CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER
20-855

Pharmacology Review(s)

Labeling Review for Mesna, NDA 20855

From: Wendelyn J. Schmidt, Ph.D., Pharmacology Reviewer

Through: David Morse, Ph.D., Pharm/Tox Team Leader

Date: 2/5/02

Drug Interactions:
Currently Reads:

Should read:

FDA still working on wording.]

Carcinogenicity, Mutagenesis, Impairment of Fertility (currently not included).
Should read:
Carcinogenesis:
No long-term studies in animals have been performed to evaluate the carcinogenic potential of Mesnex.

Mutagenesis:
Mesna was not genotoxic in the *in vitro* Ames bacterial mutagenicity assay, the *in vitro* mammalian lymphocyte chromosomal aberration assay or the *in vivo* mouse micronucleus assay.

Impairment of Fertility:
No studies on male or female fertility were conducted. No signs of male or female reproductive organ toxicity were seen in 6 month oral rat studies (at doses up to 2000 mg/kg/day) or 29 week oral dog studies (520 mg/kg/day; both studies approximately 10 fold higher than the maximum recommended human dose on a body surface area basis).

Pregnancy:

[redacted]

Should read:
Pregnancy Category B. Reproduction studies have been performed in rats and rabbits at oral doses of 1000 mg/kg in rabbit and 2000 mg/kg in rat (approximately 10 times the maximum recommended total daily iv. [redacted] human dose on a body surface area basis) and have revealed no evidence of harm to the fetus due to mesna. There are however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Overdosage:
Currently reads:
There is no known antidote for Mesnex.

Add:
Oral doses of 6.1 and 4.3 g/kg were lethal to mice and rats, respectively. These doses are approximately 15 and 22 times the maximum recommended human dose on a body surface area basis. Death was preceded by diarrhea, tremor, convulsions, dyspnea, and cyanosis.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Wendelyn Schmidt
3/19/02 12:51:01 PM
PHARMACOLOGIST

David Morse
3/19/02 03:39:59 PM
PHARMACOLOGIST

JUL - 4 1997

NDA #20-855

DIVISION OF ONCOLOGY DRUG PRODUCTS, HFD-150
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Review No.1 (original)

NDA No. 20855

Serial No(s): 000 **Letter Date(s) of Submission:** 3/20/97

Information to be Conveyed to Sponsor: Yes (X), No ( )

Reviewer: Wendelyn J. Schmidt, Ph.D.

Date of Review: 4/11/97

Sponsor: Asta Medica, Inc. **Manufacturer (if different):**

Drug Name: Primary: mesnex Other Names: mesna
Secondary:

Chemical Name: Sodium-2-mercaptoethane sulfonate

Structure: $HS\text{-}CH_2\text{-}CH_2\text{-}SO_3^- Na^+$

Molecular Weight (and Formula optional): $C_2H_5O_3S_2Na$; mw = 164.18

Referenced INDs/NDAs/DMFs: NDA 19884 for intravenous mesna; IND [redacted]

Related INDs/NDAs/DMFs: DMF # [redacted]

Class: chemoprotective

Indication: as a prophylactic agent in reducing the incidence of ifosfamide-induced hemorrhagic cystitis.

Clinical Formulation: tablet consisting of 400.0 mg mesna
[redacted] mg lactose monohydrate
mg microcrystalline cellulose
mg calcium phosphate
mg cornstarch
mg polyvidone
mg magnesium stearate
mg hydroxypropylmethyl cellulose
mg polyethylene glycol
mg titanium dioxide
mg simethicone

Route of Administration: oral

Proposed Dose and Schedule:

Previous Review(s), Date(s) and Reviewer(s): NDA 19884: Dr. Alan Taylor, 9/88.

Studies Reviewed for this submission:
1. Thermann, H. et al. electron microscopic investigations of the cyclophosphamide-induced lesions of the urinary bladder of the rat and their prevention by mesna. Urol. Int. 42: 37-43 (1987).
2. Brock, N and J. Pohl. Organ-specific detoxication of cytostatics. Comparison of mesna and N-acetylcysteine. Therapiewoche, May 1987.
3. Report # D007-093/2400000001, Report of a study on the interaction of oral mesna with the efficacy of single intraperitoneal doses of ifosfamide administered on day 1 against intraperitoneally implanted P388 lymphocytic leukemia.
4. Report # D007093/24000000002, Report of a study on the interaction of oral mesna with the efficacy of single intraperitoneal doses of ifosfamide administered on day 1 against intraperitoneally implanted L1210 murine leukemia.
5. Report # D007093/24000000003, report of a study on the interaction of oral mesna with the efficacy of a high single intraperitoneal doses of ifosfamide administered on day 1 against intraperitoneally implanted L1210 murine leukemia.
6. Report # D007093/24000000004, comparison of the antitumor activity of two preparations of ifosfamide administered on day 1 against intraperitoneally implanted L1210 murine leukemia.

Studies Not Reviewed for this NDA: none

Studies Previously Reviewed for this NDA: none

Pertinent Studies Previously Reviewed in NDA 19884 (attached):
1. Pharmacokinetics with oral administration in guinea pig, rat and dog.
2. Acute toxicity of mesna by various route in mouse, rat and dog.
3. Six month study of oral mesna in rat.
4. Six week study of intravenous mesna in rat.
5. Six week study of intravenous mesna in dogs.
6. Twenty-nine week study of oral mesna in dog.
7. Segment II reproductive toxicity study with oral mesna in rabbit.
8. Segment II reproductive toxicity study with oral mesna in rat
9. Genotoxicity of mesna as measured by the Ames test, mouse micronucleus assay, and sister chromatid exchange .

Note: Portions of this review were excerpted directly from the sponsor's submission.

PHARMACOLOGY

1. Thermann, H. et al. Electron microscopic investigations of the cyclophosphamide-induced lesions of the urinary bladder of the rat and their prevention by mesna. Urol. Int. 42: 37-43 (1987).

Male Sprague Dawley rats (14/group) were administered either 68.1 mg/kg iv cyclophosphamide (CYP) or 68.1 mg/kg iv CYP + 31.6 mg/kg iv mesna 15 minutes prior to CYP. Two rats/group were killed at the following times following CYP administration: 1, 2, 3, 6, 8, 18, and 24 hours and the bladders examined by electron microscopy.

By one hour following CYP administration, bladder epithelial cells become smoothed and detached (cellular necrosis, vacuolization, degeneration of the mitochondria cristae, condensation of chromatin, increased lysosomes). The process progresses with detachment of deeper cells, edema and formation of gaps in the intercellular connections (decrease in desmosomes) and ulceration extending down to the muscle layer and perivascular tissue with damage reaching maximal levels at 18 hours. With mesna administration, no detachment of the cells is noted at any time. Organelles remain intact and no intercellular gaps become apparent.

2. Brock, N and J. Pohl. Organ-specific detoxication of cytostatics. Comparison of mesna and N-acetylcysteine. Therapiewoche, May 1987.

Primarily a review article, this piece emphasized the following: 1) the 4-hydroxy metabolites of ifosfamide and cyclophosphamide and acrolein were responsible for bladder toxicity (hemorrhagic cystitis and secondary bladder cancer); 2) the ideal protectant couldn't interfere with antitumor effects; and 3) the compound needed to have a favorable pharmacokinetic/distribution profile to achieve local detoxification. The authors investigated the free thiol concentration in the urine (the active species for reaction with acrolein and 4-hydroxy metabolites). In both rats and humans, 50-70% of the dose of mesna is excreted as free thiol, with significant concentrations (>10 umol/kg) in the urine for at least 4 hours following administration. N-acetylcysteine does not maintain favorable levels of free thiols for as extended a period as mesna.

3. Report # D007-093/2400000001, Report of a study on the interaction of oral mesna with the efficacy of single intraperitoneal doses of ifosfamide administered on day 1 against intraperitoneally implanted P388 lymphocytic leukemia.
4. Report # D007093/24000000002, Report of a study on the interaction of oral mesna with the efficacy of single intraperitoneal doses of ifosfamide administered on day 1 against intraperitoneally implanted L1210 murine leukemia.
5. Report # D007093/24000000003, report of a study on the interaction of oral mesna with the efficacy of a high single intraperitoneal doses of ifosfamide administered on day 1 against intraperitoneally implanted L1210 murine leukemia.
6. Report # D007093/24000000004, comparison of the antitumor activity of two preparations of ifosfamide administered on day 1 against intraperitoneally implanted L1210 murine leukemia.

These studies were conducted at [redacted] in 1996. Male BD2F/BOM mice (8-10/dose) were injected ip with P388/L1210 murine leukemia lines on day 0. Ifosfamide (IF) at doses between 46.4 and 464 mg/kg ip was administered on day 1. Mesna (lot 492) was administered either by ip or oral gavage at 2.15 X the IF dose (a 3 fold molar excess of IF) either 10 minutes or 1 hour prior to IF. Animals were observed twice daily. Tumor response was measured by increase in lifespan (%ILS).

The results of the experiments listed above are shown in the following table. The highest doses of IF, 464 mg/kg was toxic to mice. Mesna at 1000 mg/kg, the highest dose tested, when given ip also increased toxicity. With IF alone, survival time was increased by 50%, with the combination of IF + mesna, maximal survival was increased to 85%. Administration of oral mesna 10 minutes prior to IF showed no early (toxic) deaths to mice and allowed 464 mg/kg IF to be tolerated. L1210 was a poor model for investigating the effect on tumor with the drug combination due to the low response to IF. The sponsor further investigated this low response of L1210 by comparing two sources of IF: both provided similarly low inhibition of tumor.

Ifosfamide dose mg/kg	ip mesna dose mg/kg 10 min prior	oral mesna dose mg/kg 10 min prior	oral mesna dose 1 hr prior	P388		L1210	
				Mean survival time in days (range)	%ILS	Mean Survival time in days (range)	%ILS
0				10	0	11	0
46.4				12.5	25	11	0
100				14	40	12	9
215				15	50	10	-9
464				13.5	35	12	20*
46.4	100			13	30	11	0
100	215			14	40	12	9
215	464			16	60	13	18
464	1000			9.5	-5	12.5	25*
46.4		100		12	20	11	0
100		215		14	40	11	0
215		464		15.5	55	10	-9
464		1000		18	80	12.5	25*
46.4			100	12	20	11	0
100			215	14	40	12.5	14
215			464	14.5	45	13.5	23
464			1000	18.5	85	13.5	35*

*L1210 with 464 mg/kg ifos + 1000 mg/kg mesna was done in a separate experiment with MST for 0 ifos=10 days (range [redacted]1)

Summary of Pharmacology

The studies submitted with this NDA further support the concept that mesna does not interfere with the effects of cytotoxics/cytostatics while protecting the urinary bladder from toxic effects. Therrman and associates detail the toxic effects in rat of cyclophosphamide metabolites (4-hydroxy-cyclophosphamide and acrolein) on the bladder epithelium which are completely ablated by iv mesna 15 minutes prior to CYP. Oral mesna did not differ significantly from ip mesna in murine leukemia treatment in combination with IF, at least when administered 10 minutes prior to IF. The particular models used for investigating tumor protection were not optimal as response to chemotherapeutic agent was minimal for both P388 and L1210; a model where cures are seen

would be optimal. However, a period of tumor regrowth following cytotoxic treatment was observed (ILS is a better measurement than tumor growth based on results observed with other chemoprotectants). From the limited data provided, it is not entirely clear whether: a) high dose ip mesna with high dose IF enhances toxicity or protects the P388 tumor, b) HD oral mesna potentiates HD IF antitumor activity against P388, or c) HD ip mesna inactivates IF.

OVERALL SUMMARY AND EVALUATION

Intravenous and oral mesna have been under investigation for several decades. Intravenous mesna has been approved for prevention of urinary bladder toxicity with ifosfamide. Mesna acts by reacting with the 4-hydroxy metabolites and acrolein from ifosfamide and cyclophosphamide in the bladder to prevent damage to the bladder epithelium. Mesna, and its disulfide form, dimesna, remain primarily in the vascular compartment and are excreted via the kidney. No evidence of protection of tumor from the effects of the antineoplastic drugs have been noted preclinically or clinically. The models used in the current submission (P388 and L1210) are less than optimal since there is at most a 50% increase in lifespan (5 days) in response to the chemotherapy in the absence of mesna, the tumors are murine, not human, and the tumors are leukemic, not solid. The rat DS carcinosarcoma (Vol. 1.8, p 15) where ifosfamide cured 60% of the rats showed no significant difference (or a possible potentiation) of tumor growth inhibition over more than 2 months.

Half- lives and bioavailability of mesna and dimesna are shown in the following table. As the plasma half-life of oral mesna is longer than that with intravenous there was some concern that the mesna could interact with ifosfamide or cyclophosphamide in the plasma. However, in plasma, mesna is oxidized to the disulfide form, dimesna, which is less reactive. Dimesna is then reduced to mesna in the kidneys via thiol transferase and glutathione reductase. As approximately 80% of the oral dose in rats was excreted in the urine within 24 hours and less than [redacted]% of the dose was recovered in the feces, bioavailability exceeds 80% in the rat. In a paper by Ormstad and colleagues, (Cancer Res. 43: 333-338, 1983) free and reducible thiols were measured in plasma and urine following iv, ip and oral administration. Cmax for reducible disulfides after iv and oral administration of mesna differed by <30%; however, peak free thiol levels from oral mesna were approximately 1/10 those with intravenous mesna in the rat. AUC's were not calculated by the authors (graphs were too imprecise to allow calculation by the reviewer).

Species	oral t½ (h)	intravenous t½ (h)
guinea pig	3.5	1.48
rat	2:6	0.18
dog	2.0 to 3.32	1.69
human--mesna		
human-dimesna		

Acute and chronic toxicity data was collected in both rodent and non-rodent species. The ratios of LD50 oral/intravenous could suggest a lower bioavailability as the LD50 was 2-3 fold higher with oral administration to rats and mice than that by the intravenous route. Alternatively, toxicity may be exposure (AUC) or Cmax dependent. The oral mouse LD50 was 18 g/m2 while the oral rat LD50 was 25 g/m2. Clinical signs included diarrhea, tremor, decreased activity , convulsions, and cyanosis. Rats administered mesna by oral gavage daily for 6 months at doses up to 2.0 g/kg/day (12 g/m2/day) showed no dose dependent changes in serum chemistry, hematology or

histopathology. Dogs administered daily oral mesna in capsules for 29 weeks had one death at week 28 in the 316/560 mg/kg group (max 11.2 g/m2/day). Findings were confined to the MD and HD dogs and included semi-liquid stools, emesis, mild anemia, and small superficial erosion of the mucosa of the colon in 1/8 HD dogs and small areas of liver fibrosis in 1/8 dogs at 31.6 and 316 mg/kg. Thus, the NOEL, based on histopathologic findings was less than 31.6 mg/kg/day or 632 mg/m2/day. As 6 weeks of daily intravenous administration of mesna (maximum dose 316 mg/kg/day) resulted in emesis, decreased glucose in 1/6 HD dogs, and isolated round cell nodules in the liver of 1/6 HD females; mesna by oral and intravenous administration had very similar toxicologic profiles with the possible addition of gastrointestinal erosion with prolonged oral exposure. Oral doses of dimesna, the main metabolite of mesna, in either rat or dog showed almost no histopathologic damage with no damage detectable by hematologic or serum chemistry examinations.

Reproductive toxicity studies with mesna were performed in both the rat and rabbit by the oral route. Maximal daily doses in the rat and rabbit were 2000 mg/kg and 1000 mg/kg respectively (12.0 g/m2 and 11.0 g/m2). No evidence of increased mortality/resorptions or malformations were noted with mesna administration. Ames test, mouse micronucleus, sister chromatid exchange and chromosomal aberrations were all negative in the presence of mesna.

RECOMMENDATION

The NDA is approvable from pharmacology/toxicology provided the following changes are made to the labeling. The recommended oral dose, 480 mg/m2, may need adjustment to comply with the available tablet size, 400 mg (contingent on MO review).

Labeling Review:

Draft

4. Overdosage:

To be marketed product issues (NDA only): Impurities, Extractables, and Excipients: none

/S/ 7/2/97
Wendelyn J. Schmidt, Ph.D.
Pharmacologist/Toxicologist

revised 6/25/97, 7/2/97
Original IND/NDA/DMF
c.c. /Division File
/PAndrews, Team Leader
/Medical Officer
/C.S.O.
/WSchmidt, Reviewer

Concur I am. /S/ 7/2/97

Attachment

Summary

PHARMACOLOGY AND TOXICOLOGY REVIEW

OF NDA 19-834

ORIGINAL SUMMARY

Sponsor: Asta Pharma AG
Weismuellerstrasse 45,
D-6000 Frankfurt 1
West Germany

Submission: NDA dated June 30, 1988, received HFD-150 July 8, 1988, assigned to reviewer July 13, 1988, review completed Sept. 6, 1988

Drug: Uromitexan (Mesna) for Injection
Sodium-2-mercapto-ethane sulfonate supplied in 200, 400, 600, 1000 mg ampoules for intravenous administration.

Indication: Prevention of ifosfamide induced hematuria

Related documents: IND's

I. Introduction and Mechanism of Action

Mesna was developed as a uroprotectant to be used with oxazaphosphorines. Mesna acts by two mechanisms to inhibit urotoxicity. It reacts with acrolein to form a stable non-toxic thio-ether and it reacts with 4-hydroxy metabolites forming relatively stable condensation products that are themselves non-toxic and prevent further breakdown to acrolein. Mesna was the best of a series of thiols tested. Other desirable properties shown by this compound were low penetration into body tissue and rapid excretion, as well as no interference with the antitumor activity of ifosfamide, cyclophosphamide or other chemotherapeutics tested.

II. Preclinical Pharmacology

A. General Properties.

Numerous reprints were included which described the pharmacologic activities of mesna and its interaction with ifosfamide/cyclophosfamide and other chemotherapeutics. The following key observations were made:

1. Mesna inhibited the urotoxicity of oxazaphosphorines in a dose-dependent manner administered orally or intravenously.

2. Two reports suggest that mesna may reduce renal and GI toxicities associated with cisplatin administration.

3. Mesna had no effect on antitumor activity or non-urological toxicities of oxazaphosphorines when given at various dose/schedule regimens in various test systems.

4. Mesna and dimesna alone showed no evidence of carcinogenic potential. However they did cause a dose-related reduction in the incidence of urinary bladder tumors associated with cyclophosphamide administration in rats.

5. Mesna had the following pulmonary and cardiovascular effects in dogs which were dose dependent and seen starting at doses of about 200 mg/kg: increased ventilation rate; transient decreases then increases in respiratory volume and pulmonary resistence; increased pulmonary elastance; transient hypotension with associated sinus bradycardia and significant ECG abnormalities.

B. Pharmacokinetics

1. Absorption and Elimination.

In rat studies mesna was analyzed colorimetrically as total thiols and dimesna as reducible disulfides after treatment with sodium borohydride. Alternatively, other rat, dog and guinea pig studies monitored ^{35}S-or ^{14}C-mesna radioactivity. Pharmacokinetic parameters and tissue distribution are given in the accompanying tables and figures. After IV administration the majority of the mesna dose was found in the kidneys. Total serum protein binding was 9.7% of the total mesna present in serum. Binding was mainly to albumin and immunoglobulin. Distribution studies performed in pregnant rats indicated that the concentrations in placenta and fetus were 60% and 17% respectively, of that in maternal blood. The half lives in placenta and fetus were 4.1 hr and 7.7 hr respectively.

Data from rats given 100 mg/kg doses IV

	Free Thiols				Free thiols plus reducible disulfides			
	Co nmol/ml	t1/2 hr	AUC in mol/ml/hr	% in urine at 6 hr	Co	t1/2	AUC	% in urine at 6 hr
Mesna	2000	0.18	1030	72.1	3100	0.31	1302	89.8
Dimesna	760	0.16	133	37.5	4420	0.20	1462	99.3

Oral administration

Animal species Dose mg/kg µmoles/animal	Guinea-pig 200 305	Rat 200 244	Dog 50 6,100
Time of maximum blood level	± 1 h.	± 1 h.	± 1 h.
Half-life time in blood	3.49 hours (calculated between 1 and 16 hours)	2.59 hours (calculated between 1 and 16 hours)	1.99 hour (calculated between 1 and 8 hours)
Half-life time in liver	4 hours (calculated between 1 and 16 hours)	4.6 hours (calculated between 1 and 16hours)	
% excreted in urine			
after 24h	79	75	63
48h	83	77	76
% excreted in faeces			
after 24h	6	8.9	2.2
48h	9	9.0	4.6
0 h blood level :			
in % of administered dose/1 ml	0.094	0.17	0.033
in µM/1 ml	0.286	0.408	2.01
Urinary metabolites	Unchanged Mesna + oxidized derivative		

2. Metabolism

The following data is derived from studies in isolated tissues or cells.

a) After PO administration, mesna (M) and dimesna (DM) are both absorbed from the intestine and DM undergoes reduction to M during intestinal absorption.

b) When present in plasma, M is rapidly oxidized to DM by a metal dependent reaction.

c) M and DM pass unchanged through the hepatic vasculature, are not taken up into liver cells, and are not excreted in bile.

d) In the kidney, DM is filtered through the glomeruli and subsequently reabsorbed, where upon, reduction to the pharmacologically active thiol form (M) forms in the renal tubular epithelium and the thiol is reexcreted into the tubular lumen.

e) Reduction of DM to M occurs by a mechanism involving cytosolic enzymes thiol transferase and glutathione reductase.

III. Preclinical Toxicology

Preclinical toxicology teratology and mutagenicity studies were all previously submitted and reviewed as parts of INDs [illegible]. Excerpts from Dr. Almon H. Coulters review of that material are given here with additional comments indicated by an asterick(*).

A summary of all the acute toxicity data is given below.

SUMMARY OF ACUTE TOXICITY STUDIES ON MESNA

Species	Route	Animals/Sex/Gp	LD_{50} (95% confidence limits) mg/kg Male	Female
Mouse	i.v.	10/sex	1900 (1593-2034)	1900 (1785-2190)
	i.p.	10/sex	2000 (1784-2240)	2030 (1783-2297)
	i.p.	2 males	>1600	—
	i.m.	2 males	>1600	—
	s.c.	2 males	>1600	—
	p.o.	10/sex	6100 (5550-6704)	>7200
Rat	i.v.	10/sex	2007 (1469-2954)	1910 (1578-1804)
	i.v.	2 males	>1600	—
	i.p.	10/sex	1305 (1282-1755)	1223 (1029-1458)
	i.p.	2 males	>800	—
	i.m.	2 males	>800	—
	s.c.	2 males	>800	—
	p.o.	10/sex	4230 (4090-4600)	4510 (4253-4781)
	p.o.	2 males	>1600	—
Dog	i.v.	2-4 (sex not specified)	>200	
	p.o.	2-4 (sex not specified)	>2000	

Strain of animals used were mice-NMRI (iv, ip, po), Swiss SPF-(ip,im,sq); rat-Wistar(iv,ip,po) Sprague Dawley (iv, po), BD II SPF (ip,im,sq); dog-mongrel(iv,po), beagle(po)

Toxic signs observed in rats and mice were - reduced motor activity, breathing difficulty, cyanosis, bristled fur, arched back, diarrhea, tremors, convulsions, and local necrosis (s.q. only). Dogs displayed diarrhea, emesis restlessness mastication, swallowing, peripheral dilation, writhing movements,

and a comatose phase which included loss of reflexes, mydriasis, cyanosis, bradycardia, opisthatonus, tetanization, tonic contractions, bloody diarrhea, and violent emesis.

B. SUBACUTE/CHRONIC TOXICITY:

1. RAT: 6 Weeks, IV

Compound: Mesna
Formulation: aqueous solution
Route IV 2.15 ml/kg and 5 ml/kg over 1 minute in tail
Dose Levels: 0,100,316,1000 mg/kg/day X 42 days
Strain: Sprague-Dawley 183-243g M, 159-189g F
Number: 15/sex/group
Study Site/Date: A sta-Werke , FRG 5/78 to 7/78

Results

Slight loss of hair on ventral side in 9 high dose animals - some tails in high dose group swollen so could not give injections on certain days - weight loss in mid dose (M 8%, F 4%); high dose group (M 34%, F16%) - food consumption decrease and in high dose males, Hb decreased in mid and high dose - REC decreased high dose - increased leukocytes, high dose anemia, high dose - absolute and relative weight increase in liver, spleen, lung, and kidney at high dose - deaths in control (3M), low (2M), mid (1M), high (3M, 2F) - sacrifices, high (1M,1F) - kidneys of high dose group developed distended tubules with high concentration of protein; glomerular capillaries contained hyaline deposits - BUN values in M and F high dose - SGOT increased 2X in high dose F.

2. RAT 6 Months, PO

Formulation: 10,20,40% solutions in deionized water
Route: gavage
Dose Levels: 0,500,1000,2000 mg/kg/day X 6 months
Strain: Mistar - 130 to 190 g M and 110 to 160 g F
Number: 25/sex/group
Control Treatment: deionized water
Study Site/Date: ork done in 1970

Results

Deaths occurred in all groups and were said to be due to dosing or spontaneous disease states - sporadic diarrhea and decreased body weight gain in high dose group - no drug related changes were said to occur in the hematology or clinical chemistry parameters - increased relative liver, spleen, kidney and gonad weights for all male treated groups - no drug related histopathologic changes were said to be observed

*Supplement dated Feb. 12, 1970 with additional anatomo-pathological data confirmed that no significant drug related lesions were seen. Inflammatory lesions in various organs, slight prevalence of red series in bone marrow, and liver steatosis was found in control and treated animals and was not related to drug treatment.

3. Dog: 12 Days, IV

Formulation - 40% aqueous solution
Route: IV over 3 minutes
Dosage Levels: 200 mg/kg/day X 12 days
Strain: Mongrels
Numbers: 4 animals - sex not indicated
Control Treatment: no control
Study Site/Date: [redacted] ork done in 1969

Results

Sedation on first day - emesis and diarrhea following injection - slight weight decrease in two of the four animals - no other data

4. DOG 6 Weeks, IV

Formulation: aqueous solution 200 mg Mesnum/ml
Route IV 1.6 ml/kg over 1 minute
Dosage Levels: 0,10,31.6,100,316 mg/kg/day X 6 weeks
Strain: Beagle - 6.9 to 10.0 kg (treated), 9.2 to 12.7kg (control)
Number: 3/sex/group
Control Treatment: Demineralized water
Study Site/Date: .....

This group (10 mg/kg) was treated only five weeks at this dose then treated at 316 mg/kg/day for a further six weeks. This was done since no toxicity was observed in the 10 mg/kg group over five weeks.

Results

Emesis occurred at 100 and 316 mg/kg at 5-15 min after injection - body weight of treated dogs increased 13-32% control weights remained relatively constant (controls were 12-13 months old, treated animals were 6-7 months old) - glucose decreased 37% in one high dose F - other chemistries, hematology, urinalysis, and fecal examination appeared normal - one control F died - no abnormalities in absolute or relative organ weights - one high dose F had isolated nodules of round cells in liver parenchyma - other pathology which was observed in treated animals also occurred in controls.

5. DOG: 29 Weeks, PO

Formulation: gelatine capsules with enteric coating in 100 g meatball
Route: oral
Dosage Levels: 0,31.6,100,316/kg/day* X 29 weeks
Strain: Beagle 9.3-12.1 kg M, 7.8-10.4 kg F, 7-9 months old
Number: 4/sex/group
Control Treatment: empty capsule
Study Site/Date: ./June 78 to Jan 79

*The high dose schedule was 316 mg/kg through week 5, then 420 mg/kg through week 25, and 560 mg/kg through week 29. This was done in order to allow animals to accept drug without vomiting.

Results

One high dose male died day 194 (week 28); necropsy not done due to autolysis and canabilism (this animal had long history of symptoms, i.e. diarrhea, apathy reduced motor activity, cardiac irregularities, weight loss, and decreased WBCs, RBc, HgB, Hct, platelets, creatinine and CPK)
- clinical signs were semiliquid stools decreased motor activity, loss of general condition, and emesis - body weight and weight gain were somewhat higher in high dose - ECGs were similar in all groups - no ophthalmoscopic abnormalities - RBC, Hct, and Hb lower in high dose males - mid and high dose groups showed slight increased in sedimentation rate, AP, and slight decrease in creatinine in all treated groups - slight changes in electrophoretic pattern - normal urinalysis - estral changes in some treated animal at macroscopic examination - one high dose animal with small superficial erosion of mucosa in colon and acute inflammatory response - reduction of lymphoid tissue in thymus of two mid dose and one control animal; also bronchial cysts - small areas of liver fibrosis in one low and high dose animal.

6. DOG: 4 Month Inhalation

Dogs were exposed to aerosol sprays of 20% and 40% UCB 3983 solutions. Three groups (3/sex/group) received either distilled water or aerosol drug, the low dose (75-129 mg/kg/day), or the high dose (142-244 mg/kg/day). All groups were exposed for 30 minutes/day for 4 months. No beagles died - emisis was dose related - coughing during exposure was dose related - no changes noted in clinical chemistry, hematology, ophthalmological, or ECG examinations - there were no drug related histopathology lesions - the high dose group showed a slight enlargement of the livers and gonads. *Vomiting was seen once in controls, 7 times at 20% and 17 times at 40% drug immediately after dosing.

Supplement dated May 3, 1970 confirmed that no significant drug related lesions were seen. Inflammatory lesions in various organs, calcium deposits in kidneys and lungs and melanic pigment deposits in lymph nodes were seen in treated and control dogs and bore no relation to drug administration.

IV. Terotology, Mutagenicity and Special Studies

A. Terotology

1. SEGMENT II: RABBIT, PO

Formulation: Aqueous solutions
Route: Oral - esophageal tube
Dosage: VC, 500, 1000 mg/kg, thalidomide 150 mg/kg (as a 15% suspension)
Strain: Local strain called "hare rabbits"
Number of Gravid Animals: 7/11 VC, 10/11 low, 10/10 high, 4/5 thalidomide
Control Treatment: Water
Study Site/Date: July 1969

Results

Some diarrhea in high dose females - a trial dose of 1500 mg/kg turned out to be toxic to gravid females - body weight was below the VC throughout the study for both drug treated groups - implantations were within the normal limits - fetal loss was high in the low dose (23%) but was not drug related; high dose fetal loss was 7.6% and within the normal limits (7.6-10%) - no gross external abnormalities observed in the drug groups - no differences between controls and mesna treated groups with respect to visceral and skeletal examinations - thalidomide was teratogenic.

2. SEGMENT II: RAT, PO

Formulation: 10%, 20%, 30%, and 40% solutions-presumably aqueous
Route: oral, via canula
Dosage Levels: VC, 500, 1000, 15000, 2000 mg/kg from day 8 through day 15, at 5 ml/kg
Strain: Wistar, 190-220 g
Number of Gravid Animals: VC 33, 18, 16, 15, 16
Control Treatment: Vehicle - presumably water
Study Site/Date: /July 1969

Results

Maternal Effects:

Soft stools at 1000 mg/kg and higher - weight gain reduced beginning day 15 in 2000 mg/kg group - drug not toxic to females.

Fetal Effects:

The fetal observations are given in the following table

Dose mg/kg	Gravid. females	% of gravid.	Implantations	Average per rat	Fetuses at term: Live	Fetuses at term: Dead	Fetuses not entered Early	Fetuses not entered Late resorptions	% loss	N° grass observ.	%	Overall body weight average of live fetuses	Malformations observed.
C.G.	309/383	81	3813	12.3	3.634	1	153	30	5.35	3	0.085	3.56	2 cleft palates 1 valgus foot
C.P.	33/39	84.6	420	12.7	400	1	17	2	5	1	0.3	3.5	1 cleft palate
500	18/20	90	221	12	211	0	10	0	4.6	0	0	3.7	
1000	16/20	80	207	12.9	193	0	6	1	3.6	0	0	3.55	
1500	13/19	78	167	10.8	157	0	9	1	6	0	0	3.6	
2000	16/20	80	189	11.8	180	0	9	0	4.7	1	0.5	3.4	1 edema-tous fetus

Viceral abnormalities [agenesis/atalectasia of lung lobe(s)] were at 7% in the 1000/kg group and 4% in the control. Misshaped lcmbar vertebra occurred in 1 high dose fetus. Misshaped dorsal vertebra were found in 2 fetuses from the 1500 mg/kg group; the low dose had 2 fetuses with delayed ossification of cranial bones. The most frequent abnormalities observed in all groups were: absence of cervical vertebra and variations in the number, shape, size, position and development of the 2nd and 5th sternebra.

3. SEGMENT II: RAT Inhalation:

Formulation: aqueous solutions
Route: Aerosol inhalation
Dosage Levels: VC, 20% (17 mg/kg/day), 40% (35 mg/kg/day) from day 8 through day 15. Exposure was 30 min./day
Strain: Wistar
Number of Gravid Animals: 18 VC, 15 low, 17 high
Control Treatment: Distilled water
Study Site/Date: /July 1969

Results

Maternal Effects:

Animals sneezed and scratched their snouts during the first half of the exposure period each day - no additional effects were observed

Fetal Effects:

Fetal observations are indicated in the table below.

Substance	Dosage mg/kg	Gravid fem.	% gravidity	Implantations	Means per rat	Fetuses arrived at activity live	dead	Fetuses not entered Early	late resorptions	% loss	No. of abnormal fetuses on external gross examination		Overall body weight average of live fetuses
control	-	18/20	90	213	11.8	208	0	5	0	2.3	0	0	3.5
3983	385	15/20	75	177	11.8	162	0	15	0	12.8	0	0	3.6
3983	402	17/20	85	189	11.1	180	0	9	0	4.7	0	0	3.6

The percentage of loss (early and late resorption loss) was higher in the low dose than was observed in the controls or high dose groups. This was said to be within the normal limits for this strain of rats. Visceral examination showed no significant increase in the number of fetuses with abnormalities. No significant increase was observed in the skeletal examination.

4. SEGMENT II: RABBIT, PO

Formulation: aqueous solutions
Route: Oral - esophageal tube
Dosage: VC, 500, 1000 mg/kg, thalidomide 150 mg/kg as a 15% suspension
Strain: Local strain called "hare rabbits"
Number of Gravid Animals: 7/11 VC, 10/11 low, 10/10 high, 4/5 thalidomide
Control Treatment: water
Study Site/Date: /July 1969

Results

High dose females showed some diarrhea. A trial with 1500 mg/kg turned out to be toxic to the gravid females. Body weight was below the VC throughout the study with the high dose group below the low dose group. Implantations were within the normal limits. There was a rather large loss (23%) of fetuses in the low dose group, but was apparently not drug related for the high dose group (7.6%) was within the normal limits (7.6%-10%). No gross external abnormalities were observed in the mesna treated groups. The thalidomide group displayed the teratogenic malformations produced by this drug. There were no differences between the control and the mesna treated groups with respect to visceral and skeletal examinations.

B. MUTAGENICITY:

No activity was observed in the Ames test or the mouse micronucleus assay. These studies were done by [redacted] cs. *Mesna did not increase the incidence of sister chromosome exchange or chromosomal abberations when studied in vitro in PHA stimulated lymphocytes. A slight comitogenic effect of mesna was observed in this system which should be taken into account when administered in preparation of patients for bone marrow transplantation.

C. Ocular and Intravenous Tolerence.

In rabbits, a 0.215 ml test solution (50, 100, 200, 400 mg/ml) introduced into the conjunctival sac produced no reddening or swelling with repeated administration. In the rabbit ear vein, irritation occured after the second and third administrations of 20% and 40% solutions with necrosis in some animals. Healing followed in three weeks.

V. Human Pharmacokinetics and Bioavailibility

Four studies sponsored by Asta Pharma and two studies published in the literature examined pharmacokinetics of mesna in patients. The concentration of mesna (free thiol groups) was measured by HPLC or spectrophotometrically at 420 nm. Disulfide groups were reduced to the free thiols with sodium borohydride prior to analysis. Disulfide levels (Dimesna) were calculated by subtracting free thiol levels from total thiol levels obtained.

Asta Pharma supported the studies of P. Luecker et al., Institute for Clinical Pharmacology, Bobenheim and Berg, Federal Republic of Germany, and H. Rogers et. al., Guy's Drug Research Unit, London, UK.

Findings in each study are summarized in the following table:

					PLASMA (free thiols)					URINE EXCRETED (Free thiols)				
Investigator (Reference No.)	Title	Dosage Form	Route of Administration	Dose	t_{max}	T_{max}	Vd (l/kg)	AUC (nmole/ml/hr)	$T_{1/2}$	C_{max} (ug/ml)	T_{max}(h)	% Excreted	Clr (l/kg/hr)	Clr (l/kg/hr)
Luecker, P.W. (1)	Bioavailability and time course of thiol group concentration in the urine after intravenous and oral administration of mesna (MNA), trade-name, Uromitexan®	2 ml à 10 ml capsules 100 mg/ml Batch 7 8202 à 1001	IV oral oral	20 mg/kg 20 mg/kg 40 mg/kg	-	-	-	-	0.8h 1.7h 2.1h	5765.63 3200.75 4152.50	0.99 1.76 2.83	48.74 52.46 57.64	-	-
Luecker, P.W. (2)	The question of the steady state concentration of thiol groups in the urinary bladder from mesna (MNA), trade-name: Uromitexan®, during repeated oral administration of 20mg/kg body weight at 2 different dosage intervals	4 ml à 10 ml capsules batch # 1010 and 1001	repeated administration 20 mg/kg IV and oral followed by 20 mg/kg oral every 4 hours for 9 doses 20 mg/kg IV and oral followed by 20 mg/kg orally every 3 hours for 7 hours 20 mg/kg IV and oral followed by 20 mg/kg orally every 3-4 hours for 4 doses	-	-	-	-	- 1.74h,(single dose) 3.88h (multiple dose) (n=8 patients) -	-	-	41.93 37.66 33.28	-	-
Luecker, P.W. (3)	The Pharmacokinetics of Uromitexan®	10 ml 100 mg/ml Batch 10005	IV oral IV	40 mg/kg 40 mg/kg 40 mg/kg x2	-	-	-	-	IV 0.08h (initial) IV 0.82h (terminal) 1.17h(dimesna)	-	-	36.00 33.00 30-40	-	-
Rogers et al (4)	Single dose absolute bioavailability study of mesna in healthy volunteers	-	IV oral	800 mg 800 mg	(1) 19.4 (nmoles/ml)	-	0.652	30.4	0.36h	-	1.17	36.0 17.6	1.23 -	- 0.413
Jones, et al (5)	Excretion of Sodium 2-mercaptoethane-sulfonate (MESNA) in the urine	-	oral	400 mg (7.5 mg/kg)	-	-	-	-	-	-	-	242 (61.5 as total thiols)	-	-
Plaa, et al* (6)	Protecting the bladder from cyclophosphamide with mesna	-	IV	20 mg/kg	-	-	-	-	-	-	2	33.00	-	-

Summary Evaluation

Mesna was developed as a uroprotectant against hemorrhagic cystitis associated with oxazaphosphorine cancer chemotherapy. Mesna was the best of a series of thiols tested which neutralized toxic metabolites, showed low penetration into body tissue, and rapid excretion. In addition mesna was shown to have no effect on the antitumor activities of oxazaphosphorines.

Acute toxicites reported in mice (LD50 IV=1900 mg/kg) and rats (LD50 IV=1850 mg/kg) were reduced motor activity, breathing difficulties, cyanosis, piloerection, arched back, diarrhea, and tremor followed by convulsions. In dogs, clinical signs were diarrhea, vomiting, restlessness, mastication and deglutition, arching of back, peripheral vasodilation and writhing movements. At lethal doses in dogs (500 mg/kg) symptoms were loss of reflexes, mydriasis, cyanosis, brady cardia, abdominal contractions, prostration, melena and dyspnea.

Subacute toxicity studies in rats (6 wks) indicated signs of weight loss, leucocytosis, anemia, increased relative organ weights of liver, lung, spleen and kidney and distended tubules in the kidney with high protein concentration and hyaline deposits at doses greater than 1000 mg/kg/d. The six week intravenous study in dogs showed toxicities including emesis, diarrhea, and minor changes in hematology/serum chemistry values with no significant histopathology finding.

Mesna showed no significant teratogenic potential in segment II studies nor mutagenic potential by standard assays. No long term carcinogenic studies were undertaken. Based on preclinical pharm/tox data, there appear to be no unusual toxicities which should preclude safe utilization of this drug. The package insert appears to voice all precautions necessary for the safe administration of mesna in association with oxazaphosphorine chemotherapy.

Recommendations:

This NDA is approvable from the standpoint of pharmacology and toxicology considerations.

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cc:
Orig. NDA 19,884
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