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APPLICATION NUMBER: NDA 20-892

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA 20,892

Submission Date: June 26, 1998

Drug Name:

Valrubicin (AD 32)

Formulation:

Solution for intravesical injection

Sponsor:

Anthra Pharmaceuticals, Inc.

P.O. Box 41361,

Memphis, TN 38174

Reviewer:

Elena V. Mishina, Ph. D.

Type of Submission:

Amendment to New Drug Application

Background:

AD 32, valrubicin (N-trifluoroacetyl-diamycin-14-valerate) is a semisynthetic derivative of the anthracycline antibiotic doxorubicin which has an anti-tumor activity against lung, breast, and urinary bladder cancer, soft tissue sarcoma, and transitional cell sarcoma of the renal pelvis. It is intended for intravesical instillation at the 800 mg dose with a two hour drug retention period in the Orphan Drug setting of refractory carcinoma in situ of the urinary bladder.

The sponsor has submitted an amendment to the NDA including the revised version of the package insert.

LABELING COMMENTS

cc: NDA 20,892 original
HFD-150 Division file
HFD-150 AStaten , GWilliams, WOdujinrin
HFD-205 FOI
HFD-850 LLesko
HFD-860 HMalinowski, MMehta, ARahman, EMishina
CDR BMurphy

STATE 2

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA 20,892	Submission Date:	December 31, 1997 March 9, 1998 March 13, 1998
Drug Name:	Valrubicin (AD 32)	
Formulation:	Solution for intravesical injection	
Sponsor:	Anthra Pharmaceuticals, Inc. P.O. Box 41361, Memphis, TN 38174	JUN -9 1998
Reviewer:	Elena V. Mishina, Ph. D.	
Type of Submission:	New Drug Application (original)	

SYNOPSIS:

AD 32, valrubicin (N-trifluoroacetyl Adriamycin-14-valerate) is a semisynthetic derivative of the anthracycline antibiotic doxorubicin which has an anti-tumor activity against lung, breast, and urinary bladder cancer, soft tissue sarcoma, and transitional cell sarcoma of the renal pelvis. It is intended for intravesical instillation at the 800 mg dose with a two hour drug retention period in the Orphan Drug setting of refractory carcinoma in situ of the urinary bladder.

The "Human Pharmacokinetics and Bioavailability" section of the NDA contains pharmacokinetic (PK) information from 5 clinical studies using the intended route of administration involving patients with transitional cell carcinoma most of whom were treated for recurrent disease. In the two pivotal clinical studies, urinary recovery of valrubicin and its metabolites were evaluated. The additional data reports are organized according to administration route: intraperitoneal (one clinical study), and intravenous (information from 4 abstracts published in the literature).

The submitted package includes data on assay methods, absorption, metabolism and excretion, and PK in special population (patients dosed immediately after trans-urethral resection of the bladder).

Two different drug products (lyophilized and liquid formulations) were used during pharmacokinetic studies A91-0101 and A92-1100. A lyophilized drug product was used in study A92-0101, and a liquid drug product was used in study A92-1100. All of the rest of the pharmacokinetic studies used a liquid drug product, which required dilution at the clinical site. Dissolution data are not applicable.

The urine and plasma concentrations of unchanged valrubicin and its metabolites (N-trifluoroacetyl Adriamycin, AD 41, and N-trifluoroacetyl Adriamycinol, AD 92) were determined by

The detection limit for each component was 5 ng/mL plasma and 5 ng/50 µL urine aliquot. The sponsor has adequately validated the applied method.

after intravesical administration. The systemic absorption of valrubicin from the bladder into the bloodstream was dependent upon the condition of the bladder. When the bladder was perforated plasma valrubicin concentrations were considerably higher ($\mu\text{g}/\text{mL}$ order of magnitude).

Following intravesical instillation of 800 mg of valrubicin, a mean of 99.0% (1-mg dose) was recovered in the urine within 24 hours as 98.6% (1-mg dose) AD 32 and 0.4% (1-mg dose) AD 41. The majority of the instilled dose was recovered in the instillate by approximately 2 hours after instillation. Negligible amount of AD 32 was metabolized to AD 41 within 24 hours in the bladder. In serum, AD 32 is rapidly metabolized to AD 41 and AD 92 following intravenous administration. Therefore, the results of these studies demonstrate that AD 32 is not entering the systemic circulation since, systemically, it would be immediately metabolized to AD 41 and the amount of AD 41 in the urine would be expected to be greater than AD 32.

In the dose escalation study (intravesical instillation of 200 - 900 mg of valrubicin), the median total anthracycline concentration in serum was not more than 16.5 nmoles/L at doses of AD 32 up to 900 mg and the median serum concentration of AD 41 was higher than AD 32 or AD 92. The mean total anthracycline $\text{AUC}_{0-6 \text{ hours}}$ did not exceed 82 nmoles/L·hr at a dose of 200 mg of AD 32. At a dose of 600 mg of AD 32, the mean $\text{AUC}_{0-6 \text{ hours}}$ was 69.6 nmoles/L·hr. There was no apparent relationship between either number of doses or amount of AD 32 administered and the transurothelial absorption of the drug over a range of 200 to 900 mg of drug administered.

Following "typical" transurethral resection (TURB), AD 41 was the major component (38 ng/mL) of total serum anthracycline (48.5 ng/mL) at 6 hours post 800-mg intravesical dose of AD 32 in 14 patients. In the group of 7 patients who had an "extensive" TURB, 2 patients had markedly higher anthracycline concentration values, but the rest of 5 patients values were just slightly higher and similar otherwise. One patient in the group of "extensive" TURB had AD 41 and total anthracyclines serum concentrations of 436 ng/mL and 553 ng/mL, respectively, 6 hours after instillation of 800 mg of AD 32 in the bladder. The highest serum concentration of AD 41 and total anthracyclines occurred in the patient with a perforated bladder (2395 ng/mL and 2981 ng/mL, respectively, 6 hours after instillation of AD 32). The mean systemic exposure to AD 32 and its metabolites was also very dependent upon the extent of the TURB ($\text{AUC}_{0-6 \text{ hours}}$ 207 nmoles/L·hr, "typical," and 1364 nmoles/L·hr, "extensive"). The greatest systemic exposure to AD 32 and its metabolites was found in the patient with a perforated bladder ($\text{AUC}_{0-6 \text{ hours}}$ 18,382 nmoles/L·hr).

Mean total anthracycline concentration in the excised bladder tissue samples decreased with increasing depth from the urothelial surface. The results were similar for samples taken from the right lateral, left lateral, and dome surfaces. AD 32 concentrations of μM (mcg/mL) killed 90% of the cells of five human bladder tumor cell lines in a clonogenic assay. The mean total anthracycline concentrations measured in the bladder tissue exceeded the 90% cytotoxic levels to a depth of at least 1,250 μm , which exceeds the depth of invasion of T₁ lesions. At depths of 1,400 to 1,800 μm , which correspond to the depth of invasion of T₂ lesions, the mean total anthracycline concentrations closely approximated the clonogenic IC₉₀ range.

In vitro data of receptor binding and metabolism are referred to Non-Clinical Pharmacology Section.

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4. LIST OF TERMS AND ABBREVIATIONS

AD	adriamycin
AUC	area under the plasma concentration versus time curve extrapolated to infinity
AUC _{0-t}	area under the plasma concentration versus time curve determined at the last measured time point
TURB	transurethral resection of the bladder
CYP	cytochrome P450
HPLC	high performance liquid chromatography
IND	investigational new drug
NDA	new drug application

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5. COMMENTS

5.1 GENERAL COMMENTS

1. The sponsor has adequately characterized the pharmacokinetics of valrubicin at the recommended dosing regimen in the targeted population. Intravesicle instillation of 800 mg dose with a two hour drug retention period in the patients with refractory carcinoma in situ of the urinary bladder may provide optimum exposure for therapeutic action of the drug. However, the conclusion that anthracycline bladder tissue concentrations (intracellular, based on the method of analysis, were higher than the values of IC_{90} measured from clonogenic assay (extracellular) is inappropriate since the IC_{90} values do not account for the intracellular drug concentration of the cancer cells.

2. In the pivotal human pharmacokinetics studies, the method for assay of unchanged valrubicin and its metabolites (N-trifluoroacetyladrubicin, AD 41, and N-trifluoroacetyladrubicinol, AD 92) in human plasma and urine was adequately validated according to current bioanalytical method validation standards.

3. In healthy volunteers, the total recovery study after intravesicle instillation of valrubicin was adequately performed. The total recovery of drug related compounds within 24 hours was 99% of the administered dose (98.6% AD 32 and 0.4% AD 41). The majority of the administered dose was recovered in the instillate at approximately 2 hours after instillation. In serum, AD 32 is rapidly metabolized to AD 41 and AD 92 following intravenous administration. The results of the pharmacokinetic studies demonstrate that AD 32 is not entering the systemic circulation where it would be immediately metabolized to AD 41 and the amount of AD 41 in the urine would be expected to be greater than AD 32. Therefore, the systemic toxicity of AD 32 after intravesicle instillation can be considered to be negligible.

5.2 LABELING COMMENTS

6. SUMMARY

6.1. Introduction

AD 32 (N-trifluoroacetyl Adriamycin-14-valerate) is a semisynthetic derivative of the anthracycline antibiotic Adriamycin® (doxorubicin) developed at the [redacted] and initially tested in programs supported by the National Cancer Institute, National Institutes of Health. AD 32 differs from doxorubicin in having a 5-carbon aliphatic ester at the 14-carbinol position and a trifluoroacetyl substitution on the glycosidic amino group. These structural modifications make AD 32 highly lipid soluble, and result in important pharmacological differences from doxorubicin. In contrast to doxorubicin, which is transported slowly into cells and becomes localized in nuclei and on chromosomes, AD 32 rapidly traverses cell membranes and accumulates in the cytoplasm. In a number of animal tumor models, AD 32 has been shown to be therapeutically superior to doxorubicin and to produce less gastrointestinal toxicity, alopecia, and local tissue damage, and an apparent lack of cardiac toxicity. Leukopenia and thrombocytopenia were the dose-limiting toxicities associated with intravenous administration of AD 32. Initial studies in selected solid neoplasms indicated that AD 32 possesses anti-tumor activity against lung, breast, and urinary bladder cancer, soft tissue sarcoma, and transitional cell carcinoma of the renal pelvis.

Recent studies have demonstrated the therapeutic benefit of direct (intravesical) administration of chemotherapeutic agents into the bladder. The agents tested to date by this route of administration include doxorubicin, mitomycin C, bacillus Calmette-Guérin (BCG) vaccine, and interferon. Although there were some differences in the side effect profiles of these agents, intravesical administration appears to be ideal for the treatment of superficial bladder cancer because it maximizes tumor exposure while minimizing systemic exposure. However, prior trials with doxorubicin were limited by poor tissue penetration and a high rate of irritation and chemical cystitis.

Animal studies have shown that systemic absorption resulting from bladder instillation of AD 32 is less than 1%. In rats, AD 32 easily penetrated tumor masses, produced no local irritation, and exhibited no potential for systemic toxicity. Thus, AD 32 administered directly into the bladder could result in minimal systemic exposure while maximizing the effect at the site of action. The intravesical administration of AD 32 was not expected to cause any of the systemic side effects associated with the vehicles used with intravenous administration. Intravesical administration of AD 32 was investigated in a phase I/II study involving 32 patients with transitional cell carcinoma, most of whom (91%) were being treated for recurrent disease. A majority of the patients (72%) had undergone three or more prior transurethral resections of bladder (TURBs) for tumor removal, and most (78%) had previously received one or two courses of intravesical therapy, primarily bacillus Calmette-Guérin (BCG). The results showed that six weekly doses of AD 32 ranging from [redacted] mg were safe and well tolerated. Systemic exposure following intravesical instillation of the drug was negligible. Complete responses to one course of AD 32 treatment--defined as no evidence of disease for at least 12 months--occurred in 13 of the 32 patients (41%), including 2 of 7 patients (29%) who had

carcinoma in situ with or without papillary tumors at baseline.

6.2. Physical/Chemical Properties of AD 32

Two physically different drug products (lyophilized and liquid formulations) were used during pharmacokinetic studies (A91-0101) and (A92-1100), a lyophilized drug product, and a liquid drug product. Rest of the pharmacokinetic studies used a liquid drug product, which required dilution at the clinical site.

6.3. Intravesical Administration

6.3.1. Absorption

AD 32 in the nanogram/mL amounts were absorbed into the systemic circulation from an intact bladder after intravesical administration. The systemic absorption of AD 32 from the bladder into the bloodstream was dependent upon the condition of the bladder. When the bladder was perforated, microgram/mL quantities of AD 32 were found in the systemic circulation during and after intravesical administration of AD 32. Details on the systemic absorption of AD 32 for all of the intravesical studies are presented below.

In a clinical study reported as abstract [Israel et al], both AD 32 and AD 41 were detected in nanogram/mL concentrations in the serum, indicating that very small amounts of AD 32 were absorbed across the bladder wall into the systemic circulation following a single intravesical instillation of 400 or 600 mg of AD 32 (Appendix I, Fig. 1.) In this study, the median serum concentrations of AD 32 after the 200 mg dose appeared to be higher than for the other doses. This could be explained by the uncertainty of assay due to the estimations of values that are close to the limit of detection. In a clinical study reported as abstract [Chai et al], after an intravesical administration of doxorubicin hydrochloride (a drug closely related to AD 32), there was insignificant systemic exposure to the drug. There was a high target site (bladder) specificity and insignificant absorption, metabolism and/or degradation of the drug.

The quantity of AD 32 and its metabolites, AD 41 and AD 92, found in the serum during and after a two-hour, 800-mg, intravesical dose of AD 32 was dependent upon the extent of the transurethral resection of the bladder (TURB) (Appendix I, Fig. 2). Following a "typical" TURB, median serum concentrations of AD 41 were higher than AD 32 or AD 92 (Appendix I, Fig. 3). The highest median serum concentration of AD 41 and total anthracycline measured was 38 ng/mL and 48.5 ng/mL, respectively, at 6 hours after administration of an 800-mg intravesical dose of AD 32 to "typical" TURB patients. Following an "extensive" TURB without bladder perforation and a 800-mg intravesical dose of AD 32, the highest median serum concentration of AD 41 measured was 388 ng/mL and the highest median serum concentration of total anthracyclines measured was 460 ng/mL at 6 hours after dosing of seven participating patients (Appendix I, Fig. 4, Table 7). These results included two patients with markedly higher level of drug. The mean data for the rest 5 patients were slightly higher but otherwise similar with the group of patients with typical TURB. One patient had serum AD 41 and total

anthracyclines values of 436 ng/mL and 553 ng/mL, respectively, 6 hours after instillation. The highest concentration of total anthracyclines in the serum occurred after an 800-mg intravesical dose of AD 32 in the patient with a perforated bladder. The serum concentration of AD 41 and total anthracyclines in this patient were 2395 ng/mL and 2981 ng/mL, respectively, 6 hours after instillation of AD 32 (Appendix I, Fig. 5, Table 8).

The mean systemic exposure (absorption $AUC_{0-6 \text{ hours}}$) to AD 32 and its metabolites was also dependent upon the extent of the transurethral resection. The greatest systemic exposure to AD 32 and its metabolites was found in the patient with a perforated bladder. The unadjusted mean systemic exposure to total anthracyclines as measured by $AUC_{0-6 \text{ hours}}$ was 207 nmoles/L·hr following "typical" TURB and 1364 nmoles/L·hr following "extensive" TURB (7 patients). In the patient with a perforated bladder, systemic exposure to total anthracyclines as measured by $AUC_{0-6 \text{ hours}}$ was 18,382 nmoles/L·hr (Appendix I, Fig. 6, Table 9).

In the PK study report for (A91-0101), blood samples were obtained from the first 16 patients enrolled in this study for measurement of serum concentrations of AD 32 and its metabolites. Nanogram/mL concentrations of unmetabolized AD 32 and metabolites, AD 41 and AD 92, were found consistently in the serum after the first, third, and sixth intravesical doses of AD 32. The median total anthracycline concentration in serum was not more than 16.5 nmoles/L at doses of AD 32 up to 900 mg and the median serum concentration of AD 41 was higher than AD 32 or AD 92. In addition, the mean total anthracycline $AUC_{0-6 \text{ hours}}$ did not exceed 82 nmoles/L·hr at a dose of 200 mg of AD 32. At a dose of 600 mg of AD 32, the mean $AUC_{0-6 \text{ hours}}$ was 69.6 nmoles/L·hr. There was no apparent relationship between either number of doses or amount of AD 32 administered and the transurothelial absorption of the drug over a range of 200 to 900 mg of drug administered. The results of this study indicate that the transurothelial absorption of AD 32 at a dose of up to 900 mg is in the nanogram range and nanogram concentrations of AD 32 and metabolites are not sufficient to cause any serious drug-related systemic toxicity.

6.3.2. Distribution

Following a single intravesical instillation of 400 or 600 mg of valrubicin, both AD 32 and AD 41 were detected in nanogram/mL concentrations in the serum, indicating that very small amounts of AD 32 were absorbed across the bladder wall into the systemic circulation. A mean of 82% of total anthracyclines was recovered in the urine voided at the completion of the drug dwell time, with >99% being recovered as AD 32 and <1% being recovered as AD 41 (Appendix I, Table 3).

In PK study (A9305) the mean total anthracycline concentration in the excised bladder tissue samples decreased with increasing depth from the urothelial surface. The results were similar for samples taken from the right lateral, left lateral, and dome surfaces of the bladder wall. The anthracycline concentrations exceeded the 90% cytotoxic levels for human bladder cell lines to a depth of at least 1,250 μm , which exceeds the depth of invasion of T₁ lesions. At depths of 1,400 to 1,800 μm , which correspond to the depth of invasion of T₂ lesions, the anthracycline

concentrations closely approximated the clonogenic IC₉₀ range. The results of this study suggested that intravesically administered AD 32 in a single dose of 800 mg penetrates human bladder tissue to a depth sufficient for treatment of superficial bladder cancer.

6.3.3. Metabolism and Excretion

Both AD 32 and AD 41 were detected in nanogram/mL concentrations in the serum, indicating that very small amounts of AD 32 were absorbed across the bladder wall into the systemic circulation following a single intravesical instillation of 400 or 600 mg. In the clinical study reported in abstract 6.10.4.1. after an intravesical administration of doxorubicin hydrochloride (a drug closely related to AD 32), there was insignificant systemic exposure to the drug. There was a high target site (bladder) specificity and insignificant absorption, metabolism and/or degradation of the drug (Appendix I, Scheme 1).

Following intravesical instillation of 800 mg of AD 32, a mean of 99.0% (792 mg) was recovered in the urine within 24 hours, with 98.6% of the dose recovered as AD 32 and 0.4% of the dose recovered as AD 41 (Appendix I, Fig. 11, Tables 4, 5). The majority of the instilled dose was recovered in the instillate at approximately 2 hours after instillation. Only 3.1 mg of the 800 mg of AD 32 instilled into the bladder was metabolized to AD 41 after 24 hours. In serum, AD 32 is rapidly metabolized to AD 41 and AD 92 following intravenous administration. Therefore, the results of these studies demonstrate that AD 32 is not entering the systemic circulation since, systemically, it would be immediately metabolized to AD 41 and the amount of AD 41 in the urine would be expected to be greater than AD 32. When the mean values for urine collected from 6 patients after a total of 14 administrations of AD 32 were calculated, 99.0% of the 800 mg of AD 32 instilled in the bladder (792 mg) was recovered within 24 hours, with 98.6% of the dose recovered as AD 32 and 0.4% of the dose recovered as AD 41.

On average, 82% of total anthracyclines was recovered in the urine at the completion of the drug dwell time following a single 400 or 600 mg intravesical dose of AD 32, with >99% being recovered as AD 32 and <1% being recovered as AD 41.

After the intravesical administration of doxorubicin hydrochloride (a drug similar to AD 32), dilution of doxorubicin hydrochloride concentration in the urine was due mainly to urine production during treatment. There was significant intra- and interpatient variation in bladder emptying by catheterization with an average residual urine volume of 13 mL (range mL).

6.4. Intraperitoneal administration

In the PK report (92-1100), when 200 to 600 mg/m² of AD 32 were administered intraperitoneally to patients, milligram/mL amounts of AD 32 were found immediately in the peritoneal instillate (Appendix I, Fig. 8, Table 11). AD 32 was the predominant anthracycline detected in the peritoneal instillate samples through 24 hours after intraperitoneal administration of AD 32. Gradually, over a 24-hour period, the quantity of AD 32 in the peritoneal instillate decreased from approximately 800 to 35 micrograms/mL after a 600 mg/m² intraperitoneal dose

of AD 32 (Appendix I, Fig. 9, Table 12). Microgram/mL amounts of AD 32 were absorbed into the systemic circulation. AD 32 absorbed into the bloodstream rapidly and completely metabolized to AD 41, AD 92, AD 48 and ADR. AD 41 was the predominant anthracycline detected in serum samples through 24 hours after intraperitoneal administration of AD 32 (Appendix I, Fig. 7, Table 10). Peak serum concentrations of AD 32 occurred 4 hours after instillation while peak serum concentrations of AD 41, AD 92, and AD 48 occurred 12 hours after intraperitoneal instillation of AD 32. The median peak concentration of total anthracyclines in the peritoneal instillate after a 600 mg/m² dose of AD 32 was 1106 µmol/L. The median peak concentration of total anthracyclines in the serum after a 600-mg/m² dose of AD 32 was 4.3 µmol/L

In PK study 6.9.2.1. (A92-1100), up to 7.4 % of a 600-mg/m² intraperitoneal dose of AD 32 was excreted in the urine as total anthracyclines in a 24-hour period after dosing. The mean amounts of anthracyclines excreted in the 24-hour urine collection after a 600-mg/m² dose of AD 32 were 17.8 mg of AD 41, 12.4 mg of AD 92 and 0.3 mg of AD 32. AD 41 was the predominant anthracycline detected in the urine.

6.5. Intravenous Administration

In a clinical study reported as abstract 6.10.2.1., plateau levels of AD 32 in the plasma were reached within 1 - 2 hours after the initiation of infusion into the systemic circulation. AD 32 rapidly disappeared from the plasma after infusion was complete. AD 32 was metabolized to AD 41 and AD 92 in the plasma. After injection of 20 mg/kg of AD 32, the plasma concentration showed a biphasic decline decreasing from 20 µg/mL at 2.5 minutes to barely detectable levels at 4 hours. Plasma levels of the principal metabolite, AD 41, were 14 µg/mL at 2.5 minutes and declined gradually thereafter but persisted throughout the study (Appendix I, Fig. 10). Lower levels of the metabolite, AD 92, were detectable at the early sampling times (4 µg/mL) but then declined to approximately the same levels as AD 32. Total anthracycline fluorescence in the plasma (AD 32 + AD 41 + AD 92 + other metabolites) declined from 40 µg/mL at 2.5 minutes to 1.6 µg/mL at 6 hours. The mean half-life for distribution of the total fluorescence was calculated to be 9 minutes and the mean half-life for elimination was calculated to be 99 minutes. Using the trapezoidal rule, the total area under the concentration-time curve (AUC_{0-6 hours}) was calculated to be 11,975 nmoles/L•hr.

After the AD 32 dose (100 mg/m²) administered as IV infusion over 24 hours, urinary anthracycline excretion within 26 hours after the initiation of infusion accounted for 4 - 9% of mostly as AD 41 and AD 92. After the IV infusion over 24 hours of AD 32 at the 400 mg/m² dose 33% of the total administered dose of AD 32 accumulated in the bile over 36 hours. Twelve and one-half (12.5) percent of the total administered dose of AD 32 accumulated in the urine over 36 hours. The principal metabolites found in bile were AD 92 (70%) and AD 41 (17%). Other metabolites found in the bile were doxorubicin hydrochloride (4%), adriamycinol (4%) and polar metabolites (4%). Unchanged AD 32 was not detected. The principal metabolites found in the urine were AD 92 (50%) and AD 41 (43%). The hepatobiliary system was the major metabolic and excretory pathway for AD 32 following intravenous administration.

Cumulative excretion of AD 32 metabolites over 36 hours in the biliary and urinary systems accounted for almost 50% of the total dose of AD 32 administered.

6.6. Summary

The concentration of AD 32 and its metabolites absorbed into the serum was dependent upon the route of administration and the condition of the bladder. After intravesical administration of AD 32 only nanogram/mL quantities of anthracyclines were detected in the serum of patients with intact bladders. If the bladder was perforated microgram/mL quantities were absorbed into the systemic circulation. After intraperitoneal and intravenous administration of AD 32, microgram/mL quantities of anthracyclines were also detected in the serum. AD 41 was the predominate anthracycline metabolite in serum. Table 1 shows the median peak concentrations of total anthracyclines and AD 41, and figures 1 and 2 show the peak concentrations of total anthracyclines and AD 41 absorbed into the systemic circulation after intravesical, intraperitoneal and intravenous administration.

Table 1. Median Serum Concentration of Total Anthracyclines and AD 41 Six Hours After Administration of the Highest Dose of AD 32

Study No. Route of Administration	6.10.1.1. IVe	6.10.3.1. IVe	6.10.4.1. IVe	6.9.1.3. IVe	6.9.1.2. IVe	6.9.2.1. IP	6.10.2.1 IV
Total Anthracyclines (nmoles/L)	10	14	†	48.5‡	14.1	1106	20000
AD 41 (ng/mL)	7	N.A.	N.A.	38	6.5	1007	14000

N.A. = Not available; † Value = 0.4 nmoles/mL; ‡ This value is in ng/mL

Figure 1. Median Serum Concentration of AD 41 Six Hours After Administration the Highest Dose of AD 32

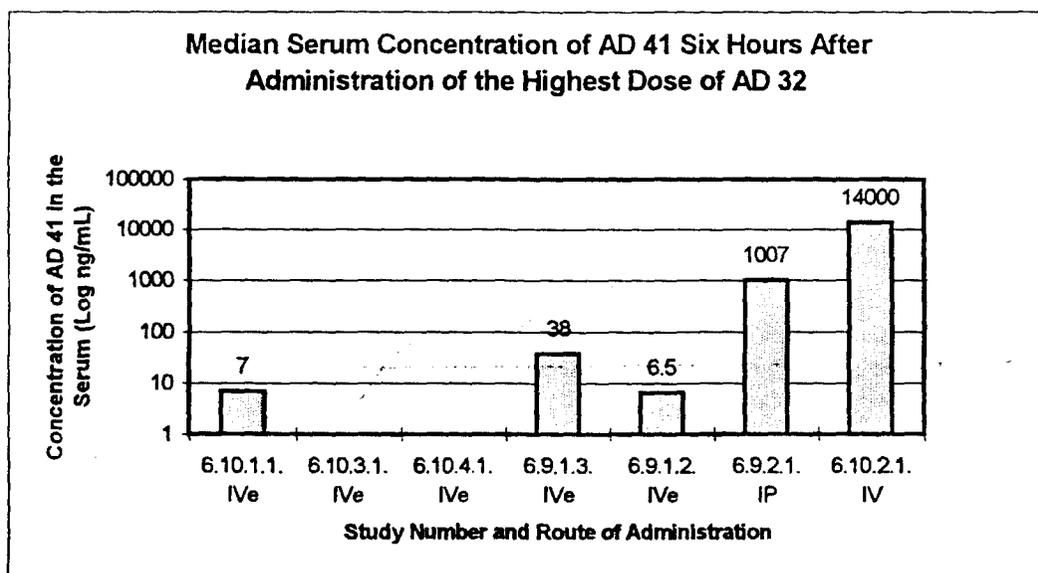
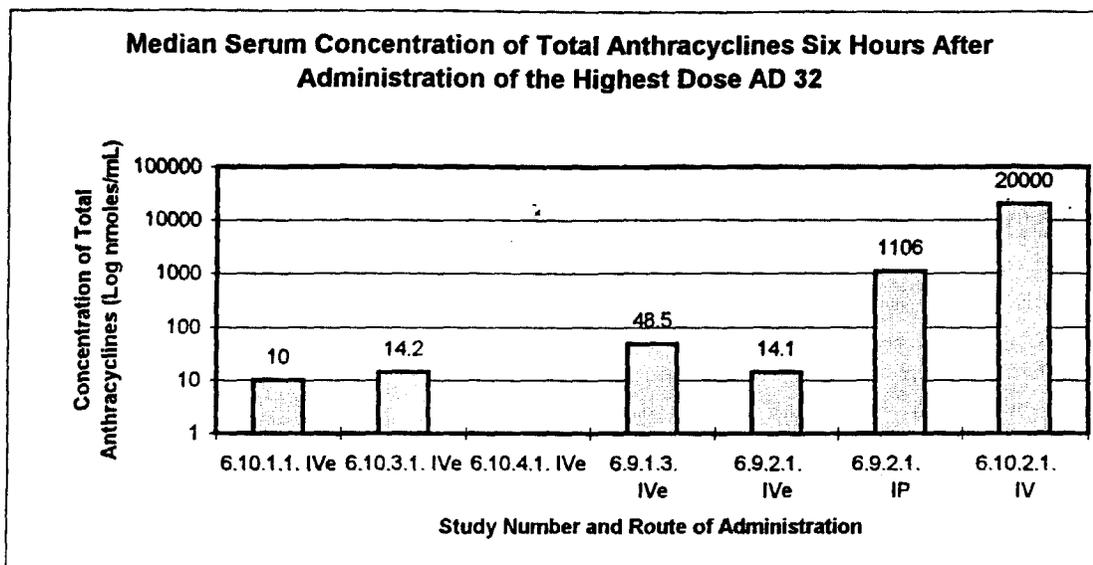


Figure 2. Median Serum Concentration of Total Anthracyclines Six Hours after Administration of the Highest Dose of AD 32



6.7. Special Populations - Pharmacokinetics of AD 32 in Patients Dosed with AD 32 Immediately After Trans-Urethral Resection of the Bladder

The quantity of AD 32 and its metabolites, AD 41 and AD 92, found in the blood during and after a two-hour, 800-mg, intravesical dose of AD 32 was dependent upon the extent of the transurethral resection. Following a "typical" TURB, median serum concentrations of AD 41 were higher than AD 32 or AD 92. The highest median serum concentration of AD 41 and total anthracycline measured was 38 ng/mL and 48.5 ng/mL, respectively, at 6 hours after administration of an 800-mg intravesical dose of AD 32. Following an "extensive" TURB without bladder perforation and a 800-mg intravesical dose of AD 32, the peak median serum concentration of AD 41 measured 388 ng/mL and the peak median serum concentration of total anthracyclines measured 460 ng/mL at 6 hours after dosing. These concentrations included one patient who had AD 41 and total anthracyclines values of 436 ng/mL and 553 ng/mL, 6 hours after instillation, respectively. The highest concentration of total anthracyclines in the serum occurred after an 800-mg intravesical dose of AD 32 in the patient with a perforated bladder. The serum concentration of AD 41 and total anthracyclines in this patient was 2395 ng/mL and 2981 ng/mL, respectively at 6 hours after instillation of AD 32.

The mean systemic exposure ($AUC_{0-6 \text{ hours}}$) to AD 32 and its metabolites was also somewhat dependent upon the extent of the transurethral resection. The greatest systemic exposure to AD 32 and its metabolites was found in the patient with a perforated bladder. The unadjusted mean systemic exposure to total anthracyclines as measured by $AUC_{0-6 \text{ hours}}$ was 207 nmoles/L·hr

following "typical" TURB and 1364 nmoles/L·hr following "extensive" TURB (7 patients). In the patient with a perforated bladder, systemic exposure to total anthracyclines as measured by $AUC_{0-6 \text{ hours}}$ was 18,382 nmoles/L·hr.

There was no significant difference in the mean systemic exposure to AD 32 and its metabolites between the "typical" and "extensive" TURB groups (5 patients) if the two patients who had unusually high serum concentrations of anthracyclines were excluded from the analysis. When the mean systemic exposure of the "extensive" TURB patients (5 patients) and the 14 "typical" TURB patients were combined the systemic exposure was only 2.4% of the systemic exposure of the perforated bladder patient. When the mean systemic exposure of the "typical" TURB patients alone was calculated, it was 2.5% of the total systemic exposure of the perforated bladder patient. This suggested the increased exposure in the "extensive" TURB group (7 patients) was probably due to the inclusion of two patients with unusually high serum levels of anthracyclines.

The peri-TURB doses of AD 32 administered in this study (400 to 800 mg) were well tolerated. A majority of the patients experienced some type of irritative bladder symptoms after the AD 32 was removed from the bladder, but most of the symptoms were mild or moderate and required no treatment. There was little evidence of a dose relationship for the occurrence of bladder symptoms or of an effect of AD 32 retention time or time between TURB and instillation. With one exception, no systemic effects attributable to AD 32 were reported.

A patient who received a peri-TURB dose of AD 32 and was later found to have had bladder perforation during the TURB had systemic exposure to the drug via extraperitoneal extravasation of the dose. This patient had the highest serum anthracycline levels measured in the study. The systemic exposure to total serum anthracyclines as measured by the $AUC_{0-6 \text{ hours}}$ was approximately 88 times higher in this patient (18,382 nmoles/L·hr) than in the patients who underwent "typical" TURBs. This patient with a perforated bladder developed Grade 4 leukopenia approximately 2 weeks after receiving AD 32.

Four patients (18%) had adverse events that were rated serious by the investigators. The only events that were considered to be related to AD 32 were post-infusion contact dermatitis in a patient who received three weekly doses, and leukopenia in the patient who had bladder perforation.

No patients died during the treatment period or within 30 days of receiving AD 32. The four patients who are known to have died subsequently had received AD 32 more than 4 months before their deaths and died of reasons unrelated to drug administration or to bladder cancer.

The quantity of AD 32 and its metabolites, AD 41 and AD 92, found in the blood and the mean systemic exposure ($AUC_{0-6 \text{ hours}}$) to AD 32 and its metabolites after a 2-hour, 800-mg intravesical dose of AD 32 administered immediately after TURB was dependent upon the extent of the transurethral resection.

Serum concentrations of AD 41 were higher than AD 32 or AD 92, indicating that AD 32 absorbed into the serum was immediately metabolized to AD 41 and then to AD 92. There was no significant difference in the mean systemic exposure to AD 32 and its metabolites between the "typical" TURB group and the "extensive" TURB group.

The patient with a perforated bladder experienced a serious drug-related adverse event (leukopenia) due to systemic exposure to high concentrations of AD 32 and its metabolites ($AUC_{0-6 \text{ hours}}$: 18,382 nmoles/L·hr). Systemic exposure to a mean concentration of total anthracyclines less than or equal to 1364 nmoles/L·hr did not cause any systemic drug-related serious adverse events.

The adjunctive administration of AD 32 immediately after TURB of papillary bladder tumors was feasible. A dose of 800 mg (an optimal therapeutic dose) was well tolerated in this setting. The extent of transurothelial absorption of AD 32 was not clinically significant in patients with "typical" and "extensive" TURBs without bladder perforation and did not cause any systemic drug-related serious adverse events. Systemic exposure to AD 32 in the patient with the perforated bladder resulted in serious drug-related, reversible, systemic toxicity.

APPEARS THIS WAY
ON ORIGINAL

ATTACHMENT II
INDIVIDUAL STUDY SYNIPSES

SYNOPSIS

<u>NAME OF SPONSOR/COMPANY:</u> Antra Pharmaceuticals, Inc.	<u>INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER</u>	<u>(FOR NATIONAL AUTHORITY USE ONLY)</u>
<u>NAME OF FINISHED PRODUCT:</u> AD 32	Volume:	
<u>NAME OF ACTIVE INGREDIENT(S):</u> N-trifluoroacetyl-diamycin-14-valerate	Page:	
Protocol Nos. and Titles of Studies: A9301/A9302 - Intravesical AD 32 in Patients With Carcinoma In Situ of the Bladder Who Have Failed or Have Recurrence Following Treatment With BCG; A9303 - A Phase II Study (A9303): Intravesical AD 32 in Patients With Transitional Cell Carcinoma of the Bladder		
Investigators and Study Center(s): These studies were conducted by 63 investigators at 61 study sites. The protocol chairmen were Dr. Robert Bahnsen, University of Pittsburgh, Pittsburgh, PA (A9301), Dr. Stanley Brosman, Santa Monica Urologic Group, Santa Monica, CA (A9302), and Dr. Richard Greenberg, Fox Chase Cancer Center, Philadelphia, PA (A9303).		
Publication (Reference): None		
Studied Period (years): 18 November 1993 to 30 April 1997 (Study A9301/A9302); 7 March 1994 to 30 April 1997 (Study A9303)		Phase of development: II/III
Objectives: To evaluate the efficacy and safety of intravesical instillations of AD 32 in patients with carcinoma in situ (CIS) who had previously been treated with intravesical bacillus Calmette-Guérin (BCG) for CIS and in whom recurrence or failure had occurred after multiple courses of intravesical treatment (Study A9301/A9302) and in patients with CIS who had either failed previous treatment with one course of BCG or not completed a prior course of BCG treatment, or in whom BCG was contraindicated (Study A9303); and to determine the concentration of anthracyclines in voided urine.		
Methodology: These were open-label, noncomparative studies. Each patient was to receive six weekly intravesical administrations of 800 mg of AD 32; a subgroup of patients received three additional doses for a total of nine intravesical administrations of AD 32. At their discretion, patients could participate in a urine recovery study, in which anthracycline concentrations were determined in urine samples obtained during the 24-hour period following dose administration. The primary disease evaluation (PDE) occurred approximately 6 weeks after the last instillation of AD 32 (approximately 3 months after the start of treatment). Subsequent evaluations for disease response occurred at 3-month intervals until the patient had a recurrence. Information about disease status was obtained approximately every 6 months for patients who did not respond to treatment or who had recurrences.		
Number of Subjects (planned and analyzed): The planned enrollment was at least 90 patients in each study; this interim report presents the results for the first 90 patients enrolled in Study A9301/ A9302 and the first 80 patients enrolled in Study A9303. Six of the 170 patients had urine recovery assessments.		
Diagnosis and Main Criteria for Inclusion: Patients in Study A9301/A9302 were required to have pathologically proven CIS with no evidence of muscle invasive disease and must have received at least two prior courses of intravesical therapy for CIS. One of the prior therapies must have been BCG. Patients could have either failed BCG therapy or had a recurrence after responding to BCG. Patients in Study A9303 were required to have pathologically proven CIS and to meet the criteria for one of the following prior treatment subgroups: Subgroup 1- patients who had undergone one 6-week course of BCG intravesical therapy for CIS with outcomes of failure or recurrence, and no subsequent treatment; Subgroup 2 - patients who had been unable to complete a 6-week course of BCG therapy due to toxicity; Subgroup 3 - patients who had never been treated with BCG because of contraindications. Patients in Subgroup 1 were randomly assigned to receive six or nine doses of AD 32. All other patients received six doses.		

SYNOPSIS (Continued)

<u>NAME OF SPONSOR/COMPANY:</u> Anthra Pharmaceuticals, Inc.	<u>INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER</u> Volume:	<u>(FOR NATIONAL AUTHORITY USE ONLY)</u>
<u>NAME OF FINISHED PRODUCT:</u> AD 32	Page:	
<u>NAME OF ACTIVE INGREDIENT(S):</u> N-trifluoroacetyladiamycin-14-valerate		
Test Product, Dose and Mode of Administration, Batch No.: AD 32 (Lot Numbers 515-44-0001, 515-44-0002, and 515-44-0003) was supplied in 5 mL vials containing 40 mg/mL AD 32 in NCI Diluent 12. For each 800 mg dose, four vials were further diluted with 55 mL sterile saline to a total instillate volume of 75 mL. The AD 32 solution was instilled by gravity flow via a catheter, and the patients were asked to retain the instillate for 2 hours.		
Duration of Treatment: One hundred sixty-two of the 170 patients enrolled in the two studies received six to nine doses of AD 32.		
Reference Therapy, Dose and Mode of Administration, Batch No.: None		
Criteria for Evaluation: Safety was assessed by monitoring the occurrence of adverse events and by evaluating changes in bladder symptoms, clinical laboratory tests, ECGs, and physical examinations. The risk of salvage therapy with AD 32 was assessed by determining the pathologic disease stage in patients who had cystectomies.		
Statistical Methods: Because this was an open-label study of a single dose level, the statistics were mainly descriptive, ie, means, medians, and distributions.		
Pharmacokinetic Parameters: Fourteen urine samples for the recovery studies were collected from six patients during the study. The total quantities of AD 32 and metabolites or total anthracyclines recovered in urine samples were calculated by summing the quantities of AD 32 and metabolites or total anthracyclines in the urine (mg/mL) and multiplying by the total volume (milliliters) of urine collected. Means for AD 32 and metabolites or total anthracyclines were calculated by summing the individual samples and dividing by the total number of samples. The mean percent recovery of AD 32 and metabolites or total anthracyclines in the urine was calculated by dividing the mean amount of AD 32 and metabolites or total anthracyclines recovered in the urine by the amount of AD 32 in the instilled dose and multiplying by 100.		
SUMMARY - CONCLUSIONS		
<p>This is an interim report presenting results to date for the first 170 patients enrolled in ongoing studies. Enrollment and follow-up of patients are continuing, and data collection and clarification are in progress. Therefore, safety data and information in this report are preliminary. However, the urine recovery data and information in the pharmacokinetic portion of the studies (Section 6.0) are final.</p>		
<p>Patients: A majority of the 170 patients were males, white, and either current or past smokers. Most patients were 60 years of age or older. Six of the 170 patients had urine recovery assessments after receiving 14 doses of AD 32.</p>		
<p>Safety: In both study, 40% to 50% of the patients had adverse events that qualified as local bladder symptoms at study entry. Those were also the most commonly reported adverse events during treatment. Urinary frequency, dysuria, and urinary urgency were the most common specific symptoms. The most commonly reported nonlocal adverse events were urinary tract infection, other urinary tract problems, and asthenia. Nineteen patients had serious adverse events and 20 patients died between 1 month and 2.5 years after receiving AD 32. One patient had serious adverse events (azotemia and reflux nephropathy) that were considered possibly related to AD 32; this patient had previously developed reflux nephropathy after receiving BCG. None of the remaining serious adverse events or deaths were related to AD 32. Intravesical administration of AD 32 had no effect on laboratory tests, ECGs, or physical examinations.</p>		

SYNOPSIS (Continued)

NAME OF SPONSOR/COMPANY: Anthra Pharmaceuticals, Inc.	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER	(FOR NATIONAL AUTHORITY USE ONLY)
NAME OF FINISHED PRODUCT: AD 32	Volume:	
NAME OF ACTIVE INGREDIENT(S): N-trifluoroacetyladiamycin-14-valerate	Page:	

Safety (continued): Thirty-four patients in Study A9301/A9302 and 20 patients in A9303 who did not respond to AD 32 or who had recurrences underwent cystectomy between 4 and 24 months after the initiation of therapy. As shown in the tables below, the risk of progression of disease due to attempting salvage therapy with AD 32 was not significant compared to the known rate of clinical understaging of disease in patients with refractory superficial disease undergoing cystectomy.

Clinical Stage at Baseline Versus Pathologic Stage at Cystectomy - A9301/A9302

		Pathologic Stage at Cystectomy								
		pT ₀	pT _a	pT _{is}	pT ₁	pT ₁ /pT _{is}	pT ₂	pT _{3b}	pT _{3b} /pT _{is}	NAV
Clinical	T _a /T _{is}	0	0	4	0	1	0	0	0	0
Stage at	T _{is}	4	1	13	2	1	2	0	1 ^a	2
Baseline	T ₁ /T _{is}	0	0	0	0	1	1	1	0	0

NAV = not available.

^a Patient had Stage pT_{3b} squamous cell disease and CIS.

Clinical Stage at Baseline Versus Pathologic Stage at Cystectomy - A9303

		Pathologic Stage at Cystectomy								
		pT ₀	pT _a	pT _{is}	pT ₁	pT ₁ /pT _{is}	pT ₂ /pT _{is}	pT _{3b}	pT _{3b} /pT _{is}	pT _{4a}
Clinical	T _a /T _{is}	0	0	1	0	1	2	0	0	0
Stage at	T _{is}	0	1	3	1	1	1	0	2	1
Baseline	T ₁ /T _{is}	1	0	2	0	1	0	1	0	1

Pharmacokinetics: Following intravesical instillation of 800 mg of AD 32, a mean of 99.0% (792 mg of the 800-mg dose) was recovered in the urine within 24 hours as 98.6% (789-mg of 800-mg dose) AD 32 and 0.4% (3.1-mg of 800-mg dose) AD 41. The majority of the instilled dose was recovered in the instillate at approximately 2 hours after instillation. Only 3.1 mg of the 800 mg of AD 32 instilled into the bladder was metabolized to AD 41 after 24 hours.

In serum, AD 32 is rapidly metabolized to AD 41 and AD 92 following intravenous administration (Appendix II).⁵ Therefore, the results of these studies demonstrate that AD 32 is not entering the systemic circulation since, systemically, it would be immediately metabolized to AD 41 and the amount of AD 41 in the urine would be expected to be greater than AD 32. Except for one patient who had serious adverse events (azotemia and reflux nephropathy) that were considered related to AD 32, no other serious systemic adverse events attributable to AD 32 were observed.

Conclusions:

Safety: The safety results from the first 170 patients enrolled in these ongoing studies revealed that the toxicity of AD 32 was acceptable. The most commonly encountered adverse effects (local bladder symptoms) were tolerable to most patients. Among those who eventually undergo cystectomy, the attempted salvage treatment did not increase their risk of disease progression. Both adverse events data and urine recovery results suggest that absorption of AD 32 from the bladder is negligible.

Pharmacokinetics: When the mean values for urine collected from 14 administrations of AD 32 were calculated, 99.0% of the 800 mg of AD 32 instilled in the bladder (792 mg) was recovered within 24 hours as 98.6% AD 32 and 0.4 % AD 41. Myelosuppression, the dose-limiting toxicity of AD 32 in the Phase I studies, was not reported in any of the patients enrolled in these studies, which indicates clinically insignificant systemic absorption of AD 32 following intravesical administration.

SYNOPSIS

<p>NAME OF SPONSOR/COMPANY: Antra Pharmaceuticals, Inc.</p> <p>NAME OF FINISHED PRODUCT: AD 32</p> <p>NAME OF ACTIVE INGREDIENT(S): N-trifluoroacetyladiamycin-14-valerate</p>	<p>INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER</p> <p>Volume:</p> <p>Page:</p>	<p>(FOR NATIONAL AUTHORITY USE ONLY)</p>
<p>Protocol No.: A91-0101 Title of Study: A Multicenter Phase I Evaluation of AD 32 Administered by Intravesical Instillation in Patients with Superficial Transitional Cell Carcinoma of the Urinary Bladder.</p>		
<p>Investigators and Study Centers: Nine investigators at eight study centers. Principal Investigator: Peter O'Dwyer, Fox Chase Cancer Center</p>		
<p>Publications (References):</p> <ol style="list-style-type: none"> 1. Sweatman TW, Payne C, Israel M, Gianantonio B, O'Dwyer P, Greenberg R, Bamberger M, Bellingham C, Edson M, Wood D. Clinical pharmacology of intravesical (IVe) N-trifluoroacetyladiamycin-14-valerate (AD 32) [abstract]. Proc ASCO 1993;12:162. 2. Greenberg R, O'Dwyer P, Patterson L, Bahnson R, Edson M, Wood D, Bellingham C, Childs S, Steinberg G, Bamberger M, Sweatman T, Israel M. Intravesical AD 32 (N-trifluoroacetyladiamycin-14-valerate) in the treatment of patients with refractory bladder carcinoma - clinical efficacy, pharmacology, and safety [abstract]. Proc Amer Urol Assoc 1995;153:233A. 3. Greenberg RE, Bahnson RR, Wood D, Childs SJ, Bellingham C, Edson M, Bamberger MH, Steinberg GD, Israel M, Sweatman T, Gianantonio B, O'Dwyer P. Initial report on intravesical administration of N-trifluoroacetyladiamycin-14-valerate (AD 32) to patients with refractory superficial transitional cell carcinoma of the urinary bladder. Urology 1997;49:471-5. 		
<p>Studied Period (years): 27 January 1992 to 22 June 1993</p>	<p>Phase of development: I</p>	
<p>Objectives: The primary objectives were to determine the safety and tolerance of AD 32 given by intravesical administration to patients with urinary bladder cancer, to define dose-limiting toxicity, and to determine the extent of systemic absorption.</p>		
<p>Methodology: This was an open-label, ascending-dose study. Each patient was to receive six doses of AD 32 at weekly intervals. The dose for the first three patients was 200 mg, and subsequent groups of three patients each were to receive incrementally higher doses only if no dose-limiting toxicity occurred with the previous dose. Blood samples were collected before and at intervals up to 6 hours after the first, third, and sixth doses.</p>		
<p>Number of Subjects (planned and analyzed): The planned maximum enrollment was 24 patients. The actual enrollment was 32 patients.</p>		
<p>Diagnosis and Main Criteria for Inclusion: All patients were to have transitional cell carcinoma of the urinary bladder with a stage of T_{CS}, T_a, or T₁, confirmed by positive cytology and to be recovered from previous transurethral resections, chemotherapy, radiotherapy, and immunotherapy. Patients with CIS had to have recurrent disease after treatment with bacillus Calmette-Guérin (BCG). All patients had to have adequate bone marrow, hepatic, and renal function and no serious cardiovascular conditions.</p>		
<p>Test Product, Dose and Mode of Administration, Batch No.: AD 32 (Lot Numbers 515090001 and 515090002) was initially supplied as a lyophilized product (200 mg/vial). Separate vials contained 5 mL of the diluent (NCI Diluent 12: 50% Cremophor EL®, 50% ethanol). To allow for complete solubilization, the higher doses of AD 32 could be dissolved in an additional volume of absolute alcohol before addition of the diluent. The drug was reformulated during the study, and the 800 mg dose was supplied as a liquid containing 40 mg/mL AD 32 in NCI Diluent 12 (Lot Number 515440001). This formulation contained the same concentration of drug and diluent as the original reconstituted lyophilized product.</p>		

SYNOPSIS (Continued)

NAME OF SPONSOR/COMPANY: Anthra Pharmaceuticals, Inc.	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER	(FOR NATIONAL AUTHORITY USE ONLY)
NAME OF FINISHED PRODUCT: AD 32	Volume:	
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<p>Test Product, Dose and Mode of Administration, Batch No. (Continued): With both the reconstituted product and the liquid product, an appropriate volume of sterile physiologic saline was added to yield a total volume of 75 mL. This volume was instilled intravesically through a catheter by gravity flow. The catheter was then removed and the patient was asked to retain the drug for 2 hours before voiding.</p> <p>The doses administered in this study were 200 mg (3 patients), 400 mg (10 patients), 600 mg (4 patients), 800 mg (6 patients), and 900 mg (5 patients). Four additional patients began treatment with 900 mg but had their doses reduced to 750 mg because of toxicity.</p>		
<p>Duration of Treatment: Thirty-two patients received one course of six weekly instillations of AD 32 at doses ranging from 200 to 900 mg. One of the patients received a second course of treatment, and another patient received two additional courses. Long-term follow-up of the patients ranged from 12 to 38.5 months.</p>		
<p>Criteria for Evaluation: Safety and tolerance were assessed by evaluating the occurrence and severity of adverse events and by evaluating changes in clinical laboratory tests, vital signs, and physical examinations. Systemic absorption of AD 32 was evaluated by analyzing blood samples for concentrations of AD 32 and its metabolites using reversed-phase, high-performance liquid chromatography.</p>		
<p>Pharmacokinetic Parameters: Median values for serum concentrations of AD 32 and metabolites were calculated for each point in time. AUCs were calculated in nanomoles using the area under the serum concentration time curve from 0 to the time of the last measurable serum concentration.</p>		
<p>SUMMARY - CONCLUSIONS</p> <p>PHARMACOKINETICS</p> <p>Blood samples were obtained from the first 16 patients enrolled for measurement of serum concentrations of AD 32 and its metabolites. Nanogram concentrations of unmetabolized AD 32 and metabolites, AD 41 and AD 92, were found consistently in the serum after the first, third, and sixth intravesical doses of AD 32. The median total anthracycline concentration in serum was not more than 16.5 nmoles/L at doses of AD 32 up to 900 mg and the median serum concentration of AD 41 was higher than AD 32 or AD 92. In addition, the mean total anthracycline AUC_{0-6 hours} did not exceed 82 nmoles/L·hr at a dose of 200 mg of AD 32. At a dose of 600 mg of AD 32, the mean AUC_{0-6 hours} was 69.6 nmoles/L·hr. There was no apparent relationship between either number of doses or amount of AD 32 administered and the transurothelial absorption of the drug over a range of 200 to 900 mg of drug administered. These results showed that systemic absorption of AD 32 was in nanogram amounts following intravesical administration.</p> <p>It was not possible to determine if a correlation existed between the serum concentrations observed at the higher doses of AD 32 and ethanol concentrations of $\geq 15\%$ in the formulation.</p>		

SYNOPSIS (Continued)

<p><u>NAME OF SPONSOR/COMPANY:</u> Antra Pharmaceuticals, Inc.</p> <p><u>NAME OF FINISHED PRODUCT:</u> AD 32</p> <p><u>NAME OF ACTIVE INGREDIENT(S):</u> N-trifluoroacetyladiamycin-14-valerate</p>	<p><u>INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER</u></p> <p>Volume:</p> <p>Page:</p>	<p><u>(FOR NATIONAL AUTHORITY USE ONLY)</u></p>
<p>SUMMARY - CONCLUSIONS (CONTINUED)</p> <p>SAFETY</p> <p>The results of the safety analysis supported the conclusion concerning minimal systemic exposure following intravesical administration of AD 32. Systemic signs and symptoms were uncommon, relatively minor, and resolved without treatment. Twenty-nine of the 32 patients (91%) had adverse events. The most common adverse events were localized to the lower urinary tract and consisted of irritative effects on the bladder and urethra leading to dysuria, frequency, and urgency. These symptoms persisted for several days after a dose was administered but had usually resolved before the next dose was given. Five patients had interruptions in their treatment regimens because of these symptoms, and four required reductions in dose from 900 to 750 mg.</p> <p>All of the patients who received 900 mg doses containing 21% ethanol required reductions in dose, whereas dose reduction was not required when patients received 900 mg doses containing 15% ethanol. The patients who received doses containing <15% ethanol (regardless of AD 32 concentration) had a markedly lower incidence of irritative symptoms and fewer Grade 2 (moderate) and Grade 3 (severe) symptoms than did the group who received doses containing \geq15% ethanol. For example, among the 23 patients who received doses containing <15% ethanol, the rates of dysuria, frequency, and urgency were 39%, 26%, and 13%. The corresponding rates among the 9 patients who received doses containing \geq15% ethanol were 89%, 44%, and 22%. Four of 23 patients (17%) in the "low" ethanol concentration group had Grade 2 or 3 irritative symptoms, compared with 6 of 9 (67%) in the "high" ethanol concentration group. In addition, individual patients who received doses containing the same level of AD 32 but different levels of ethanol generally had fewer irritative symptoms and less severe symptoms as the alcohol content decreased. These findings suggest that the frequency and grade of irritative bladder symptoms were primarily related to the ethanol concentration of the doses. The 800 mg dose (13.3% ethanol) was the highest dose of AD 32 with an ethanol concentration of <15%. Therefore, this dose was considered to be the maximum tolerated dose and was selected for use in all subsequent clinical studies of intravesical administration of AD 32.</p> <p>No evidence of cardiotoxicity was observed. No patient experienced a serious adverse event that was related to AD 32, and there were no deaths during the study. Two patients died during long-term follow-up, 11 months and 19 months after receiving their last doses of AD 32. Neither of these deaths was considered to be related to study drug administration. There were no clinically significant changes in laboratory test results, vital signs, or physical examination for any patients.</p> <p>OVERALL CONCLUSIONS</p> <p>The results of this study indicate that the transurothelial absorption of AD 32 at a dose of up to 900 mg is in the nanogram range and nanogram concentrations of AD 32 and metabolites are not sufficient to cause any serious drug-related systemic toxicity.</p>		

SYNOPSIS

<u>NAME OF SPONSOR/COMPANY:</u> Anthra Pharmaceuticals, Inc. <u>NAME OF FINISHED PRODUCT:</u> AD 32 <u>NAME OF ACTIVE INGREDIENT(S):</u> N-Trifluoroacetyladiamycin-14-valerate	<u>INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER</u> Volume: Page:	<u>(FOR NATIONAL AUTHORITY USE ONLY)</u>
Protocol No.: A9501 Title of Study: Pilot Study of Intravesical AD 32 Administered Immediately Following Transurethral Resection in Patients With Superficial Transitional Cell Carcinoma of the Bladder		
Investigators and Study Centers: R. Greenberg, Fox Chase Cancer Center, Philadelphia, PA; A.L. Patterson, VA Medical Center, Memphis, TN; W.L. Weems, Mississippi Urology Clinic, Jackson, MS		
Publication (Reference): None		
Studied Period (years): 24 July 1995 to 13 March 1996	Phase of development: I/II	
Objectives: To assess the safety and tolerance, extent of systemic absorption, technical feasibility, and optimal dose of intravesical AD 32 administered immediately after transurethral resection of papillary bladder tumors (TURB).		
Methodology: This was a pilot study in patients with transitional cell carcinoma of the bladder. Each patient was to receive a single dose of AD 32 immediately after undergoing TURB of papillary bladder tumors. The first 3 patients were to receive peri-TURB doses of 400 mg. If none of those patients developed dose-limiting toxicity during an observation period of at least 7 days, the next 3 patients were to receive peri-TURB doses of 600 mg. If none of those patients developed dose-limiting toxicity during an observation period of at least 7 days, the next 3 patients were to receive peri-TURB doses of 800 mg. Up to 15 more patients could receive peri-TURB doses of 800 mg. Patients with Stage T _{is} or T ₁ disease, or with Grade 3 tumors, could receive five additional doses of 800 mg at weekly intervals at the discretion of the investigator. Blood samples (10 mL) for determining the serum concentrations of AD 32 and its metabolites were to be obtained immediately before TURB (baseline) and at 0.5, 1, 2, 4, and 6 hours after AD 32 administration. Additional samples were to be obtained at 8, 12, and 24 hours when the investigator thought that the patient was at an increased risk for extravasation due to the extent of the TURB. Serum samples were analyzed using reversed-phase high-performance liquid chromatography with flow fluorescence detection. The sponsor's medical officers retrospectively classified patients as having had "extensive" resections if a review of their surgical reports revealed evidence of extravasation, denuding or fulguration of a surface area of > 3 cm ² , more than 5 tumors resected and / or sites biopsied, or qualitative comments suggesting that a "considerable amount" of tissue had been removed or fulgurated.		
Number of Subjects (planned and analyzed): The planned enrollment was up to 24 patients. The actual enrollment was 21 patients. One patient enrolled a second time after having a recurrence.		
Diagnosis and Main Criteria for Inclusion: All patients were to have newly diagnosed or recurrent papillary tumors of the bladder (Stage T _{is} or T ₁) with no evidence of carcinoma in situ (Stage T _{is}) or of muscle invasive disease (Stage T ₂ or greater). Patients had to be scheduled to undergo complete resection of papillary tumors prior to administration of AD 32.		
Test Product, Dose and Mode of Administration, Batch No.: AD 32 (Lot Number 515-44-0002) was supplied in 5 mL vials containing 40 mg/mL AD 32 in NCI Diluent 12. For each dose, the appropriate number of vials were further diluted with sterile saline to a total instillate volume of 75 mL. This volume was instilled intravesically through a catheter by gravity flow. The patients were asked to retain the peri-TURB dose for 1 to 2 hours. The peri-TURB doses administered in this study were 400 mg (3 patients), 600 mg (5 patients), and 800 mg (14 patients). Four patients received additional weekly doses of 800 mg.		
Duration of Treatment: Twenty-one patients each received a single peri-TURB dose, and one of these patients enrolled a second time after having a recurrence and received another single peri-TURB dose. Because the latter patient received different doses during the two enrollments, he is counted twice in the tables that summarize the study results. Four patients received additional doses of AD 32 at weekly intervals. Two of these patients received the scheduled five doses, but the remaining 2 patients received three doses each. Both stopped treatment prematurely because they could not retain the dose for the required 2 hours due to bladder spasms.		

SYNOPSIS (Continued)

<u>NAME OF SPONSOR/COMPANY:</u> Anthra Pharmaceuticals, Inc. <u>NAME OF FINISHED PRODUCT:</u> AD 32 <u>NAME OF ACTIVE INGREDIENT(S):</u> N-Trifluoroacetyladiamycin-14-valerate	<u>INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER</u> Volume: Page:	<u>(FOR NATIONAL AUTHORITY USE ONLY)</u>
<p>Criteria for Evaluation: Safety and tolerance were assessed by recording the occurrence of bladder symptoms and adverse events and by evaluating changes in hematologic parameters, vital signs, and physical examinations. Systemic absorption of AD 32 was assessed by determining the concentrations of AD 32 and its metabolites in blood samples obtained before and after administration of the peri-TURB doses.</p>		
<p>Pharmacokinetic Parameters and Statistical Analysis: Median values for serum concentrations of AD 32 and metabolites were calculated for each point in time. AUCs were calculated in nanomoles using the area under the serum concentration time curve from 0 to the time of the last measurable serum concentration at or prior to 6 hours after the dose was administered by the linear trapezoidal method. Statistical analysis was performed using AUC_{0-6 hours} values for patients with "typical" TURB, patients with "extensive" TURB, patients with "extensive" TURB who had unusually high serum levels of anthracyclines, and a patient with a perforated bladder.</p>		
<p>SUMMARY - CONCLUSIONS</p> <p>This was a pilot study designed to investigate the feasibility of administering AD 32 immediately after TURB of papillary bladder tumors. The goal of peri-TURB administration (adjunctive therapy) is to prevent implantation of tumor cells that may lead to recurrence or progression of disease.</p> <p>Pharmacokinetics: The quantity of AD 32 and its metabolites, AD 41 and AD 92, found in the blood during and after a two-hour, 800-mg, intravesical dose of AD 32 was dependent upon the extent of the transurethral resection. Following a "typical" TURB, median serum concentrations of AD 41 were higher than AD 32 or AD 92. The highest median serum concentration of AD 41 and total anthracycline measured was 38 ng/mL and 48.5 ng/mL, respectively, at 6 hours after administration of an 800-mg intravesical dose of AD 32. Following an "extensive" TURB without bladder perforation and a 800-mg intravesical dose of AD 32, the highest median serum concentration of AD 41 measured 38 ng/mL and the highest median serum concentration of total anthracyclines measured 46 ng/mL at 6 hours after dosing. These concentrations included one patient who had AD 41 and total anthracyclines values of 436 ng/mL and 553 ng/mL 6 hours after instillation, respectively. The highest concentration of total anthracyclines in the serum occurred after an 800-mg intravesical dose of AD 32 in the patient with a perforated bladder. The serum concentration of AD 41 and total anthracyclines in this patient was 2395 ng/mL and 2981 ng/mL, respectively at 6 hours after instillation of AD 32.</p> <p>The mean systemic exposure (AUC_{0-6 hours}) to AD 32 and its metabolites was also very dependent upon the extent of the transurethral resection. The greatest systemic exposure to AD 32 and its metabolites was found in the patient with a perforated bladder. The unadjusted mean systemic exposure to total anthracyclines as measured by AUC_{0-6 hours} was 207 nmoles/L·hr following "typical" TURB and 1364 nmoles/L·hr following "extensive" TURB. In the patient with a perforated bladder, systemic exposure to total anthracyclines as measured by AUC_{0-6 hours} was 18,382 nmoles/L·hr.</p>		

SYNOPSIS (Continued)

<u>NAME OF SPONSOR/COMPANY:</u> Anthra Pharmaceuticals, Inc.	<u>INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER</u>	<u>(FOR NATIONAL AUTHORITY USE ONLY)</u>
<u>NAME OF FINISHED PRODUCT:</u> AD 32	Volume:	
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SUMMARY - CONCLUSIONS (continued)		
<p>Safety: The peri-TURB doses of AD 32 administered in this study (400 to 800 mg) were well tolerated. A majority of the patients experienced some type of irritative bladder symptoms after the AD 32 was removed from the bladder, but most of the symptoms were mild or moderate and required no treatment. There was little evidence of a dose relationship for the occurrence of bladder symptoms or of an effect of AD 32 retention time or time between TURB and instillation. With one exception, no systemic effects attributable to AD 32 were reported.</p>		
<p>A patient (, who received a peri-TURB dose of AD 32 and was later found to have had bladder perforation during the TURB had systemic exposure to the drug via extraperitoneal extravasation of the dose. This patient had the highest serum anthracycline levels measured in the study. The systemic exposure to total serum anthracyclines as measured by the $AUC_{0-6 \text{ hours}}$ was approximately 88 times higher in this patient (nmoles/L·hr) than in the patients who underwent "typical" TURBs. This patient with a perforated bladder ; developed Grade 4 leukopenia approximately 2 weeks after receiving AD 32. However, he had no symptoms related to the leukopenia, and his WBC and neutrophil counts began to recover within 1 week.</p>		
<p>Four patients with a greater risk of recurrence (Stage T_{1b} or T₁ disease, or Grade 3 tumors) received additional weekly doses of AD 32 beginning 2 to 3 weeks after the peri-TURB dose. Two of these patients tolerated the additional doses well and received the scheduled five doses. The remaining two patients were unable to complete the five-dose course due to bladder spasms.</p>		
<p>Four patients (18%) had serious adverse events. The only events that were considered to be related to AD 32 were post-infusion dermatitis in one of the patients who could not retain the five weekly doses, and leukopenia in the patient who had bladder perforation.</p>		
<p>No patients died during the treatment period or within 30 days of receiving AD 32. The four patients who are known to have died subsequently had received AD 32 more than 4 months before their deaths and died of reasons unrelated to drug administration.</p>		
<p>Conclusions: The quantity of AD 32 and its metabolites, AD 41 and AD 92, found in the blood and the mean systemic exposure ($AUC_{0-6 \text{ hours}}$) to AD 32 and its metabolites after a 2-hour, 800-mg intravesical dose of AD 32 administered immediately after TURB was dependent upon the extent of the transurethral resection.</p>		
<p>Serum concentrations of AD 41 were higher than AD 32 or AD 92, indicating that AD 32 absorbed into the serum was immediately metabolized to AD 41 and then to AD 92.</p>		
<p>The patient with a perforated bladder experienced a serious drug-related adverse event (leukopenia) due to systemic exposure to high concentrations of AD 32 and its metabolites ($AUC_{0-6 \text{ hours}}$: 18,382 nmoles/L·hr). Systemic exposure to a mean concentration of total anthracyclines less than or equal to 1364 nmoles/L·hr did not cause any systemic, drug-related, serious adverse events.</p>		
<p>Overall Conclusions: The results of this study indicate that adjunctive administration of AD 32 immediately after transurethral resection of papillary bladder tumors is feasible. A dose of 800 mg, which has already been selected as the optimal therapeutic dose, was well tolerated in this setting. The extent of transurothelial absorption of AD 32 was not clinically significant in patients with "typical" and "extensive" TURBs without bladder perforation and did not cause any systemic, drug-related, adverse events. Systemic exposure to AD 32 in the patient with the perforated bladder resulted in serious, drug-related, reversible, systemic toxicity.</p>		

SYNOPSIS

NAME OF SPONSOR/COMPANY: Antra Pharmaceuticals, Inc.	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER	(FOR NATIONAL AUTHORITY USE ONLY)
NAME OF FINISHED PRODUCT: AD 32	Volume:	
NAME OF ACTIVE INGREDIENT(S): N-trifluoroacetyl Adriamycin-14-valerate	Page:	
Protocol No.: A9305		
Title of Study: An Evaluation of AD 32 Penetration into Urinary Bladder Tissue Following Intravesical Instillation in Patients With Carcinoma of the Bladder Scheduled for Total Bladder Resection		
Investigators: A.L. Patterson, M.D.		
Study Center(s): University of Tennessee, Memphis, TN		
Publication (Reference): Proc Amer Assoc Cancer Res 1996;37:182		
Studied Period (years): 30 December 1993 - 20 April 1994		Phase of development: I
Objectives: To determine the extent of bladder penetration of AD 32 when the drug is administered intravesically.		
Methodology: This open-label, single-center study was conducted in adult patients with carcinoma of the urinary bladder who were scheduled for total bladder resection. The patients received a single intravesical dose of 800 mg of AD 32 at the beginning of the cystectomy procedure. The AD 32 was withdrawn 1 to 2 hours later, immediately before bladder removal. Portions of the resected bladder were retained for AD 32 tissue penetration studies.		
Number of Subjects (planned and analyzed): Six patients (planned and analyzed).		
Diagnosis and Main Criteria for Inclusion: Patients were eligible for inclusion if bladder removal had already been scheduled based on determination by a urologist or medical oncologist that a total urinary bladder resection was necessary for the optimal management of the patient's disease. Patients must have recovered from the inflammatory effects of previous therapy. Also required were adequate bone marrow function, adequate hepatic and renal function, and sufficient bladder volume to accommodate the 75 mL instillate.		
Test Product, Dose and Mode of Administration, Batch No.: AD 32 (Lot Number 515-44-0001) was supplied in 5 mL vials containing 40 mg/mL AD 32 in NCI Diluent 12. For each dose, four vials of AD 32 (total dose, 800 mg) were further diluted with 55 mL sterile saline to a total instillate volume of 75 mL. Immediately before dose administration, patients had their bladders catheterized and the bladder contents drained. The AD 32 solution was instilled by gravity flow via the catheter during the patient's preparation for the surgical removal of the bladder. The AD 32 was withdrawn from the bladder, by use of the catheter, during the cystectomy procedure immediately before bladder removal.		
Duration of Treatment: Single dose of 800 mg instilled in the bladder for 1 - 2 hours		
Reference Therapy, Dose and Mode of Administration, Batch No.: None		
Criteria for Evaluation: For the assay of bladder penetration, tissue samples from the left lateral, right lateral, and dome surfaces were frozen immediately after excision. Sequential 20 µm slices of tissue were obtained starting at the luminal surface. Tissue samples were sonicated, and anthracyclines were extracted. The extracts were analyzed by ^{with} flow fluorescence detection, using N-trifluoroacetyl Adriamycin-14-octanoate as the internal standard. The resulting data were expressed as nanograms of total anthracycline per gram wet weight of bladder tissue for various tissue depths.		
For comparison purposes, an in vitro clonogenic assay to determine the inhibitory concentrations of AD 32 was performed using the following human bladder cancer cell lines: HTB 1, HTB 3, HTB 5, CRL 1472, and CRL 1473.		
Safety was assessed by monitoring the occurrence of adverse events and by evaluation of changes in clinical laboratory tests.		

SYNOPSIS (Continued)

<p>NAME OF SPONSOR/COMPANY: Antra Pharmaceuticals, Inc.</p>	<p>INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER</p>	<p>(FOR NATIONAL AUTHORITY USE ONLY)</p>
<p>NAME OF FINISHED PRODUCT: AD 32</p>	<p>Volume:</p>	
<p>NAME OF ACTIVE INGREDIENT(S): N-trifluoroacetyladiamycin-14-valerate</p>	<p>Page:</p>	
<p>Statistical Methods: Because this was a pilot study with only six participants, no summary statistics were generated for demographic or safety data. Mean values were calculated for total anthracycline content per gram wet weight of bladder tissue samples from the left lateral, right lateral, and dome surfaces. The latter results were compared with the 90% lethal concentrations of AD 32 against five human bladder tumor cell lines in vitro.</p>		
<p>SUMMARY - CONCLUSIONS</p> <p>Mean total anthracycline concentration in the excised bladder tissue samples decreased with increasing depth from the urothelial surface. The results were similar for samples taken from the right lateral, left lateral, and dome surfaces.</p> <p>AD 32 concentrations of _____ μM killed 90% of the cells of five human bladder tumor cell lines in a clonogenic assay. The mean total anthracycline concentrations measured in the bladder tissue exceeded the 90% cytotoxic levels to a depth of at least 1,250 μm, which exceeds the depth of invasion of T_1 lesions. At depths of 1,400 to 1,800 μm, which correspond to the depth of invasion of T_2 lesions, the mean total anthracycline concentrations closely approximated the clonogenic IC_{90} range.</p> <p>During the cystectomy procedure, the surgeon inadvertently perforated the posterior bladder wall of one patient before the study medication was drained, and AD 32 leaked into the patient's abdominal cavity. The abdominal cavity was irrigated with normal saline, and the bladder was removed. This patient had no adverse events and no changes in WBC or platelet counts.</p> <p>Only one adverse event (mildly elevated liver function test results) was reported in a patient with a history of heavy alcohol intake. The investigator attributed these abnormalities to anesthesia and prior alcohol intake.</p> <p>The only intercurrent illness reported was slight nervousness in another patient, which the investigator attributed to alcohol withdrawal. This patient stated on his medical history that he drank socially.</p> <p>One patient died of an apparent heart attack 10 days after receiving AD 32. The investigator considered this death to be unrelated to AD 32 administration.</p> <p>There were no clinically significant or abnormal laboratory test results, except for those reported in the patient with mildly elevated liver function test results.</p> <p>In conclusion, the results of this study suggest that intravesically administered AD 32 in a single dose of 800 mg penetrates human bladder tissue to a depth sufficient for treatment of superficial bladder cancer.</p>		

APPENDIX

Median Serum Concentrations of AD 32 After the First Intravesical Dose of AD 32

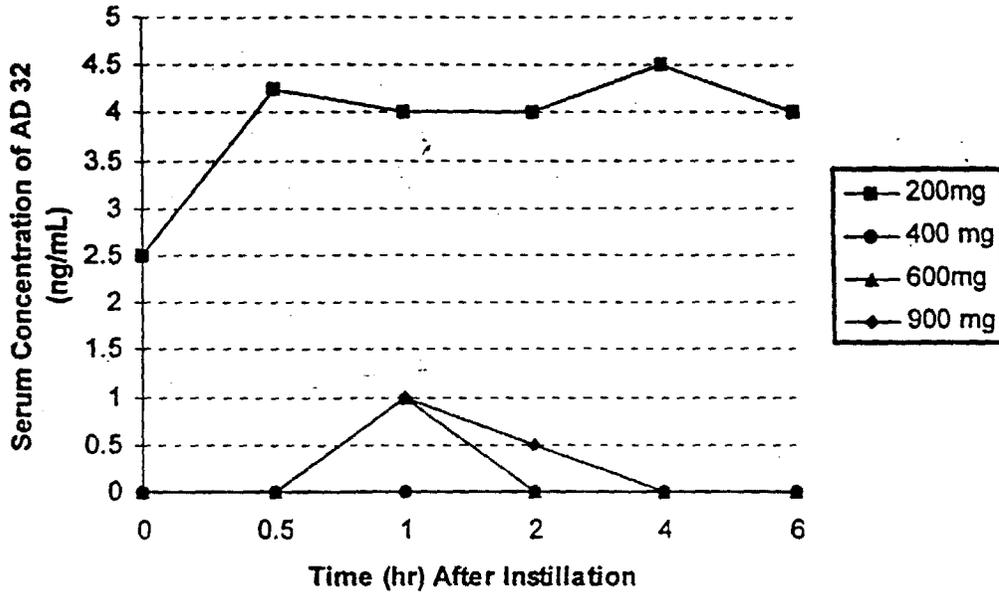


Figure 1. Median Serum Concentrations of AD 32 After Intravesical Instillation of the First Dose of AD 32

Median Serum Concentrations of AD 41 After the First Intravesical Dose of AD 32

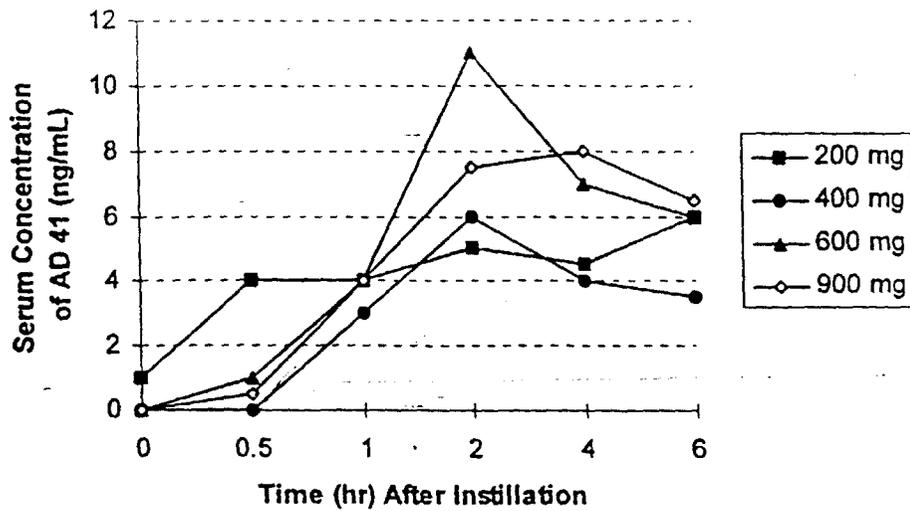


Figure 2. Median Serum Concentrations of AD 41 After Intravesical Instillation of the First Dose of AD 32

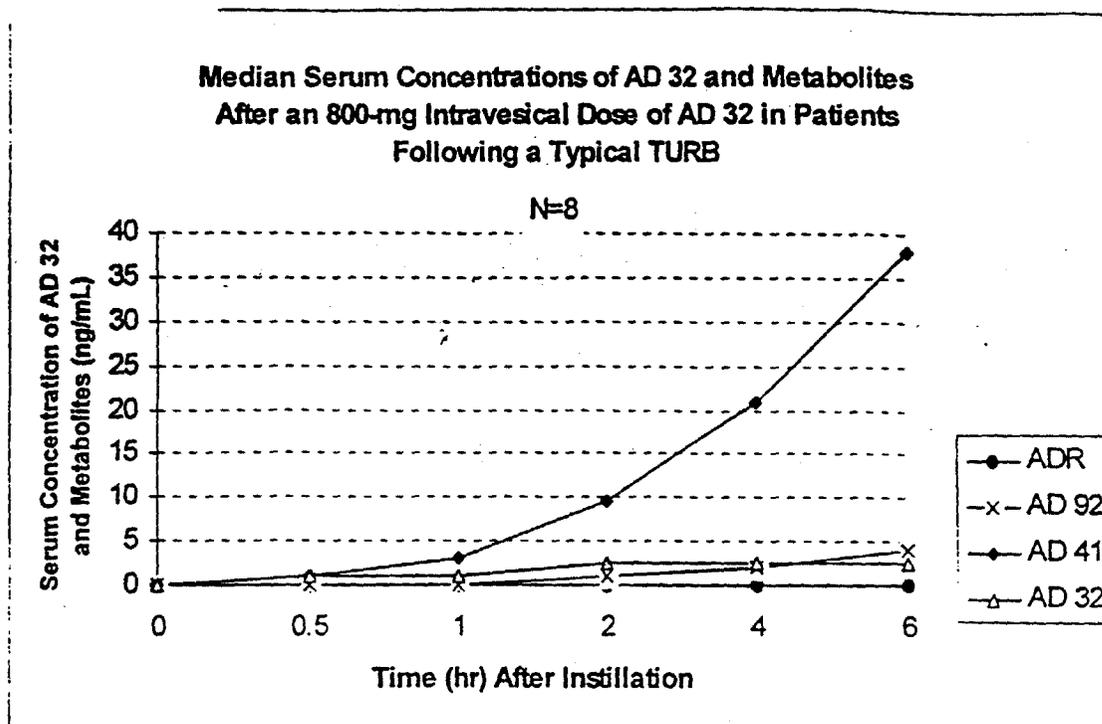


Figure 3. Median Serum Concentrations of AD 32 and Metabolites After an 800-mg Intravesical Dose of AD 32 in Patients Following a Typical TURB.

Following a "typical" TURB, median serum concentrations of AD 41 were higher than AD 32 or AD 92. The highest median serum concentration of AD 41 and total anthracycline measured was 38 ng/mL and 48.5 ng/mL, respectively, at 6 hours after administration of an 800-mg intravesical dose of AD 32 (n=8).

Following an "extensive" TURB without bladder perforation and a 600-mg intravesical dose of AD 32 (n=2), the highest median (or mean) serum concentration of AD 41 measured 388 ng/mL and the highest median (or mean) serum concentration of total anthracyclines measured 460 ng/mL at 6 hours after dosing. These high values were almost entirely due to one of the two patients. AD 41 and total anthracyclines values for this patient were 771 ng/mL and 912 ng/mL, respectively. AD 41 and total anthracycline values for the other patient dosed at 600-mg were 4 ng/mL and 7 ng/mL, respectively.

Following an "extensive" TURB without bladder perforation and a 800-mg intravesical dose of AD 32 (n=5), the highest median serum concentration of AD 41 measured 38 ng/mL and the highest median serum concentration of total anthracyclines measured 46 ng/mL at 6 hours after

dosing. These concentrations included one patient who had AD 41 and total anthracyclines values of 436 ng/mL and 553 ng/mL 6 hours after instillation, respectively.

Table 7. Median Serum Concentrations (ng/mL) of AD 32 and Metabolites After an 800-mg Intravesical Dose of AD 32 in Five Patients Following Extensive TURB

Time (hr)	ADR	AD 92	AD 41	AD 32	Total
0	0	0	0	0	1
0.5	0	0	1	1	2
1	0	0	3	1	5
2	0	1	11	5	17
4	0	3	29	5	37
6	0	5	38	3	46

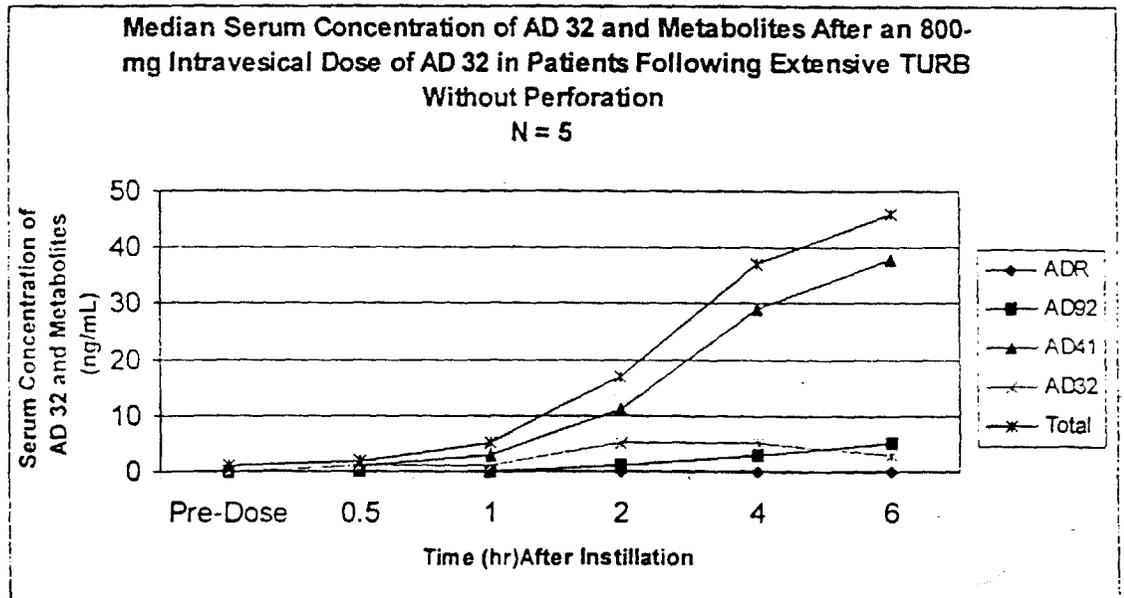


Figure 4. Median Serum Concentrations of AD 32 and Metabolites After an 800-mg Intravesical Dose of AD 32 in Five Patients Following Extensive TURB

Two (800 mg) and (600 mg) of seven patients with a retrospective assessment of extensive TURB (600 and 800 mg dose levels) had unusually high serum levels of AD 41 or total anthracyclines. Serum levels of AD 41 and total anthracyclines in the other five patients were similar to patients with "typical" TURB.

Table 8. Serum Concentrations of AD 32 and Metabolites After an 800-mg Intravesical Dose of AD 32 in Patient With a Perforated Bladder Following TURB

Time (hr)	Serum Concentration (ng/mL)				
	ADR	AD 92	AD 41	AD 32	Total
0					
0.5					
1					
2					
4					
6					

ns = No sample

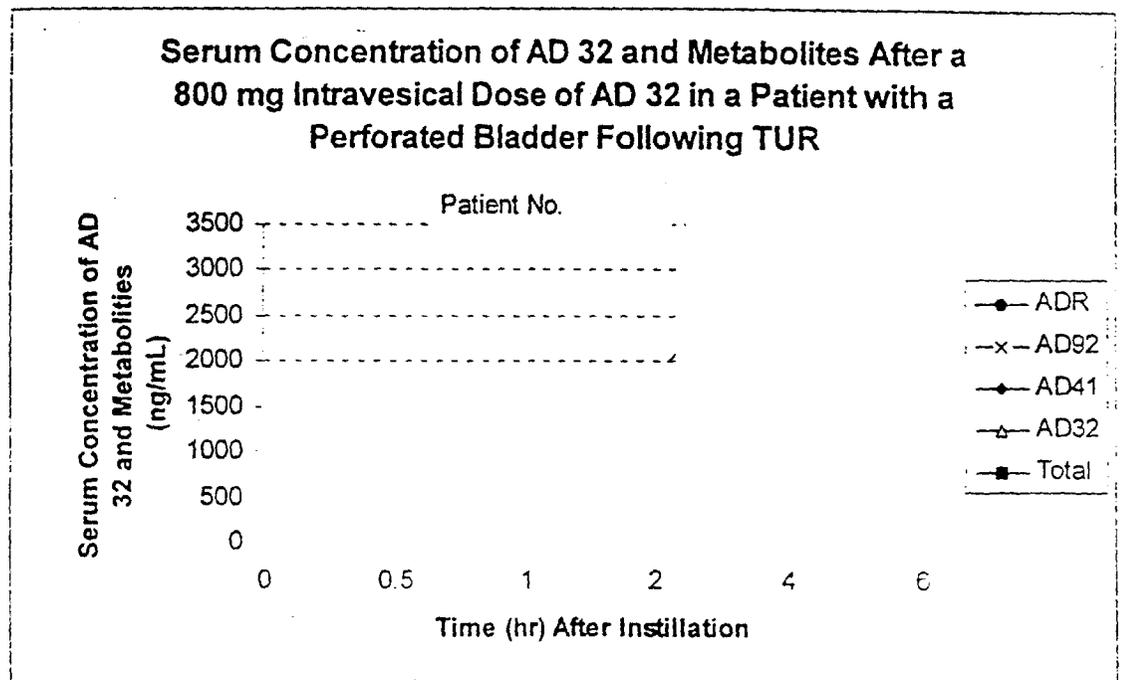


Figure 5. Serum Concentrations of AD 32 and Metabolites After an 800-mg Intravesical Dose of AD 32 in Patient With a Perforated Bladder Following TURB.

The highest concentration of total anthracyclines in the serum occurred after an 800-mg intravesical dose of AD 32 in the patient with a perforated bladder as shown in Table 8 and Figure 5. The serum concentration of AD 41 and total anthracyclines in this patient were 2395 ng/mL and 2981 ng/mL, respectively at 6 hours after instillation of AD 32.

of the 800-mg dose was 99 % for total anthracyclines, 98.6 % for AD32 and 0.4 % for AD 41. Only 3.1 mg of 800 mg of AD 32 instilled into the bladder was metabolized to AD 41 after 24 hours.

Table 3. Recovery of AD 32 and Metabolites (mg) in Urine Within 24 Hours After an 800-mg Intravesical Dose of AD 32

Patient (Dose)	AD 41	AD 32	AD 92	Catheter Wash*	Total Anthracyclines
(2)	7				
(3)					
(4)					
(5)					
(7)					
(9)					
(5)					
(9)					
(6)					
(2)**					
(3)**					
(4)**					
(5)**					
(6)**					
Mean	3.1	780.6	0.0	8.4	792

* Wash contained only AD 32.

** This patient was enrolled after the NDA cut-off date and is not included in the clinical section of the NDA.

AD 41 = N-trifluoroacetyladiamycin

AD 32 = N-trifluoroacetyladiamycin-14-valerate

AD 92 = N-trifluoroacetyladiamycinol

Table 4. Percent Recovery of Total Anthracyclines in Urine Within 24 Hours After an 800-mg Intravesical Dose of AD 32

Patient (Dose)	Collection Time (Hours)	mg Total Anthracyclines	Percent Recovered
1 (2)	0-4.6		
(3)	0-3.6		
(4)	0-4.0		
(5)	0-2.0		
2 (7)	0-24.0		
3 (9)	0-24.0		
4 (5)	0-3.0		
(6)	0-3.8		
5 (6)	0-17.9		
6 (2)*	0-24.0		
(3)*	0-24.0		
(4)*	0-24.0		
(5)*	0-24.0		
(6)*	0-24.0	820.1	
Mean		792	99.0

* This patient was enrolled after the NDA cut-off date and is not included in the clinical section of the NDA.

Table 5. Mean Percent Recovery of AD 32 and Metabolites in Urine Within 24 Hours After an 800-mg Intravesical Dose of AD 32

Compound	Mean Percent Recovered
AD 32	98.6
AD 41	0.4
Total Anthracyclines	99.0

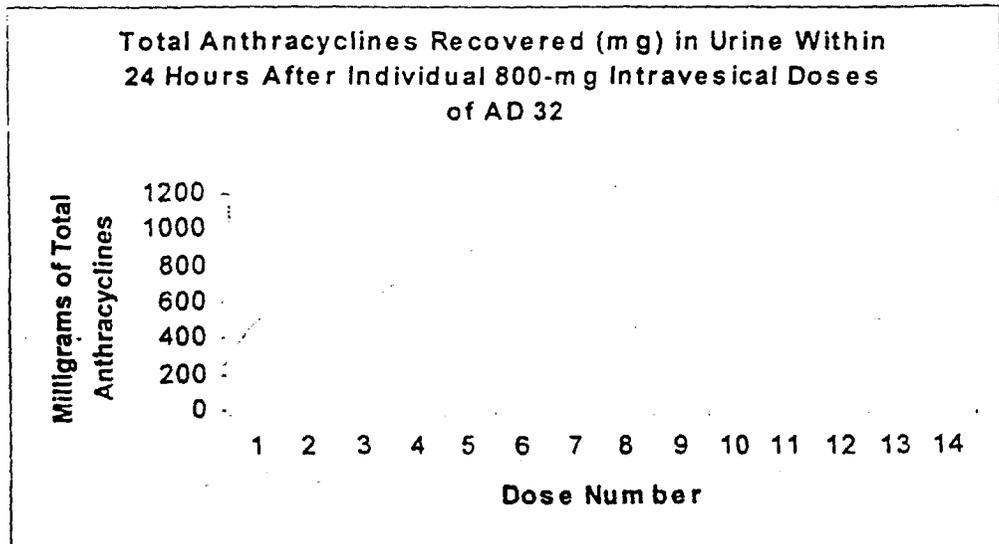


Figure 1. Total Anthracyclines Recovered (mg) in Urine Within 24 Hours After Individual 800-mg Intravesical Doses of AD 32

Table 0. Median Serum Concentrations ($\mu\text{g}/\text{mL}$) of AD 32 and Metabolites After the First $600\text{ mg}/\text{m}^2$ Intraperitoneal Dose of AD 32 (n = 14)

Time (hr)	ADR	AD 92	AD 41	AD 48	AD 32
0.0	0.000	0.000	0.013	0.000	0.005
0.5	0.000	0.001	0.044	0.001	0.019
1.0	0.000	0.007	0.180	0.005	0.048
2.0	0.000	0.030	0.383	0.019	0.066
4.0	0.002	0.113	0.839	0.067	0.088
6.0	0.012	0.203	1.007	0.091	0.062
8.0	0.010	0.427	1.765	0.175	0.080
12.0	0.010	0.582	2.222	0.214	0.046
24.0	0.006	0.290	1.256	0.188	0.014

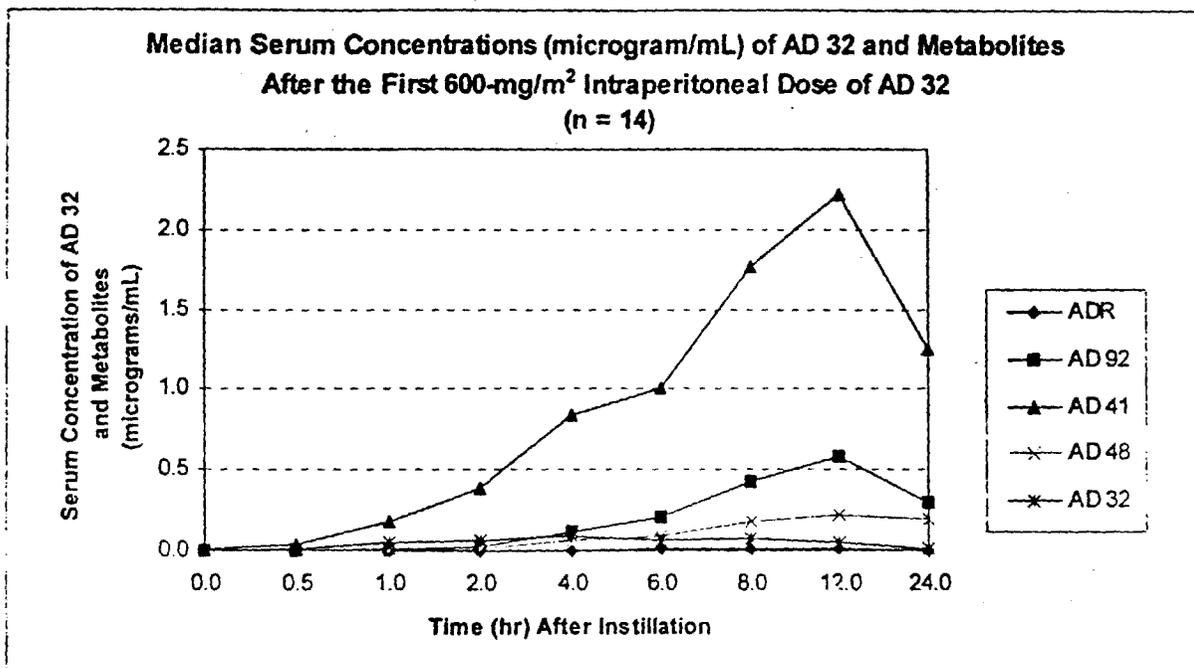


Figure 7. Median Serum Concentrations ($\mu\text{g}/\text{mL}$) of AD 32 and Metabolites After the First $600\text{-mg}/\text{m}^2$ Intraperitoneal Dose of AD 32

Table II. Median Serum Total Anthracyclines (micromoles/L) After the First Intraperitoneal Dose of AD 32

Time (hr)	200 mg/m ²	400 mg/m ²	600 mg/m ²
0.0	0.016	0.078	0.028
0.5	0.350	0.270	0.108
1.0	0.426	0.262	0.365
2.0	0.603	2.067	0.731
4.0	1.202	1.685	1.669
6.0	NS	6.720	3.720
12.0	NS	4.744	4.731
24.0	0.538	1.494	3.261

NS = No samples

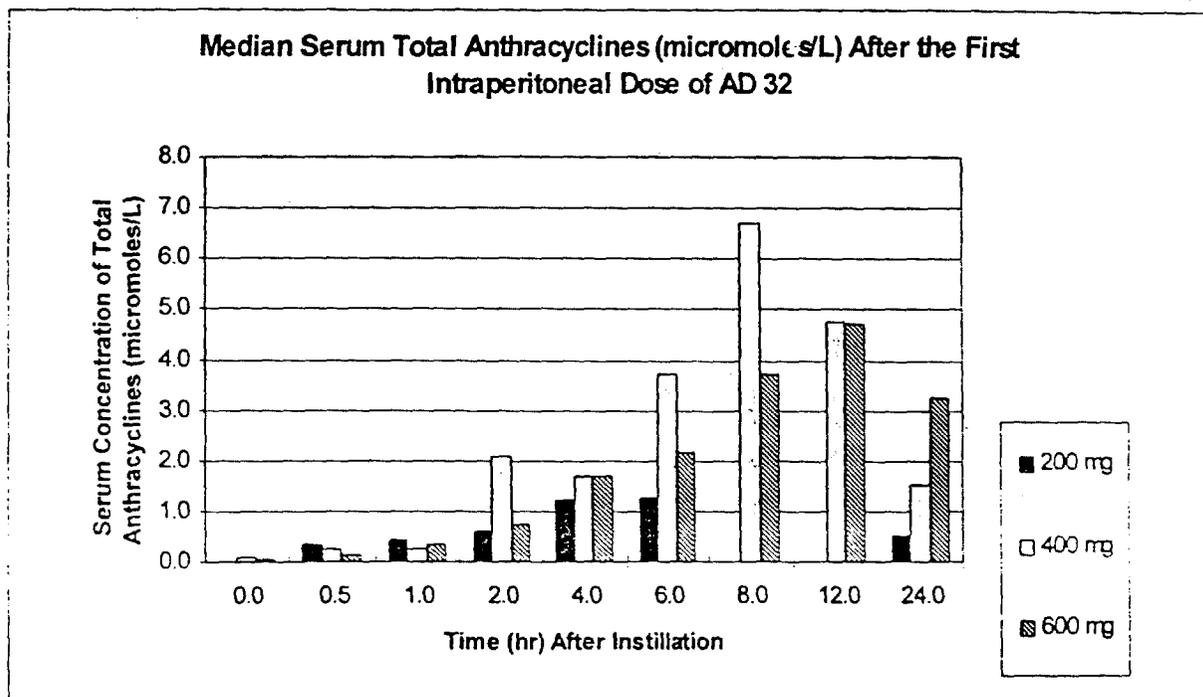


Figure 8. Median Serum Total Anthracyclines (μ moles/L) After the First Intraperitoneal Dose of AD 32

Table 12. Median Peritoneal Instillate Concentrations ($\mu\text{g}/\text{mL}$) of AD 32 and Metabolites After the First 600 mg/m^2 Intraperitoneal Dose of AD 32 (n = 12)

Time (hr)	AD 92	AD 41	AD 32
Pre-Dose	0.000	1.839	3.924
0.0	0.000	0.000	800.566
0.5	0.000	3.905	389.296
1.0	0.000	8.857	352.807
2.0	0.000	13.999	401.613
3.0	0.000	16.835	356.044
4.0	0.000	20.821	365.672
6.0	0.000	31.195	361.397
8.0	0.000	29.367	222.601
12.0	0.000	39.564	137.003
24.0	2.934	45.520	34.525

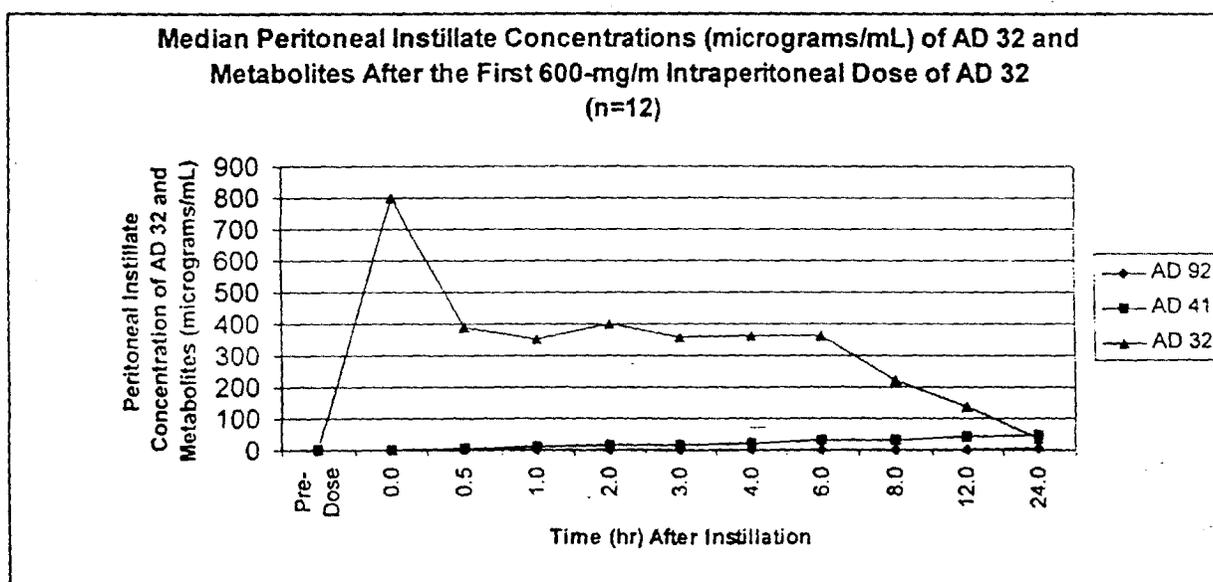
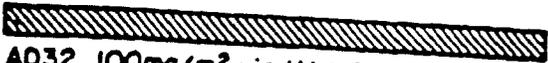


Figure 9. Median Peritoneal Instillate Concentrations (micrograms/mL) of AD 32 and Metabolites After the First 600 mg/m^2 Intraperitoneal Dose of AD 32.

Plasma Levels Of AD 32 And Metabolites
During 24 Hour Continuous I.V. Infusion


AD32, 100mg/m² via I.V. Infusion

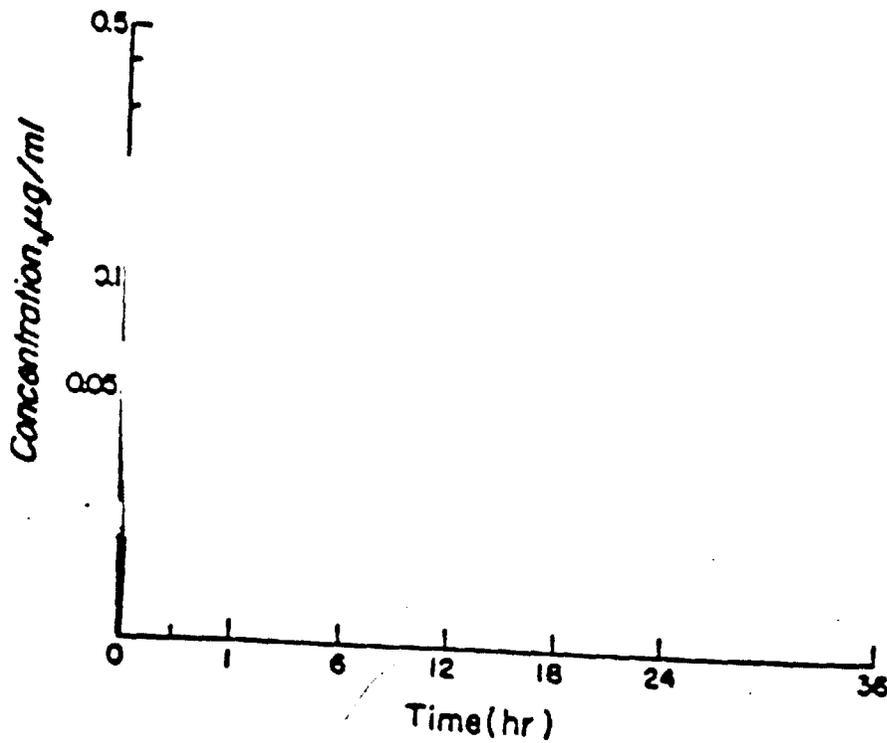


Figure 10