Eur J Cancer Clin Oncol, Vol. 18, No. 12, pp. 1377–1387, 1982.
Printed in Great Britain.

0277-5379/82/121377-10$03.00/0

Studies on the Urotoxicity of Oxazaphosphorine Cytostatics and its Prevention—III. Profile of Action of Sodium 2-mercaptoethane Sulfonate (Mesna)*

NORBERT BROCK,† JÖRG POHL,† JURIJ STEKAR† and WOLFGANG SCHEEF‡

†*Asta-Werke AG Degussa Pharma Gruppe, Abteilung Experimentelle Tumorforschung, D-4800 Bielefeld 14, F.R.G. and* ‡*Janker Klinik, D-5300 Bonn, F.R.G.*

Abstract—*Mesna is a pharmacologically unremarkable, physiologically largely inert and almost totally non-toxic thio compound. It is rapidly eliminated renally and only slightly permeates into tissues. It has been shown experimentally that the bladder damage inducible in the rat by administration of oxazaphosphorine cytostatics can be successfully prevented by quite small doses of mesna. The detoxifying action of mesna is limited to the kidneys and the efferent urinary tract. The systemic effects of the oxazaphosphorines, however, remain unaffected. This applies particularly to the curative oncocidal efficacy of these compounds. It has also been shown experimentally that mesna does not affect the curative effects of other cytostatic drugs (doxorubicin, BCNU, methotrexate, vincristine). The efficacy of the cardiac glycoside proscillaridin is also not impaired by mesna.*

INTRODUCTION

IT HAS already been reported how the development of a standardised experimental test model on the rat made it possible to study the causes of the urotoxicity of oxazaphosphorine compounds and to identify the renally excreted carriers of this severe side-effect [1–3]. The identification of the urotoxic oxazaphosphorine metabolites enabled a rational search for compounds having a detoxifying action which should, even after systemical administration, be limited as far as possible to the kidneys and the efferent urinary tract.

These investigations led to mesna (sodium 2-mercaptoethane sulfonate), a compound which ideally satisfies the requisite conditions by virtue of its high uroprotective efficacy and exceptionally low toxicity [4].

Figure 1 illustrates the uroprotective efficacy of mesna [4, 5]. An intravenous injection of 68.1 mg/kg ifosfamide induced in rats a cystitis manifesting itself macroscopically as a blue coloration (extravasation of intravenously administered trypan blue) and as oedema of the bladder tissue (bottom row). An intravenous injection of only 21.5 mg/kg mesna, administered 15 min before the urotoxic dose of ifosfamide was given, completely protected the bladder of the test rats against the urotoxic effects of the renally excreted ifosfamide metabolites (middle row). The bladders of these rats fully matched those of untreated controls both macroscopically and microscopically (top row).

Further investigations on the problem of prophylaxis of urotoxicity concerned the question of a possible intrinsic pharmacodynamic action of mesna and experimental studies of possible interactions of mesna with oxazaphosphorines and with other cytostatic agents. In view of the particular clinical importance, the question regarding a possible interference of mesna with the action of cardiac glycosides was also studied experimentally.

MATERIALS AND METHODS

Chemical compounds

Mesna, cyclophosphamide, ifosfamide and proscillaridine were procured from Asta-Werke AG, Degussa Pharma Group, Bielefeld. BCNU was kindly provided by the National Cancer Institute, Bethesda, MD; other compounds used

Accepted 28 July 1982.
*This work was supported by funds of the German Federal Ministry for Research and Technology.

originated from the producers: doxorubicin from Farmitalia Carlo Erba, Freiburg, vincristine from Eli Lilly, Bad Homburg v.d.H., 5-fluorouracil from Hoffmann-La Roche, Grenzach-Wyhlen, and methotrexate from Cyanamid-Lederle, Wolfratshausen. All compounds were tested for identity and purity by the chemical laboratories of Asta-Werke under the guidance of Drs Niemeyer, Scheffler and Weigert.

Animals

Mice (NMRI, Swiss albino, DBA2, CD_2F_1) weighing 18–27 g, both sexes, and rats (Sprague–Dawley and Wistar) weighing 150–280 g, both sexes, were obtained from professional breeder companies in SPF-conditions and were housed standardly, fed with Altromin®1324 standard diet and had free access to tap-water. BD II and BD IX rats and beagle dogs were obtained from our own breeding facilities and housed accordingly.

Procedures

Acute toxicity studies with mesna (i.v., i.p., p.o.) on mice and rats comprised 10 animals for each dose level and sex; LD_{50} values were calculated by the probit method according to Fisher.

Repeated i.v. administration with mesna was carried out for six weeks on rats ($n = 10$ per dose and sex) and on beagle dogs ($n = 3$ per dose and sex) with three dose levels, including one toxic dose. In long-term experiments on rats ($n = 10$ per dose and sex) mesna was administered orally for 6 months as a 40% solution in doses of 500, 1000 and 2000 mg/kg respectively.

Mutagenicity studies with mesna were conducted by the Ames' test on strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 of *Salmonella typhimurium* with and without activation by Aroclor-induced rat liver microsomal enzyme preparations (dose range: 1.0–10000.0 μg/plate).

Studies on the problem of interference of mesna with the activity of antitumour agents covered investigations on acute and subacute toxicity of oxazaphosphorines, on antitumour effectiveness of cytostatics and on leucotoxicity and immunosuppression of oxazaphosphorines. All tumours used for evaluation of antitumour activity have been handled according to WHO descriptions [6]. Experimental details will be presented with the results of the different investigations.

RESULTS

Pharmacodynamics of mesna

Mesna has been subjected to extensive pharmacological investigations, including studies of its intrinsic effects tested on various experimental models and studies of possible antagonistic effects [7]. Some of the results particularly important for the assessment of mesna are summarised below.

In the mouse (NMRI) very high doses of mesna (1000 mg/kg i.p.) induced slight excitation. Doses of up to 21.5 mg/kg i.v. induced no mydriasis. Doses of up to 100 mg/kg i.p. had no effect on the tremor or on the hypersalivation induced by tremorin. Doses of up to 164 mg/kg p.o. had no effect on the model of acetic acid analgesia and doses of up to 328 mg/kg p.o. had no potentiating effect on pentobarbital anaesthesia.

In the rat (Wistar) doses of up to 164 mg/kg p.o. had no effect on the carrageenin oedema. Doses of up to 164 mg/kg i.p. had no effect on the righting reflex and induced no catatonia, and doses of up to 328 mg/kg p.o. had no effect in the rotating rod test.

In the guinea-pig doses of up to 2.5 mg/kg i.v. had no histamine-antagonistic effect. In numerous other model experiments on mice, rats and guinea-pigs, mesna showed no noteworthy pharmacological effects, even after administration of very high doses (up to 1000 mg/kg) [7].

In circulation experiments on the dog unspecific reactions of the cardiovascular system (mainly drops in the blood pressure and bradycardia) were observed after doses of 200 mg/kg i.v. and higher. In the cat the blood pressure and heart rate were unaffected by doses of up to 100 mg/kg i.v.

Mesna doses of up to 500 mg/kg i.v. induced no changes in the cortical EEG of the rabbit. Doses of 1000 mg/kg i.v., however, induced slight signs of irritation in the EEG pattern.

Summing up, it can thus be stated that mesna is pharmacologically rather unremarkable; no specific effects were observed in the extensive pharmacological testing even after administration of very high doses.

Toxicology of mesna

Acute toxicity experiments were carried out on mice (NMRI and Swiss albino), rats (Wistar and Sprague–Dawley) and dogs (mongrels and beagles) [7]. The LD_{50} values determined for mice and rats after single intravenous, intraperitoneal and oral doses of mesna are specified in Table 1. In dogs death was observed after intravenous doses of 400 mg/kg and higher, but not after oral doses of up to 2000 mg/kg.

The low toxicity of mesna was confirmed in experiments with repeated administration. In a 6-week toxicity experiment rats (Sprague–Dawley) tolerated daily intravenous doses of up to 1000 mg/kg. The earliest sign of toxicity under

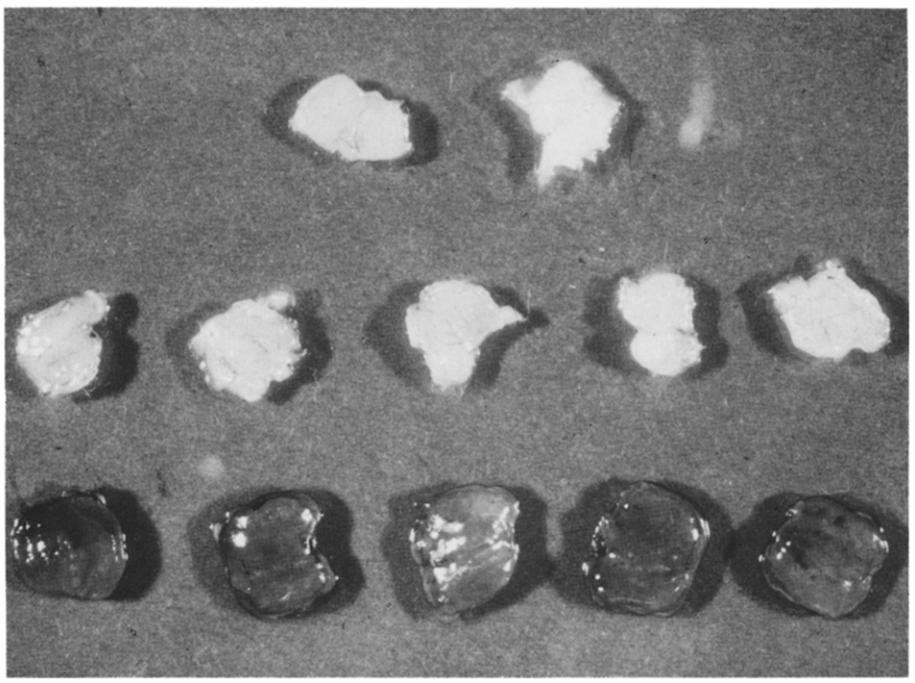

Fig. 1. *Macroscopic findings of urinary bladders of rats following i.v. administration of trypan blue. First row: controls without administration of ifosfamide, normal bladders; second row: treatment with* 68.1 mg/kg *ifosfamide i.v. with concomitant administration of the antidote mesna* (21.5 mg/kg *i.v.*); *third row: administration of* 68.1 mg/kg *ifosfamide i.v. without antidote.*

Table 1. Single-dose LD_{50} values of mesna in mice and rats determined by different routes of administration

Species	Sex	LD_{50} (mg/kg) i.v.	i.p.	p.o.
Mouse	m	1887 (1734–2053)	2005 (1732–2322)	6102 (5583–6669)
Mouse	f	2048 (1798–2334)	2089 (1893–2036)	>7200
Rat	m	2098 (1906–2310)	1529 (1369–1708)	4440 (4216–4675)
Rat	f	1683 (1377–2056)	1251 (1119–1399)	4679 (4438–4932)

The figures in parentheses are the 95% confidence limits.

this highest dosage was a retardation of the weight development. In a similar 6-week toxicity experiment dogs (beagles) tolerated daily intravenous doses of up to 316 mg/kg. The only toxic symptoms were vomiting and diarrhoea. In the 100 mg/kg group these symptoms vanished after the first 2 weeks of administration, whilst in the 316 mg/kg group they occasionally persisted to the end of the experiment. No compound-specific effects were brought to light by macroscopic and histological examinations.

In long-term toxicity experiments on rats (oral administration of a 40% mesna solution for 6 months) daily doses of up to 2000 mg/kg were tolerated without compound-linked mortality or morbidity.

In embryotoxicity studies no evidence of adverse effects of mesna on the reproduction process was found in rats at daily oral doses of up to 2000 mg/kg from day 8 to day 15 of gestation inclusive, and in rabbits at daily oral doses of up to 1000 mg/kg from day 7 to day 17 of gestation inclusive.

No evidence of mutagenicity of mesna was found in the AMES' test on strains of *Salmonella typhimurium*.

The local tolerance of mesna was tested on the eye and on the ear vein of the rabbit. In single-dose tests 40% solutions of mesna were tolerated in the conjunctival sac and in the marginal vein of the ear. With repeated injections into the ear vein on 5 consecutive days 40% mesna solutions were not tolerated, 20% solutions caused moderate irritation symptoms, whilst 10% solutions were tolerated without any local irritation symptoms.

Thus it can be concluded that the toxicity of mesna is very low. In the rat the doses required for effective prophylaxis against bladder damage induced experimentally with oxazaphosphorine cytostatics are of the order of 1% of the LD_{50} values. It may thus be stated that the therapeutic range of mesna is very wide; accordingly, this compound is well suited for clinical use.

Pharmacokinetics of mesna

The special position occupied by mesna with respect to its uroprotective efficacy amongst numerous thio compounds is based on the peculiarities of its pharmacokinetic behaviour [4, 8]. Some of the more important findings are briefly summarised below.

Systemically administered mesna shows almost no penetration into tissue but remains predominantly in the intravasal space. In experiments with radioactively labelled mesna (^{35}S, ^{3}H) no enrichment in activity in comparison with the blood was found in any of the organs except the kidneys [9]. Mesna is very rapidly eliminated through the kidneys and accumulates in the urine. In the rat over 80% of the administered dose is recovered in the urine within 3 hr after intravenous administration [7].

The only known metabolite of mesna is mesna disulfide, which is formed spontaneously by autooxidation. In the blood mesna is found predominantly in the form of the disulfide. This is eliminated through the kidneys by glomerular filtration, being partly reduced to mesna during excretion [10]. Some 30–60% (average 45%) of the administered dose is found in the urine in the form of mesna, the reactive thiol.

The renal excretion of other thio compounds is substantially slower and generally much less complete. Thus the amounts recovered in the rat urine within 6 hr after intravenous administration of high doses (100–316 mg/kg) of various thio compounds amounted to only 1.7% of the dose in the case of carboxymethylcysteine, to 3.3% for disulfiram, to about 6.5% for the disulfiram metabolite DDTC and to about 6% for *N*-acetyl-L-cysteine.

These low urinary excretion rates of other thio compounds correlate well with their experimentally proven low or absent uroprotective efficacy [4].

Interactions of mesna with oxazaphosphorines and with other cytostatics

The doses of mesna required to prevent haemorrhagic cystitis induced experimentally with oxazaphosphorine cytostatics are very small, of the order of 1% of the LD_{50}. In quantitative terms the doses of mesna required for successful uroprophylaxis are about 30% of the oxazaphosphorine doses, corresponding to a molar mesna: oxazaphosphorine ratio of about 0.5:1.

Accordingly, this molar ratio was used as a basis for the dosage in the experiments aimed at testing possible interferences of mesna with the toxic and chemotherapeutic effects of oxazaphosphorines. In some experiments higher molar proportions of mesna were applied as well.

Interference of mesna with the acute toxicity of ifosfamide and cyclophosphamide

In order to study the question of interaction of mesna with oxazaphosphorine cytostatics, the acute toxicity of ifosfamide on its own and of cyclophosphamide on its own was compared to that of various combinations of each of these two cytostatics with mesna in parallel experiment series on male rats ($n = 10$ per dose; Sprague-Dawley).

In a first series of experiments the lethality of various doses was determined at the uroprotectively effective constant molar cytostatic: mesna ratio of 1:0.5.

In a second series of experiments mesna was combined with each of the two oxazaphosphorines in equal absolute doses (w/w), i.e. the molar oxazaphosphorine: mesna ratio was 1:1.6.

In order to test the effects of different elimination kinetics of the two compounds, the experiments with mesna in conjunction with ifosfamide were conducted in two variants in both series of experiments. In the first variant mesna was administered simultaneously, in the second variant it was administered one hour after the administration of ifosfamide. The observation period was 14 days in all the experiment series. The LD_{50} was determined by the probit method according to Fisher.

The results of the individual experiment series were evaluated statistically by probit analysis, with a chi-square test for parallelism of the dose-response curves and a chi-square test for homogeneity for the investigation material. The dosage scheme and the LD_{50} values with their 95% confidence limits are shown in Table 2. The two chi-square tests showed that the dose-response curves of the experiment series are parallel to each other and that the composition of the investigation material is homogeneous. The probit analysis showed no significant separation of the individual test groups at the 5% level.

The time-course of the mortality also showed no differences between the individual experiment series. Both the mean and the median survival times were practically the same in the individual experiment series.

Studies on the interaction of mesna with ifosfamide in toxicity experiments on rats and dogs with i.v. administration for 6 weeks

In the experiments on rats ifosfamide was administered intravenously for 6 weeks in daily doses of 1.0, 3.16, 10.0 and 31.6 mg/kg. In a parallel series of experiments this treatment was combined with the concurrent i.v. administration of mesna in doses 10 times higher than those of ifosfamide (molar ratio = 16). Each dosage group in each series of experiments consisted of 15 male and 15 female rats.

The experiments on dogs were similar with ifosfamide doses of 1.0, 2.15, 4.64 and 10.0 mg/kg. Each dosage group in each series of experiments consisted of 3 male and 3 female beagles.

The toxicity symptoms observed with the rats and the dogs, and the mortality in the higher dosage groups, were due solely to ifosfamide. No evidence was found of any influence of the relatively high mesna doses on the toxicity symptoms, particularly on the haematochemical and haematological parameters and the body weight.

Studies on the question of an influence of mesna on the chemotherapeutic efficacy of cytostatics

The influence of mesna on the curative efficacy of cyclophosphamide and of ifosfamide was studied in parallel series of experiments with various experimentally induced tumours, including leukaemia L5222, Yoshida's sarcoma and solid DS carcinosarcoma of the rat and leukaemia L1210 of the mouse.

In these parallel experiment series mesna was combined with ifosfamide in molar ratios of 0.5:1, 1.6:1 and up to 21.5:1. In addition, in order to study possible effects of the different pharmacokinetics of the two compounds the relative

Table 2. Comparison of the acute toxicity of single i.v.-administered oxazaphosphorines with and without concomitant i.v. administration of mesna: dosage scheme and LD_{50} *values* (mg/kg)

Combination	Doses (mg/kg)					LD_{50} (mg/kg)
Ifosfamide	316	383	464	562		424 (380–517)
+Mesna, MR = 0.5 simultaneously	99.3	120.5	146.0	176.7		442 (395–517)
1 hr after IF	99.3	120.5	146.0	176.7		438 (408–472)
+Mesna, MR = 1.6 simultaneously	316	383	464	562		383 (348–414)
1 hr after IF	316	383	464	562		410 (354–465)
Cyclophosphamide	178	215	261	316	383	272 (252–294)
+Mesna, MR = 0.5 simultaneously	55.9	67.6	82.1	99.4	120.4	272 (255–290)
+Mesna, MR = 1.6 simultaneously	178	215	261	316	383	260 (238–285)

In parentheses: 95% confidence limits. MR = molar ratio mesna: oxazaphosphorine; IF = ifosfamide.

times of administration were varied, with mesna being administered 15 min before, simultaneously or up to 120 min after the administration of ifosfamide.

The results of all these parallel experiments were quite clear-cut. They all show that mesna does not affect the curative efficacy of ifosfamide or of cyclophosphamide. Further, similar parallel experiments have shown that mesna does not affect either the efficacy of other cytostatics or the pharmacological action of the cardiac glycoside proscillaridine.

Leukaemia L5222 of the rat

Cyclophosphamide and ifosfamide both have a dose-dependent curative effect. The treatment was administered only once on day 5 after implantation, i.e. at an advanced stage of the leukaemia. Mesna had no effect on the curative efficacy (Table 3).

Table 3. Rat BD IX, leukaemia L5222 (3×10^6 cells i.p.), single i.v. administration on day 5: results of oxazaphosphorine treatment, with and without concomitant administration of mesna

Dosage (mg/kg)			Cured rats
Cyclophosphamide	Ifosfamide	Mesna	(x/n)
0.464	—	—	0/6
0.464	—	0.464	0/6
1.00	—	—	3/6
1.00	—	1.00	5/6
2.15	—	—	3/5
2.15	—	2.15	5/5
—	4.64	—	10/12
—	4.64	1.46	6/6
—	4.64	4.64	6/6
—	10.0	—	10/12
—	10.0	3.15	5/6
—	10.0	10.0	5/6
—	10.0	21.5	6/6
—	10.0	46.4	6/6
	10.0	100	6/6
—	10.0	215	12/12
Controls			0/24

Yoshida's ascites sarcoma AH 13s of the rat

In a dosage range from 0.63 to 2.5 mg/kg (example: cyclophosphamide) the cytostatic treatment increases the median survival time. This chemotherapeutic effect is not influenced by high doses of mesna (100 mg/kg).

At higher dosages (>2.5 mg/kg; example: ifosfamide) definitive cures are achieved. The curative efficacy of the two oxazaphosphorines is also unaffected by high overdoses of mesna (Table 4).

Solid DS carcinosarcoma of the rat

Untreated, subcutaneously implanted solid DS carcinosarcoma kills all the tumour-bearing animals when an average tumour weight of about 80 g has been attained (mean survival time: 26 days). A single high dose of ifosfamide (215 mg/kg i.v.) induces inhibition of the tumour growth in all the test rats. Some 35% of the test rats are cured. In the others the average survival time is prolonged from 26 to 71 days. These therapeutic results are not impaired by the simultaneous administration of mesna at a molar dose ratio of 0.5:1 or of 1.6:1 compared to the dose of ifosfamide (Table 5). In fact, Table 5 shows a slight improvement of the therapeutic effect in the groups treated with mesna, but these differences are not significant.

Table 4. Rat Sprague-Dawley, Yoshida's ascites sarcoma AH 13s (10^6 cells i.p.), single i.v. administration on day 1: curative efficacy of cyclophosphamide and ifosfamide, with and without concomitant administration of mesna

Dosage (mg/kg)			Cured rats	MST
Cyclophosphamide	Ifosfamide	Mesna	(x/n)	(days)
Controls	—	—	0/6	6.0
0.63	—	—	0/6	7.5
0.63	—	100	1/6	7.0
1.25	—	—	0/6	11.0
1.25	—	100	0/6	10.0
2.5	—	—	1/6	22.0
2.5	—	100	1/6	32.0
—	4.64	—	11/12	
—	4.64	1.46	6/6	
—	4.64	4.64	6/6	
—	10.0	—	12/12	
—	10.0	3.15	5/6	
—	10.0	10.0	6/6	
—	10.0	21.5	6/6	
—	10.0	46.4	6/6	
—	10.0	100	6/6	
—	10.0	215	5/6	

MST = median survival time.

Leukaemia L1210 of the mouse

A single intravenous administration of cyclophosphamide or ifosfamide induces a dose-dependent prolongation of the median survival time. This therapeutic effect is not impaired by simultaneous administration of mesna (Table 6).

Other cytostatics

Doxorubicin and BCNU, like the oxazaphosphorine cytostatics, induce dose-dependent cures of the leukaemia L5222 of the rat (Table 7). In the leukaemia L1210 of the mouse, a dose-dependent prolongation of the median survival time can be induced with vincristine, 5-fluorouracil, methotrexate and *cis*-platinum. The chemotherapeutic efficacy of these cytostatics is not impaired by mesna (100 mg/kg, administered simultaneously; Table 8).

Cardiac glycosides

The effect of mesna on the mean lethal medication rate was investigated in guinea-pigs, according to Lenke [11]. In the case of i.v. infusion of proscillaridine this rate amounts to 39.6 mg/kg per min without mesna and to 37.2 mg/kg per min in combination with 100 mg/kg mesna. The difference is not significant statistically at the 5% level.

Table 5. Solid DS-carcinosarcoma of the rat, BD II (~ 5 × 10⁶ cells i.m.): therapy with ifosfamide, with and without mesna, by i.v. injection on day 7 or 8 after implantation, at a tumour weight of 1–2 g

Ifosfamide dose (mg/kg)	Mesna dose (mg/kg)	Number of rats (*n*)	Weight of the rats ($\bar{x} \pm$ S.D.) (g)	Weight of the tumour (g) on day 7*	14	21	28	MST† (days)	Cured rats (*x*/*n*)
—	—	25	312 ± 48	1.64	15.8	48.3	81.2‡	25.8	0/25
215	—	25	328 ± 30	1.75	1.18	1.49	2.39	70.8	7/25
215	67.6	10	304 ± 31	1.25	0.42	0.44	0.84	80.4	5/10
215	215	10	327 ± 45	1.73	0.69	1.46	2.14	77.5	5/10

*On the day of treatment.
†Mean survival time of the rats dying from the tumour.
‡Weight of the tumour on day 25 after implantation.

Table 6. Mouse CD_2F_1, leukaemia L1210 (10^5 cells i.p.): cyclophosphamide treatment (single i.v. administration on day 1), with and without simultaneous i.v. administration of mesna (n = 6 mice per dosage group)

Dosage (mg/kg) Cyclophosphamide	Mesna	MST (days)
Controls		11
31.6	—	12
31-6	31.6	11
68.1	—	15
68.1	68.1	14
147.0	—	18
147.0	147.0	17

MST = median survival time in days.

Table 7. Rat BD IX, Leukaemia L5222 (3 × 10⁶ cells i.p.), single administration on day 5: results of treatment with BCNU (i.p.) or doxorubicin (i.v.), with and without concomitant administration of 100 mg/kg mesna

Compound	Dosage (mg/kg)	Cured rats (*x*/*n*) without mesna	with mesna
Control	—	0/6	0/6
BCNU	0.100	1/6	2/6
	0.464	6/6	6/6
	1.00	6/6	6/6
Doxorubicin	1.00	0/6	0/6
	2.15	6/6	5/6
	4.64	6/6	5/6

Table 8. Mouse CD_2F_1, leukaemia L1210 (10^5 cells i.p.): results of i.p. treatment with 5-fluorouracil (5-FU), methotrexate (MTX), vincristine (VCR) and cis-platinum (DDP), with and without concomitant administration of 100 mg/kg mesna (n = 6 mice per dosage group)

	Dosage single dose (mg/kg)	Days of treatment	Total dose (mg/kg)	Mesna dose (mg/kg)	Without mesna MST	cured	With mesna MST	cured
Control	—	—	—	—	10.0	0/8	10.0	0/8
5-FU	10.00	1-9	90.00	9 × 100	15.0	0/6	15.5	0/6
	21.50	1-9	193.50	9 × 100	21.5	2/6	22.5	1/5
	46.40	1-4 (tox)	185.60	4 × 100	9.0	0/6	10.0	0/6
MTX	0.32	1-9	2.84	9 × 100	15.0	0/12	14.0	0/12
	1.00	1-9	9.00	9 × 100	22.0	1/12	19.0	1/12
	3.16	1-9 (tox)	28.44	9 × 100	23.0	2/12	21.0	1/12
VCR	0.10	1-9	0.90	9 × 100	18.0	0/6	17.0	1/6
	0.32	1-9	2.84	9 × 100	17.5	0/6	16.0	0/6
	1.00	1-4 (tox)	4.00	4 × 100	7.0	0/6	8.0	0/6
DDP	2.15	1		100	11.0	0/6	11.0	0/6
	4.64	1		100	12.5	0/6	12.0	0/6
	10.00	1		100	14.0	0/6	13.5	0/6

MST = median survival time in days; cured = 60-day survivors.

Investigations on the subject of a possible interaction of mesna with the leucotoxic and the immunosuppressive effects of ifosfamide

At doses above 21.5 mg/kg i.v. the oxazaphosphorine cytostatics induce in the mouse and the rat a dose-dependent leucopenia with a nadir on day 3 and a rapid overshooting regulation up to day 10.

Mesna on its own has no effect on the peripheral leucocyte counts and it also has no effect on the typical picture of the leucotoxic potency of ifosfamide (Fig. 2). This has been confirmed statistically by multiple variance analysis.

Concurrently with the leucotoxic effect, ifosfamide and cyclophosphamide exert a dose-dependent inhibiting effect on the formation of humoral antibodies after immunisation of the mouse with sheep erythrocytes and of the rat with *Brucella*. The intensity of the immunosuppression in these two models is not affected by combining the two cytostatics with mesna, even in very high doses (Fig. 3).

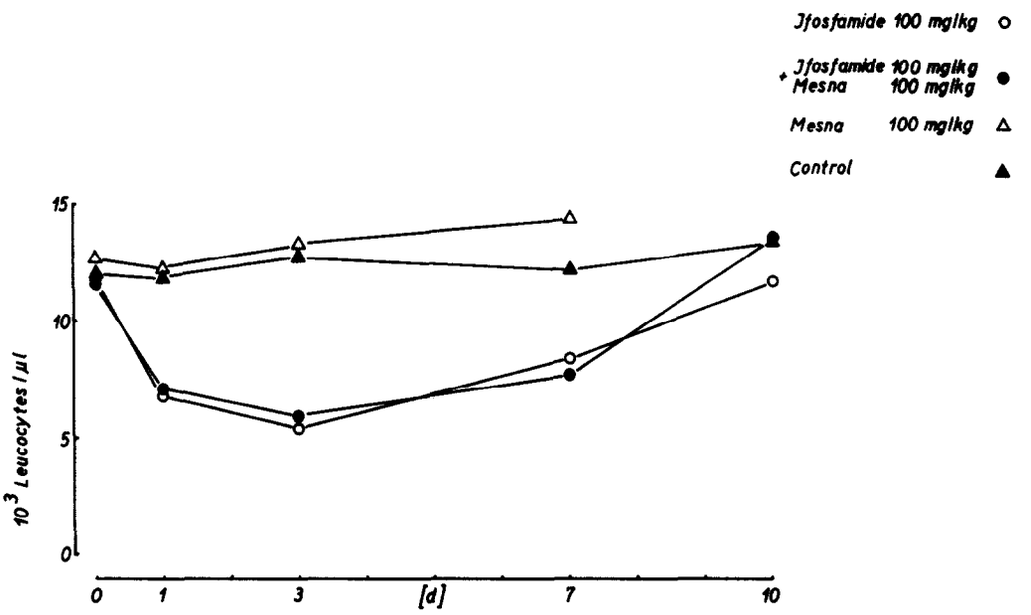

Fig. 2. The leucotoxic potency of 100 mg/kg *ifosfamide in rats* (○———○) *is not influenced by simultaneous administration of* 100 mg/kg *mesna* (●———●). *Mesna itself* (100 mg/kg, △———△) *does not change the number of peripheral leucocytes (untreated control,* ▲———▲).

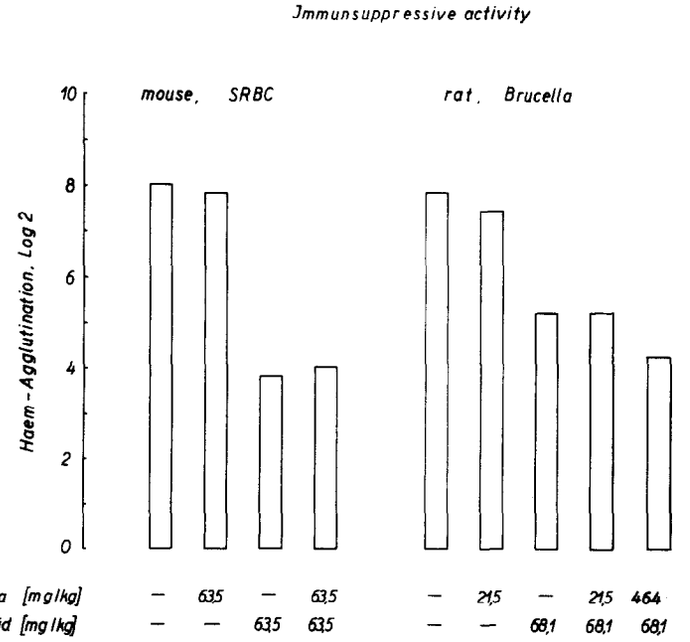

Fig. 3. Immunosuppressive efficacy of ifosfamide in the sheep erythrocyte immunisation of the mouse (SRBC) and in the Brucella *immunisation of the rat. The efficacy of ifosfamide is not attenuated by combination with mesna.*

DISCUSSION

In view of the insufficient tumour-specificity of most cancer chemotherapeutics, the dosage of these agents has to be pushed to the limit of tolerance, particularly in the treatment of solid tumours. This limit is determined by the occurrence of organotropic toxicity, for example in the gastrointestinal tract, and/or of specific myelo-, haemato-, pneumono-, hepato-, cardio- or nephrotoxicity.

With the oxazaphosphorine cytostatics cyclophosphamide, ifosfamide and trofosfamide, the predominant therapy-limiting factor is their urotoxicity [12]. The carriers of this urotoxicity are the 4-hydroxy-oxazaphosphorines, and especially acrolein, which is formed spontaneously from these metabolites [2, 3, 13]. This regionally limited toxicity prompted the search for a detoxification method which would also be regionally limited and would not affect the known, desirable or undesirable, systemic effects of these cytostatics.

Data on the chemical reactivity of the urotoxic oxazaphosphorine metabolites led to studies of thio compounds as regional detoxification agents [14]. The thio compounds characterised by rapid distribution in the organism, high penetration into tissues and cells and a slow renal elimination were found to be unsuitable for the protection of the kidneys and the efferent urinary tract. This group includes almost all the compounds of this general class which have found clinical uses as radiation protection compounds, as hepato-protective agents or as heavy metal antidotes and chelating agents.

It has been shown experimentally [4] that with these compounds, because of their slow renal elimination, urinary concentrations sufficient to ensure partial detoxification can only be achieved with extremely high doses which are often in the toxic range. Furthermore, the high penetration of these compounds into tissues and cells raises the risk of unpredictable interference with the chemotherapeutic effects and with the systemic toxicity of cytostatics. In some cases this interference may reduce the systemic toxicity and increase the therapeutic range of cytostatic agents in the treatment of some experimentally induced tumours [15], whereas other thio compounds cause a substantial increase in the systemic toxicity of cytostatics [16, 17].

Only a few of the thio compounds tested are eliminated renally and sufficiently rapidly to detoxify the toxic oxazaphosphorine metabolites excreted with the urine [4]. Amongst these few compounds mesna occupies a special place because it hardly penetrates into tissues at all, is particularly rapidly eliminated through the kidneys, is pharmacologically inert and is almost completely non-toxic. The exclusively regional detoxification achievable with mesna is based on the pharmacokinetics and metabolic peculiarities of this compound which have been thoroughly investigated and will be reported in detail elsewhere [18].

From the clinical viewpoint mesna is a drug by the means of which the urotoxicity of oxazaphosphorine cytostatics can be reliably prevented. This makes it possible to increase the dosage of these cytostatics up to the systemically tolerable limit and thus to achieve better therapeutic results.

Clinical experience has confirmed the good tolerance of mesna, the reliable protection of the efferent urinary system and, up to now, also the absence of interactions with the systemic oncocidal effects of the chemotherapy [19–23].

Acknowledgements—The authors wish to express their thanks for the painstaking technical assistance in the pursuit of these investigations provided by J. Dost, U. Dreier, M. Elliger, W. Holtei, M. Iselt, R. Kämpfer, E. Kampherm, J. Kottmeier, U. Laufkötter, P. Licher, B. Piening and U. Riemer.

REFERENCES

1. BROCK N, POHL J, STEKAR J. Studies on the urotoxicity of oxazaphosphorine cytostatics and its prevention—I. Experimental studies on the urotoxicity of alkylating compounds. *Eur J Cancer* 1981, **17**, 595–607.
2. ALARCON RA, MEIENHOFER J. Formation of the cytotoxic aldehyde acrolein during *in vitro* degradation of cyclophosphamide. *Nature New Biol* 1971, **233**, 250–252.
3. COX PJ. Cyclophosphamide cystitis—identification of acrolein as the causative agent. *Biochem Pharmacol* 1979, **28**, 2045–2049.
4. BROCK N, POHL J, STEKAR J. Studies on the urotoxicity of oxazaphosphorine cytostatics and its prevention—II. Comparative study on the uroprotective efficacy of thiols and other sulfur compounds. *Eur J Cancer Clin Oncol* 1981, **17**, 1155–1163.
5. BROCK N, STEKAR J, POHL J, SCHEEF W. Antidot gegen urotoxische Wirkungen der Oxazaphosphorin-Derivate Cyclohosphamid, Ifosfamid und Trofosfamid. *Naturwissenschaften* 1979, **66**, 60–61.

6. WHO. Descriptions of systems used in experimental screening of anti-cancer preparations in sixteen countries. *WHO Monogr* 1975, CAN/75.6.
7. POHL J. Toxicology, pharmacology and interactions of Uromitexan. In: BURKERT H, NAGEL GA, eds. *New Experience with the Oxazaphosphorines with Special Reference to the Uroprotector Uromitexan®*. Basel, Karger, 1981, 12–20.
8. POHL J, BROCK N, SCHNEIDER B, WETZELSBERGER K. Zur Pharmakokinetik von Uromitexan®. *Meth Find Clin Pharmacol* 1981, 3 (Suppl 1), 95–101.
9. GOBERT J, RISACK L, CLOSE J. Etudes biochimiques, métaboliques et pharmacologiques d'un mucolytique: le Mistabron. *Acta Anaesth Belg* 1971, **22**, 85–114.
10. BROCK N, STEKAR J. Verhütung urotoxischer Wirkungen von Cyclophosphamid und Ifosfamid durch Dimesna (Vorläufige Mitteilung). *Arzneim Forsch* 1982, **32**, 486–487.
11. LENKE D. Zur Frage der Standardisierung von deutschem Bulbus Scillae-Standard und Proscillaridin A am Meerschweinchen. *Arzneim Forsch* 1967, **17**, 1241–1242.
12. VAN DYK JJ, FALKSON HC, VAN DER MERWE AM. Unexpected toxicity in patients treated with iphosphamide. *Cancer Res* 1972, **32**, 921–924.
13. BROCK N, STEKAR J, POHL J, NIEMEYER U, SCHEFFLER G. Acrolein, the causative factor of urotoxic side-effects of cyclophosphamide, ifosfamide, trofosfamide and sufosfamide. *Arzneim Forsch* 1979, **29**, 659–661.
14. BROCK N, HOHORST HJ. The problem of specificity and selectivity of alkylating cytostatics: studies on *N*-2-chloroethylamidooxazaphosphorines. *Z Krebsforsch* 1977, **88**, 185–215.
15. GOLDIN A, VENDITTI JM, KLINE I, WODINSKY I, SCHABEL FM, JR. In: BURKERT H, ed. *Preclinical Investigations with Ifosfamide in Relation to Cyclophosphamide. Proceedings of the International Holoxan Symposium.* Bielefeld, Asta-Werke AG, 1977, 19–28.
16. HABS H, HABS M, SCHMÄHL D. Influence of pretreatment with disulfiram (DSF) on the acute toxicity of cyclophosphamide (CP) in male Sprague Dawley rats. *Arzneim Forsch* 1981, **31**, 530–531.
17. KELLY MG, RALL DP, TRIVERS GE, O'GARA RW, ZUBROD CG. Actions of S,2-aminoethylisothiuronium Br.HBr (AET). Toxicity and protective effect against nitrogen mustard toxicity. *J Pharmacol Exp Ther* 1960, **129**, 218–230.
18. ORMSTAD K, ORRENIUS S, LÅSTBOM T *et al.* Pharmacokinetics and metabolism of sodium 2-mercaptoethane sulfonate (mesna) in the rat. *Cancer Res* In press.
19. BURKERT H, SCHNITKER J, FICHTNER E. Verhütung der Harnwegstoxizität von Oxazaphosphorinen durch einen "Uroprotektor". *Munch Med Wochenschr* 1979, **121**, 760–762.
20. SCHEEF W, KLEIN HO *et al.* Controlled studies with an antidote against the urotoxicity of oxazaphosphorines. Preliminary results. *Cancer Treat Rep* 1978, **63**, 501–505.
21. KLEIN HO, WICKRAMANAYAKE P, COERPER C, CHRISTIAN E. Experimental and clinical studies on the significance of the uroprophylactic sodium 2-mercaptoethane sulfonate (Uromitexan) in cytostatic therapy with oxazaphosphorines. In: BURKERT H, NAGEL GA, eds. *New Experience with the Oxazaphosphorines with Special Reference to the Uroprotector Uromitexan®*. Basel, Karger, 1981, 25–29.
22. BRYANT B, JARMAN M, FORD H, SMITH J. Prevention of isophosphamide induced urothelial toxicity with 2-mercaptoethane sulfonate sodium (mesna) in patients with advanced carcinoma. *Lancet* 1980, **ii**, 657–659.
23. SCHEULEN M, NIEDERLE N, SEEBER S. Results of a phase II study on the use of ifosfamide in refractory malignant diseases. Comparison of the uroprotective effect of Uromitexan and forced diuresis with alkalization of the urine. In: BURKERT H, NAGEL GA, eds. *New Experience with the Oxazaphosphorines with Special Reference to the Uroprotector Uromitexan®*. Basel, Karger, 1981, 40–47.