplastic findings after single dose administration of 3 anthracyclines are summarized in Table I. Mammary tumors were the main cause of death in most groups, with the exception of the rats in group B treated with Doxo, chronic progressive nephrosis (CPN) was the predominant cause of mortality in 9/20 affected rats. Other important finding included was cardiomyopathy that was evident on histological examination and biochemical changes. All three findings were recognized as the typical chronic systemic effects for these compounds. All compounds caused an increase incidence of mammary tumors in both 7-week old and 13-week old rats, when compared to controls (Table II).

In 7-week old rats, drug-induced increase was due to occurrence of fibroadenomas whilst a slight increased incidence of adenocarcinomas was also seen in the 13-week old rats.

Mortality and non-neoplastic findings after multidose anthracyclines are summarized in Table III. Due to excessive mortality of the two higher doses, the low dose group was the only one that could be effectively compared between drugs. The cumulative dose of this group was 2.5 mg/kg for Doxo and EPI and 0.6 mg/kg IDA. These dose levels were similar to those in the single dose study. As in the previous experiment, the expected pathology findings were observed and included atrophic changes in the testes and epididymides.

Table II. Study of single dose administration of anthracyclines to female rats of different ages with 12-month observation: Neoplastic findings.

Compounds	Dose (mg/kg)		Percentage of rats with mammary tumors (%)	Mammary tumor types (%)			Pituitary tumors (%)
				Fibroadenoma	Adenoma	Adenocarcinoma	Adenoma
Controls	0	A	19	17	0	6	29
		B	25	22	0	6	36
DOXO	3	A	80	80	5	5	10
		B	67	67	8	25	10
EPI	3.6	A	45	45	0	0	20
		B	65	65	0	15	50
IDA	0.75	A	45	35	5	5	30
		B	74	63	5	21	40

A, younger rats (7 weeks old); B, older rats (13 weeks old); Control group, 36 rats; Treated groups, 20 rats/group; Percentages are calculated on the number of tissues actually examined.

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Table III. Study of multiple dose administration of anthracyclines to male and female rats with 18-month observation: Causes of death.

Compounds	Dose (mg/kg)	Sex	No. of decedents	Kidney disease (%)	Mammary tumors (%)	Pituitary tumors (%)
Controls	-	M	14/35	-	-	50
		F	12/35	8	-	75
DOXO	0.25	M	15/20	40	-	-
		F	9/20	-	33	56
DOXO	0.5	M	16/20	88	-	-
		F	12/20	25	33	17
DOXO	1	M	15/20	93	-	-
		F	16/20	100	-	-
EPI	0.25	M	10/20	10	-	20
		F	9/20	-	22	78
EPI	0.5	M	16/20	63	-	6
		F	15/20	7	27	53
EPI	1	M	16/20	100	-	-
		F	16/20	75	-	13
IDA	0.06	M	7/20	14	0	29
		F	12/20	-	25	50
IDA	0.125	M	8/20	25	-	25
		F	10/20	-	20	60
IDA	0.250	M	16/20	75	-	6
		F	15/20	20	40	20

M, males; F, females.

In neoplastic findings, mammary gland tumors and subcutaneous tumors were observed as in Table IV. The time of onset for mammary tumors was earlier and the severity was greater than that of controls. Deaths attributed to mammary tumors occurred in three deaths given 0.25 mg/kg and four deaths given 0.5 mg/kg of Doxo, in two rats given 0.25 mg/kg and four rats given 0.5 mg/kg EPI, in three, two and six rats given 0.06, 0.125 and 0.25 mg/kg IDA, respectively. There was no increased incidence of mammary tumors in males or females treated with EPI (Table III).

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Table IV. Study of multiple dose administration of anthracyclines to male and female rats with 18-month observation: Neoplastic findings.

Compounds and doses	No. rats with mammary tumors (%)	Mammary tumors			Subcutaneous tumors	
		Fibroadenoma (%)	Adenocarcinoma (%)	Fibroma (%)	Fibroma (%)	Fibrosarcoma (%)
Controls						
M	1 (4)	0	0	1 (4)	5 (14)	0
F	22 (65)	20 (59)	6 (18)	0	0	0
DOXO 0.25						
M	2 (12)	1 (6)	1 (6)	0	8 (40)	2 (10)
F	15 (79)	12 (63)	3 (16)	1 (5)	3 (15)	0
DOXO 0.5						
M*	0	0	0	0	3 (15)	2 (10)
F	16 (80)	14 (70)	3 (15)	0	4 (21)	2 (10)
DOXO 1						
M*	0	0	0	0	0	0
F	2 (12)	2 (12)	0	0	0	0
EPI 0.25						
M	1 (8)	1 (8)	0	0	9 (45)	1 (5)
F	10 (50)	9 (45)	1 (5)	0	1 (5)	0
EPI 0.5						
M*	1 (5)	0	1 (5)	0	15 (75)*	2 (10)
F	10 (50)	10 (50)	1 (5)	0	3 (15)	3 (15)
EPI 1						
M*	0	0	0	0	1 (5)	0
F	8 (42)	7 (37)	3 (16)	0	0	0
IDA 0.06						
M	0	0	0	0	7 (35)	0
F	11 (55)	10 (50)	1 (5)	0	4 (20)	0
IDA 0.125						
M	0	0	0	0	5 (25)	4 (20)
F	11 (55)	10 (50)	3 (15)	1 (5)	0	2 (10)
IDA 0.25						
M*	0	0	0	0	1 (5)	0
F	16 (80)	15 (75)	3 (15)	0	2 (11)	1 (6)

Percentages are calculated on the number of tissues actually examined. Control group, 35 rats; Treated groups, 20 rats/group; M, males; F, females; *Early termination; *Statistically significant, $P < 0.001$.

Treatment was associated with an increase in incidence of fibrous tumors in subcutaneous tissue which was exclusively in the proximity of mammary tissue. The only incidence that achieved statistical significance of $P < 0.05$ or greater, was that of the benign fibromas in males receiving EPI. The statistical impact is due to the prolonged survival (665 days) of EPI-exposed animals receiving 0.5 mg/kg when compared with Doxo at the same dose level (Table V). In control males there was a 14% incidence of fibromas and no fibrosarcomas. In treated groups, the highest incidences were seen with EPI at the 0.5 mg/kg dose level with 75% fibromas.

Table V. Study of single and multiple dose administration of anthracyclines to rats: Mortality.

Compounds	Dose mg/kg	Single dose study (1 year)		Dose mg/kg	Cumulative dose (mg/kg)	Multiple dose study (2 years)			
		Mortality (%)				Mortality (%)		Day of early termination	
		A	B			M	F	Males*	Females*
DOXO	3	25	50	0.25	2.5	75	45	-	-
				0.5	5	80	60	day 536	-
				1	10	75	80	day 206	day 420
EPI	3.6	5	20	0.25	2.5	50	45	-	-
				0.5	5	80	75	day 665	-
				1	10	80	80	day 249	day 609
IDA	0.75	5	20	0.06	0.6	35	60	-	-
				0.125	1.25	40	50	-	-
				0.25	2.5	80	75	day 644	-

A, younger rats (7 weeks old); B, older rats (13 weeks old); *7 weeks old at dosing.

Overall Summary and Evaluation:

Epirubicin (4'-epi-doxorubicin (4'-epi-adriamycin); IMI 28; EPI) is a new anthracycline, a doxorubicin derivative, which was rationally synthesized to increase the therapeutic index of doxorubicin by Pharmacia & Upjohn (formerly Farmitalia, Farmitalia Carlo Erba, Pharmacia). The precise mechanism of action of epirubicin is not fully understood, however, many studies indicate that epirubicin binds to DNA by intercalation and inhibits nucleic acid synthesis and function. This action may be attributed to interfere with topoisomerase-DNA cleavable complex and helicase activity by anthracyclines. Epirubicin is a cell cycle non-specific anthracycline, with maximal cytotoxic effects on the S and G₂ phases.

The in vitro cytotoxicity tests of epirubicin showed that the compound possesses cytotoxic effect essentially similar to that of doxorubicin against a variety of animal and human cell lines. Epirubicin was active against murine leukemias (P388, L1210, Gross leukemia), murine solid tumors (S180, mammary ca., B16 melanoma, Lewis lung carcinoma) and human tumor xenograft models. In particular, epirubicin was active against breast cancer, epidermoid cancer, oat-cell carcinoma of the lung, prostate and ovarian carcinoma and human melanoma.

Pharmacokinetic studies of epirubicin have been performed in mice, rats, rabbits and dogs. Following rapid intravenous administration, epirubicin declined triexponentially with terminal half-lives of 12-16 h in mice, 17-21 h in rats, 21-23 h in rabbits and 35-77 h in dogs. The terminal half-life of doxorubicin was ~32% longer than that of epirubicin in rats (15.6 h vs 49.7 h). However, peak plasma drug concentrations were similar following intravenous administration of equimolar doses of epirubicin and doxorubicin.

Epirubicin undergoes extensive tissue distribution and high clearance. The volume of distribution values were high and variable, but similar to those reported for doxorubicin. AUC values adjusted for dose were 30 to 70% higher for doxorubicin than epirubicin following single dose intravenous administration. Epirubicin is eliminated mainly by biliary excretion (~48%), and ~15% of the dose in the urine as unchanged drug and metabolites. Like doxorubicin, epirubicin is rapidly metabolized to epirubicinol (the C-13-carbinol derivative, adriamycinol or 4-epi-adriamycinol), unidentified metabolites (polar metabolites), and aglycone derivatives. After epirubicin administration to different animal species and man, concentrations of epirubicin and its metabolites were determined. Beside man, small amounts of glucuronides were detected only in the rabbits.

Toxicity studies of epirubicin were conducted in mice, rats, rabbits and dogs. In acute studies, epirubicin toxicity was evaluated in mice, rats and dogs, often in comparison with doxorubicin. Chronic toxicity studies were conducted: 6 week i.v. toxicity studies in rabbits and dogs, and 13 week i.v. toxicity studies in rats and dogs. Overall the toxicological profile of epirubicin was qualitatively similar to that of doxorubicin. When tested in comparison with doxorubicin, epirubicin appeared to be less toxic.

The acute intravenous toxicity of epirubicin (PFS, RTU) was compared in mice and rats with different formulations of LCF (test 1), ADF (test 2), and LYO (test 3). No mortality occurred at single dose level of 18.00 mg/kg, 20.5 mg/kg and 10 mg/kg for the test 1, 2 & 3, respectively. Among the toxic effects of epirubicin, all three formulations produced marked reduction in size of thymus, testes at doses ≥ 22.78 mg/kg in test 1, at ≥ 18 mg/kg in test 2, and ≥ 12 mg/kg in test 3. Overall, toxic effects induced by EPI RTU were qualitatively or quantitatively similar to those with LCF, ADF or LYO.

In special toxicity studies, when hematotoxicity of epirubicin was compared with doxorubicin in rats, both compounds induced similar dose-related decrease in WBC with nadir on day 8. Epirubicin 6 mg/kg and doxorubicin 3 mg/kg were equitoxic on leukocytes. Also, slight to marked decreases in platelets were noted in both compound at high doses. In bone marrow toxicity study, decreases in WBC, platelet, RBC, Hb, Hct values were observed in male dogs treated with epirubicin and doxorubicin both at 1 mg/kg. The immune response of epirubicin was compared with adriamycin in mice. The effect in IgG was more pronounced with epirubicin than adriamycin, irrespective of the treatment schedule used. Epirubicin had a slight immunosuppressive effect on delayed hypersensitivity reactions. The potential immunogenicity of epirubicin was evaluated in guinea pigs. No passive systemic anaphylaxis was induced by epirubicin, however, a delayed skin hypersensitivity reaction was observed.

The cardiotoxicity study of epirubicin was compared with doxorubicin after single and multiple dose regimens in rats. After single dose regimen (6 & 9 mg/kg IMI 28, 3 & 6 mg/kg doxorubicin) in rats, epirubicin induced a multifocal degenerative cardiomyopathy qualitatively similar to that observed after intravenous doxorubicin. However, its cardiotoxic effects were considerably less severe than those of doxorubicin in rats. In a multidose regimens, male rats received epirubicin and doxorubicin at equi-myelotoxic dose of 1 mg/kg/week for 7. Both compounds induced cardiomyopathy with myocardial lesions and renal impairments. The myocardial damage was moderate for epirubicin and severe for doxorubicin.

In a Segment I fertility study, epirubicin caused decreases in size/weight of testes, epididymides, and hypospermatogenesis in male rats while in females, epirubicin had no effect on precoital, mating performance, or fertility. In Segment II reproductive studies, pregnant rats were given i.v. doses of 0, 2 or 4 mg/kg/day on gestation days 9 and 10. Both doses of epirubicin resulted in maternal toxicities and fetal malformations. Pregnant rabbits received i.v. doses of 0, 1 or 3 mg/kg/day on days 10-12 of gestation. Both doses induced 80% and 100% abortion, maternal toxicities but no teratogenic effects. In a Segment III peri-postnatal toxicity study, i.v. doses of epirubicin ranging from 0.03 to 2 mg/kg/day were given from day 17 of pregnancy to day 7 after delivery. Two high doses of 1 and 2 mkd were lethal to dams. Epirubicin exposure caused maternal toxicities and reduced nursing ability. In another Segment III peri-postnatal study, i.v. doses of epirubicin ranging from 0.05 to 0.5 mg/kg/day were given from day 17 of pregnancy to day 21 after delivery. High dose of 0.5 mkd resulted in maternal toxicities characterized by significant decreases in body weight and food consumption, gross alterations (lymphoid organs, ovaries, uteri), and reducing nest building and nursing ability. Beside reduced litter size/weight, no noteworthy changes were observed in the offsprings. Progeny weights and viability index were lower, developmental delays and reduced physical activity was noted in the newborns whose mothers were exposed to HD epirubicin in utero. Epirubicin had no effect on reproduction parameters, progeny survival to weaning, and functional and behavioral tests.

The mutagenic potential of epirubicin was investigated using in vitro tests and in vivo experiments. Epirubicin was mutagenic in 3 strains of *S. typhimurium*, TA 1538, TA 100 and TA 98 with and without metabolic activation. Epirubicin was clastogenic without metabolic activation in vitro in the gene mutation test on V79 Chinese hamster lung cells. Epirubicin induced both chromatid and chromosome aberrations on human lymphocytes. Epirubicin induced chromosome aberrations in mouse bone marrow in vivo.

In carcinogenicity studies, s.c. doses of 0.75 and 1 mg/kg/day epirubicin given to newborn rats increased the incidence of tumors increased 21% (control) to 80-85% in the treated females and 66-81% in treated males. In long-term toxicity study, single (3 mg/kg dox; 3.6 mg/kg epi) and multidose i.v. doses ranging from 0.06 to 0.5 mg/kg epirubicin was given to 7

and 13 weeks old female rats. Mammary gland tumors and subcutaneous tumors were observed. These findings suggest that epirubicin appears to be carcinogenic.

Recommendation: The pharmacologic/toxicologic data in this NDA supports approval with revisions to the labeling as indicated in a separate review.

/S/

Doo Y. Lee Ham, Ph. D.

cc Original NDA 21-010
HFD-150/Division File
/LeeHam
/Andrews
/Honig
/Guinn
DYLH/MW

Division of Oncology Drug Products, HFD-150
Review and Evaluation of Pharmacology and Toxicology Data
Labeling Review

Keywords: labeling, epirubicin
NDA: 21-010 and 50-778
Serial #: 000 **Type:** NDA
CDR date: 12/15/98

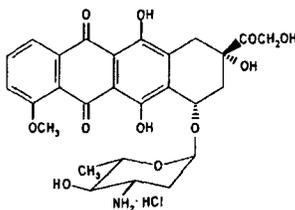
Information to be conveyed to sponsor: Yes

Reviewer: Doo Y. Lee Ham, Ph. D.

Date Review Completed: 6/8/99

Applicant: Pharmacia & Upjohn
 Kalamazoo, MI 49001

Drug: Code Name: IMI 28, EPI
 Generic name: Epirubicin Hydrochloride for Injection (4'-epi-doxorubicin; 4-epi-adriamycin hydrochloride)
 Trade Name: Ellence
 Chemical Name: (8S, 10S)-10-[(3-amino-2, 3, 6-trideoxy- α -L-arabinohexopyranosyl)oxy]-8-glycoloyl-7, 8, 9, 10-tetrahydro-6, 8, 11-trihydroxy-1-methoxy-5, 12-naphthacenedione hydrochloride
 CAS Registry Number: 56390-09-1
 Molecular formula/weight: $C_{27}H_{29}NO_{11} \cdot HCl$; 579.99
 Structure:



Related IND & NDA: IND NDA

Class: Anthracycline cytotoxic agent

Indication: Epirubicin is indicated for the treatment of the patients with advanced breast cancer. The recommended clinical dose is 100 to 120 mg/m².

Formulation:

The complete formulation of the freeze dried dosage form (10 and 50 mg) is detailed in Table 9. This is the only formulation to date which has been used clinically.

Table 9: Formulation of Epirubicin Hydrochloride freeze dried

Strength	Formulation No.	Ingredient	Quantity per vial	Function	Reference to Standard
10 mg	F1 7701/F1	Epirubicin Hydrochloride	10 mg	Active Pharmaceutical Ingredient	In-house
		Lactose	50 mg	Bulking agent	NF
50 mg	F1 7701/F2	Epirubicin Hydrochloride	50 mg	Active Pharmaceutical Ingredient	In-house
		Lactose	250 mg	Bulking agent	NF

Route of Administration: Intravenous

Previous Reviews, Dates and Reviewers:

NDA — (Epirubicin HCl for Injection)	8/13/85	JSun
NDA 21-010 (Epirubicin HCl for Injection)	6/15/99	DYLeeHam

Studies Reviewed Previous Submissions: see current NDA review

Studies Reviewed with This Submission: see current NDA review

Studies Not Reviewed: see current NDA review

* Portions of this review were excerpted directly from the sponsor's submission

INTRODUCTION AND DRUG HISTORY

Epirubicin (4'-epi-doxorubicin, IMI 28) is a doxorubicin derivative which was synthesized in an effort to increase the therapeutic index of the parent compound. Doxorubicin HCl is the most widely used cancer drug as a single agent or in combination with other anti-neoplastic agents to treat for both hematologic and non-hematologic malignancies since its approval 1970s.

NDA — for epirubicin was submitted by Farmitalia in 1984 for the treatment of advanced breast cancer. This NDA was not approved. Farmitalia transferred the right for Epirubicin to Pharmacia & Upjohn.

NDA 21-010 for epirubicin was re-submitted for the same indication by Pharmacia & Upjohn in 1998. Pharmacia & Upjohn references NDA — for NDA 21-010 with regard to all nonclinical pharmacology, ADME and toxicology data. Besides reproductive toxicity studies, additional data regarding pharmacokinetics, mutagenic and carcinogenicity have been submitted.

Labeling Comments:

Labeling generally conforms to the format specified under CFR 21. Part 201. Subpart B dated April 1, 1998. The proposed labeling describes the preclinical observations for the most part. However, the revisions indicated under "Draft Letter to Sponsor" below are requested:

Recommendation:

The pharmacologic/toxicologic data in this NDA supports approval with revision of the labeling as indicated in this review.

Draft Letter to the sponsor:

2 pages redacted from this section of
the approval package consisted of draft labeling

Doo Y. Lee Ham, Ph. D.

cc: Orig. NDA 21-010
HFD-150/Division file
 /LeeHam
 /Andrews
 /Honig
 /Guinn
DYLH/MW