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Perspectives in Cancer Research

Studies on the Urotoxicity of Oxazaphosphorine Cytostatics and its Prevention.*
2. Comparative Study on the Uroprotective Efficacy of Thiols and Other Sulfur Compounds

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Abstract—*Of the large number of thiols and other thio compounds which were tested in this connection, only a few possess the chemical, pharmacological and pharmacokinetic properties required for successful regional protection of the kidneys and urinary bladder against the urotoxicity of oxazaphosphorine cytostatics. Sodium-2-mercapto-ethane sulfonate (INN: Mesna, Uromitexan®) far excels all the other compounds tested with regard to uroprotective efficacy, low intrinsic toxicity, favorable pharmacokinetics and absence of systemic interactions with oxazaphosphorines. With correctly timed administration of mesna it is possible to completely obviate regionally the urotoxicity of oxazaphosphorines, which often constitutes a therapy-limiting factor, without impairing the therapeutically desirable systemically mediated oncotoxic effects of these drugs. Amongst the compounds tested in this connection, a few further compounds were also found to possess uroprotective efficacy, but only in high doses. Their therapeutic range is so narrow that toxic complications are likely to arise in clinical use. Thio-amino acids, for example, show a modest uroprotective effect only in very high, sub-toxic doses. The mechanism of the uroprotective efficacy of mesna is based on the formation of non-toxic compounds with the 4-hydroxy metabolites of oxazaphosphorines and with the acrolein which is formed in the urine from 4-hydroxy-oxazaphosphorines.*

INTRODUCTION

IT HAS been shown in Part 1 of this communication [1] that the urotoxic potency of oxazaphosphorine cytostatics (e.g., of cyclophosphamide and ifosfamide) is due to the renally excreted 4-hydroxy metabolites and especially to acrolein which is formed from these metabolites by β-elimination. The directly alkylating phosphoric acid diamides and other metabolites have a very much lower urotoxic potency or none at all [2, 3]. In the past, various measures have been recommended for the prevention of the urotoxic effects of oxazaphosphorine cytostatics, particularly for the prevention of hemorrhagic cystitis [4]. These measures included a high fluid intake, administration of diuretics, alkalization of the urine and, as an example of local detoxification, instillation of mercapto compounds into the urinary bladder [5–7]. The efficacy and practicability of this "standard prophylaxis", however, were unsatisfactory. We therefore embarked on the development of an antidote which could be administered parenterally or orally, would reliably prevent damage to the kidneys and urinary bladder, but would not impair the antitumoral efficacy of oxazaphosphorines (regional urotropic detoxification). The desired uroprotector should be capable either of stabilizing the 4-

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hydroxy metabolites of oxazaphosphorines in the urine, thus preventing the release of acrolein, or of detoxifying the already released acrolein.

Approaching the problem from the standpoint of chemical reactivity, we concentrated our attention on thio compounds, especially on thiols, as they match the dual aim defined above [2, 8, 9].

Thio compounds have been developed and tested medically for various indication domains. Very extensive studies have been carried out with these compounds as protective compounds, e.g. for protection against radiation damage (*N*-acetyl-homocysteine and cysteine) and for protection of the liver (cysteine, *N*-acetyl-cysteine, *N*-acetyl-homocysteine, methionine, α-lipoic acid). Further important uses of these compounds are for detoxification in cases of heavy metal poisoning (D-penicillamine, dimaval, dimercaprol), in the therapy of rheumatism (D-penicillamine) and in the treatment of alcoholism (disulfiram). In recent years these compounds are increasingly being recommended as mucolytic agents (*N*-acetyl-L-cysteine, mesna, carboxymethyl-cysteine).

It is interesting to consider the many attempts made in the past, aimed at reducing the high toxicity of alkylating compounds used in the chemotherapy of cancer by administration of thio compounds, and thus increasing the selectivity of the antitumoral action of these alkylating agents [10, 11]. This aim has not been achieved so far. As a rule, the reduction in toxicity was accompanied by an attenuation of the therapeutic efficacy [12, 13].

The same applies to a specific prevention of urotoxic side effects because the thio compounds available in the past, e.g., *N*-acetyl-cysteine, reduced not only the urotoxicity but also the antitumoral efficacy of alkylating agents [12–14].

The assumption that the toxicity of oxazaphosphorine cytostatics is reduced by thio compounds is only valid with limitations. Several sulfur compounds in fact increase the toxicity of oxazaphosphorines [15, 16]. This possibility should always be borne in mind when assessing interactions between alkylating agents and sulfur compounds.

In our experiments aimed at developing a regional detoxifying agent we tested many sulfur compounds of various structural classes. The uroprotective efficacy was tested on the model of the oxazaphosphorine-induced cystitis of the rat (see Part 1 of this communication). This model makes it possible to determine the uroprotective efficacy under standardized conditions [1].

Besides uroprotection as such, we also studied the pharmacokinetics, the intrinsic toxicity and interactions with the oxazaphosphorine toxicity (see part 3 of this communication).

MATERIALS AND METHODS

Thio compounds

The following test compounds were used: sodium thiosulfate, $Na_2S_2O_3$, Merck, Darmstadt, No. 6528; L-methionine, Merck, Darmstadt, No. 5707; L-cysteine, Fluka, cysteine No. 152254; cysteamine hydrochloride, Merck, Darmstadt, No. 802835; *N*-acetyl-L-cysteine, Merck, Darmstadt, No. 12422; D-penicillamine, batch M 21-79, Chemiewerk Homburg, Frankfurt/Main; *S*-carboxymethyl-cysteine, Chemiewerk Homburg, Frankfurt/Main; sodium-2,3-dimercapto-1-propane sulfonate (dimaval), Heyl, Berlin; tetraethylthiuram disulfide (disulfiram), Merck, Darmstadt, No. 8106; sodium-diethyl-dithio-carbaminate (DDTC), Merck, Darmstadt, No. 6689.

The following compounds were supplied by the chemical research laboratories of the Asta-Werke, Bielefeld (Dr. Scheffler and Dr. Niemeyer): sodium-2-mercapto-ethane sulfonate (INN: Mesna Uromitexan®); disodium-2,2′-dithio-bis-ethane sulfonate (INN: Dimesna); sodium-3-mercapto-2-methyl-1-propane sulfonate (Asta 7082); sodium-3-mercapto-propane sulfonate (Asta 7100); sodium-6-mercapto-1-hexane sulfonate (Asta 7277); 2,3-dimercapto-succinic acid (Asta 7512).

The following compounds were made available by the chemical laboratories of the firm Degussa, Frankfurt (Dr. Martens): 3-mercapto-2-methyl-propionic acid (DA 1); 3-acetthio-2-methyl-propionic acid (DA 2); 3,3′-dithio-bis-(2-methyl-propionic) acid (DA 4); mercapto-2-methyl-propionyl-proline (DA 5); mercapto-2-methyl-propionyl-1, 2, 3, 4-tetrahydroisoquinolino-3-carboxylic acid (DA 6, DA 7); (DL)-thiazolidine-4-carboxylic acid (DA 8, DA 9); (DL)-5,5′-dimethyl-thiazolidine-carboxylic acid (DA 10, DA 11); *N*-(3-mercapto-2-methyl-propionyl)-glycine (DA 12); *N*-(3-mercapto-2-methyl-propionyl)-homocysteine (DA 13); α-lipoic acid (6,8-dithio-octanoic acid).

Beta-mercapto-ethane-phosphonic acid was synthesized in the chemical research laboratories of the firm Henkel, Düsseldorf, by Dr. Budnowski and his team and was kindly made available for these studies.

The agent used for the experimental in-

duction of haemorrhagic cystitis in rats was ifosfamide (Holoxan®), Asta-Werke, Bielefeld.*

Test animals

Species: rat

Strains: Sprague–Dawley (SPF) and BD II (SPF)

Breeders: Asta-Werke, Bielefeld, and Mus Rattus AG, Brunnthal

Sex: ♂ and ♀

Weight: about 250 g

Food: altromin® 1324, no deprivation, water *ad libitum*

Housing: standard conditions

Groups: 5 or 10 rats per test group

Dosage: screening dose 100 mg/kg, raised or lowered in steps with a factor of 2.15.

Procedure

Experimental induction of haemorrhagic cystitis in the rat. The test rats were given an i.v. dose of 68 mg/kg ifosfamide. In a few experiments ifosfamide was also used in doses of 100 and 147 mg/kg.

Investigations of the uroprotective efficacy. The investigated compounds were administered either i.v. 15 min before ifosfamide or orally by stomach tube 60 min before the injection of ifosfamide. In orienting experiments the time interval between the administration of the test drug and that of ifosfamide was varied for the purpose of optimization.

For a comparative assessment of the test compounds the dose was increased, if the intrinsic toxicity of the compound allowed this, until complete protection of the urinary bladder was achieved. In the case of test compounds with a good protective efficacy, the smallest reliably uroprotective dose was determined.

Investigations of the pharmacokinetics. In the case of some of the test compounds the excretion kinetics were investigated in order to determine the biological availability of the compound in the urinary bladder. The free sulfhydryl compounds and the thiol compounds, detectable by reduction of the disulfide bonds, were assayed photometrically at 405 nm by a method developed by Ellman and modified by Stekar [17, 18], based on a colour reaction with 5,5'-dithio-bis-(2-nitrobenzoic acid).

*We wish to thank Drs. Scheffler, Niemeyer, Martens and Budnowski for the provision of test compounds.

Evaluation

The uroprotective efficacy of each test drug was evaluated 24 hr after the administration of ifosfamide. The rats were killed with CO_2, laparatomized and the urinary bladder was excised.

A detailed description of the evaluation procedure has already been given elsewhere [1].

RESULTS

Experimental induction of haemorrhagic cystitis in the rat

The severity of the inflammation of the bladder after i.v. administration of ifosfamide is dose-dependent. An ifosfamide dose of 68 mg/kg always induces an approximately 2-fold increase in the wet weight of the bladder and a damage score of about 2. Some of the rats also showed bladder haemorrhages. The scoring system used takes into account two parameters: firstly, increased capillary permeability (which is demonstrable objectively by the extravasal occurrence of intravenously injected trypan blue) and secondly, the increase in the weight of the bladder, which can also be assessed macroscopically as a swelling of the bladder [1]. In some selected groups the bladder damage and the extent of bladder protection were also investigated histologically.

Investigations of the uroprotective efficacy

All the test compounds were administered in the first instance intravenously or intraperitoneally at the screening dose level of 100 mg/kg. With some test compounds, belonging to various structural classes, it proved possible to prevent completely the bladder damage caused by 68 mg/kg ifosfamide. The uroprotective effect was dose-dependent. It was reflected in a reduced increase in the bladder weight and reduced extravasation of trypan blue, and it was also demonstrable histologically. In the case of the test compounds with a demonstrable uroprotective effect we determined the lowest dose ensuring reliable uroprotection. The results of these investigations of the uroprotective efficacy of compounds of various structural classes are summarized in Tables 1, 2 and 3.

Uroprotective efficacy of mercapto-alkyl compounds. Table 1 summarizes the findings with mercapto-alkane-sulfonic acids of various chain lengths, all carrying a terminal sodium sulfonate group. In one case a phosphonic acid group was introduced. Also included in Table 1 for comparison is sodium thiosulfate, which has a similar structure.

Table 1. Uroprotective action of mercapto-alkane sulfonates and analogs

Compound	Structural formula	Dose mg/kg	Animals n	Assessment of urinary bladder: Inflamm. x/n	Bleeding x/n	Weight (mg) Mean ± S.E.	Score 0–3
Untreated controls		—	105	0	0	81.0 ± 12.0	0
Ifosfamide		68.1 i.v.	100	100	63	165.0 ± 35.0	2.3
Mesna	$HS-CH_2-CH_2-SO_3Na$	6.81 i.v.	10	6	1	97.3 ± 22.6	1.5
		10.0 i.v.	10	2	0	88.8 ± 5.4	0.5
		14.7 i.v.	10	0	0	77.5 ± 10.9	0.3
		21.5 i.v.	10	0	0	72.6 ± 10.3	0
Dimesna	$S-CH_2CH_2-SO_3Na$ / $S-CH_2CH_2-SO_3Na$	21.5 i.v.	10	6	3	137.5 ± 29.1	1.4
		31.6 i.v.	10	3	0	101.6 ± 12.4	0.3
		46.4 i.v.	10	1	0	86.6 ± 12.4	0.1
		68.1 i.v.	10	0	0	77.5 ± 10.9	0
Asta 7100	$HS-(CH_2)_3-SO_3Na$	21.5 i.v.	5	5	0	138.4 ± 26.5	2.0
		68.1 i.v.	5	3	0	80.4 ± 7.4	0.5
		215.0 i.v.	5	1	0	77.0 ± 10.1	0.1
Asta 7277	$HS-(CH_2)_6-SO_3Na$	100 i.v.	5	5	0	137.4 ± 47.1	1.7
Asta 7082	$HS-CH_2-CH(CH_3)-CH_2-SO_3Na$	10.0 i.v.	5	5	1	n.d.	2.2
		31.6 i.v.	5	4	0	n.d.	1.6
		100.0 i.v.	5	3	0	92.8 ± 20.6	1.1
Dimaval	$HS-CH_2-CH(SH)-CH_2-SO_3Na$	10.0 i.v.	10	8	1	139.9 ± 25.5	1.3
		21.5 i.v.	10	7	0	121.0 ± 22.9	1.1
		46.4 i.v.	10	5	0	116.8 ± 27.1	0.8
		100.0 i.v.	10	0	0	93.6 ± 11.4	0
Mercaptoethane-phosphonic acid	$SH-CH_2-CH_2-PO_3H_2$	2.15 i.v.	10	5	1	116.8 ± 39.4	0.9
		4.64 i.v.	10	1	0	78.0 ± 12.0	0.1
		10.0 i.v.	10	2	0	86.7 ± 20.3	0.2
		21.5 i.v.	10	0	0	72.7 ± 9.8	0
		46.4 i.v.	10	0	0	87.0 ± 13.0	0 (toxic)
Sodium thiosulfate	$Na_2S_2O_3$	261 i.p.	5	5	2	n.d.	2.4
		681 i.p.	5	5	1	n.d.	1.5
		1780 i.p.	5	1	0	n.d.	0.2

n.d. = not determined.

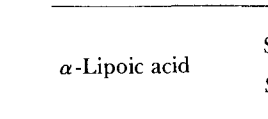
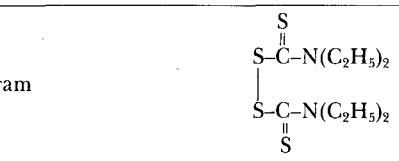
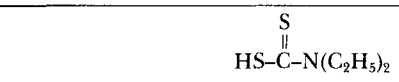
In this group of compounds we found a certain dependence of the uroprotective efficacy on the length of the chain. The highest efficacy, with a reliably uroprotective dose of 21.5 mg/kg, was found with the sodium salt of mercapto-ethane-sulfonic acid (mesna) and with the mercapto-ethane-phosphonic acid. The mesna disulfide (dimesna) is only slightly less effective, the reliably uroprotective dose being 46.4 mg/kg [19]. The longer-chain homologues, Asta 7100, Asta 7082 and Asta 7277, also exert a uroprotective effect at doses of about 100 mg/kg and more. The introduction of a second mercapto group, as in dimaval, does not improve the uroprotective efficacy (the uroprotective dose is also of the order of 100 mg/kg). The uroprotective efficacy of sodium thiosulfate is extremely low. The highest dose tested was 1780 mg/kg and showed only an indication of a uroprotective effect.

Uroprotective efficacy of mercapto-carboxylic acids. Table 2 presents the results of tests with various mercapto-carboxylic acids and their derivatives, one mercapto-amine and one dimercapto-alcohol. We found a notably high efficacy (uroprotective dose 31.6 mg/kg) in the case of 2,3-dimercapto-propanol (BAL), which is used therapeutically as a heavy-metal chelating agent. Cysteamine hydrochloride also has a good uroprotective efficacy (uroprotective dose 46.4 mg/kg).

The other mercapto-carboxylic acids listed in Table 2 were almost totally ineffective or were too toxic for these tests. A compound which was found to be extremely toxic is 3-acetthio-2-methyl-propionic acid (DA 2). This compound could only be tested up to a dose of 10 mg/kg and showed no uroprotective action at this dosage level. A slight uroprotective action was found with dimercapto-succinic acid (above 147 mg/kg) and with α-lipoic acid (above 215 mg/kg).

Uroprotective efficacy of mercapto-amino acids. The mercapto-amino acids and their derivatives lis-

Table 2. Uroprotective action of mercapto-carboxylic acids and other thio compounds

Compound	Structural formula	Dose mg/kg	Animals *n*	Assessment of urinary bladder: Inflamm. *x/n*	Bleeding *x/n*	Weight (mg) Mean ± S.E.	Score 0–3
Untreated controls		—	105	0	0	81.0 ± 12.0	0
Ifosfamide		68.1 i.v.	100	100	63	165.0 ± 35.0	2.3
Asta 7512	HS–CH–COOH | HS–CH–COOH	46.4 i.v.	10	8	3	150.6 ± 48.1	1.6
		68.1 i.v.	10	6	3	120.8 ± 29.9	1.1
		100 i.v.	10	6	1	117.6 ± 29.1	0.7
		147 i.v.	10	5	1	120.6 ± 30.4	0.6
DA 1	$HS-CH_2-CH(CH_3)-COOH$	46.4 i.v.	5(1)*	4	0	n.d.	1.4
DA 4	$S-CH_2-CH(CH_3)-COOH$ | $S-CH_2-CH(CH_3)-COOH$	100 i.v.	5	5	1	n.d.	1.8
α-Lipoic acid		100 i.v.	5	0	0	n.d.	tox.
		46.4 p.o.	10	4	0	111.1 ± 35.0	0.4
		100 p.o.	10	0	0	95.2 ± 12.5	0
		215 p.o.	10	1	0	97.7 ± 15.3	0.1
DA 2	$S-CH_2-CH(CH_3)-COOH$ | $CO-CH_3$	10.0 i.v.	2	2	0	n.d.	1.5
		46.4 i.v.	3(3)*	—	—	n.d.	lethal
Cysteamine·HCl	$HS-CH_2-CH_2-NH_2 \cdot HCl$	10.0 i.v.	5	5	1	141.2 ± 27.7	1.9
		21.5 i.v.	10	8	2	120.1 ± 36.9	1.5
		31.6 i.v.	10	5	1	89.4 ± 25.1	0.9
		46.4 i.v.	10	0	0	77.3 ± 14.2	0
		100 i.v.	10	0	0	71.6 ± 11.1	0(toxic)
		215 i.v.	5(3)*	0	0	72.5 ± 9.2	lethal
Dimercaprol	$HS-CH_2-CH(SH)-CH_2OH$	10.0 i.v.	10	4	0	104.0 ± 32.8	0.6
		21.5 i.v.	10	1	0	82.5 ± 9.8	0.2
		46.4 i.v.	10	0	0	76.4 ± 10.2	0(toxic)
		100 i.v.	10(5)*	0	0	72.2 ± 7.1	lethal
Disulfiram		100 i.p.	5	4	1	123.2 ± 22.4	1.2
DDTC		100 i.v.	5	5	0	144.6 ± 16.1	1.6

( )* = number of toxic deaths.
n.d. = not determined.

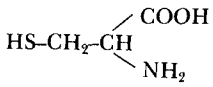
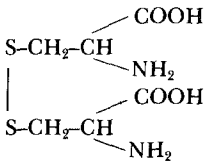
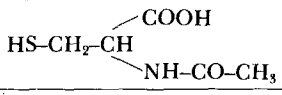
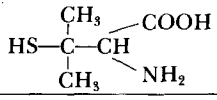
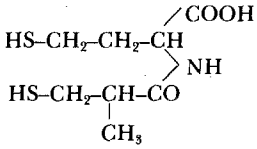
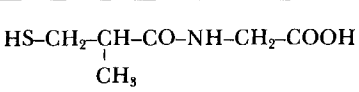
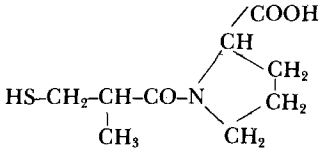
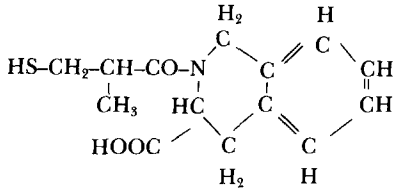
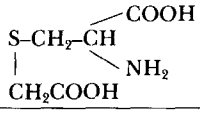
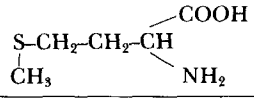
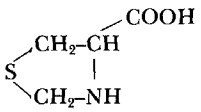
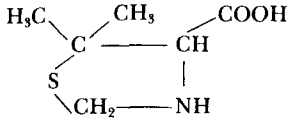
ted in Table 3 show uroprotective efficacy only in extremely high doses. As a rule a uroprotective effect is observed only at doses of about 500 mg/kg (orally) and more.

Comparative evaluation. Our findings show that the compounds listed in Table 1 (mercapto-alkane-sulfonates and mercapto-alkane-phosphonic acid) and some of the compounds listed in Table 2 (dimercapto-succinic acid (Asta 7512), α-lipoic acid, cysteamine, dimercaprol) possess a high uroprotective efficacy, whilst other mercapto-carboxylic acids shown in Table 2, sodium thiosulfate (Table 1) and practically all mercapto-amino acids (Table 3) show only a very low uroprotective efficacy or none at all.

It should be noted, however, that the uroprotective efficacy of dimercaprol and cysteamine is observed only in the toxic, sub-lethal dosage range and that toxic accompanying symptoms are also observed with uroprotectively effective doses of dimercapto-succinic acid and of α-lipoic acid. The assessment of *N*-acetyl-cysteine is also not much better. This compound exerts a vesico-protective effect when instilled directly into the bladder, but with systemic administration a sufficient degree of protection of the bladder can only be achieved with doses in excess of the very high level of 464 mg/kg (i.v.). *S*-Carboxymethyl-cysteine showed no uroprotective effect up to 316 mg/kg (i.v.).

Amongst the compounds studied, the two most potent uroprotective agents were found to be mesna and mercapto-ethane-phosphonic acid, both with a reliably uroprotective dosage

Table 3. Uroprotective action of mercapto-amino acids and derivatives

Compound	Structural formula	Dose mg/kg		Animals *n*	Assessment of urinary bladder: Inflamm. *x/n*	Bleeding *x/n*	Weight (mg) Mean ± S.E.	Score 0–3
Untreated controls		—		105	0	0	81.0 ± 12.0	0
Ifosfamide		68.1	i.v.	100	100	63	165.0 ± 35.0	2.3
L-Cysteine		234	i.v.	5	5	0	n.d.	2.0
		343	i.v.	5	5	0	n.d.	1.0
		504	i.v.	5	4	0	n.d.	0.5
		740	i.v.	5	0	0	n.d.	0
L-Cystine		511	i.p.	5	5	2	n.d.	2.0
		750	i.p.	5	4	3	n.d.	2.0
N-Acetyl-L-cysteine		215	i.v.	10	4	1	133.9 ± 38.3	0.7
		316	i.v.	10	1	0	109.6 ± 32.4	0.4
		464	i.v.	10	2	0	97.5 ± 20.2	0.2
DL-Penicillamine		21.5	p.o.	10	9	1	158.4 ± 28.9	1.7
		46.4	p.o.	10	6	0	119.4 ± 29.9	1.0
		100	p.o.	10	6	0	120.1 ± 48.8	1.05
DA 13		10.0	p.o.	10	8	1	164.5 ± 28.4	1.55
		21.5	p.o.	10	7	1	114.4 ± 36.1	1.1
		46.4	p.o.	10	2	0	87.6 ± 17.6	0.2
		100	p.o.	10	0	0	76.9 ± 7.9	0
DA 12		21.5	i.v.	10	5	0	112.8 ± 28.8	0.75
		46.4	i.v.	10	6	1	140.5 ± 35.6	1.05
		100	i.v.	10	2	0	95.1 ± 23.3	0.4
		215	i.v.	10	0	0	80.0 ± 12.2	0
DA 5		10.0	i.v.	10	10	1	132.5 ± 29.7	1.65
		21.5	i.v.	10	7	0	106.6 ± 27.0	1.0
		46.4	i.v.	10	4	0	96.4 ± 18.5	0.5
		100	i.v.	10	3	0	89.5 ± 16.2	0.25
		215	i.v.	10	5	0	88.0 ± 7.3	0.45
DA 6, DA 7		46.4	p.o.	10	10	2	174.4 ± 51.5	1.9
		68.1	p.o.	10	10	0	137.2 ± 28.3	1.6
		100	p.o.	10	8	0	149.3 ± 53.9	1.35
		147	p.o.	10	9	0	126.7 ± 46.2	1.1
		215	p.o.	10	10	0	149.0 ± 28.7	1.9
S-Carboxymethyl-cysteine		316	i.v.	5	5	0	129.6 ± 32.2	2.0
L-Methionin		1000	p.o.	10	10	1	111.0 ± 29.4	1.6
		1470	p.o.	10	6	0	105.8 ± 25.5	1.1
DA 8, DA 9		100	i.v.	5	5	0	n.d.	1.2
		100	p.o.	5	5	2	n.d.	1.6
DA 10, DA 11		100	p.o.	5	5	3	n.d.	1.9

n.d. = not determined.

level of 21.5 mg kg. Mercapto-ethane-phosphonic acid, however, is about 20 times as toxic for the rat as mesna (LD_{50} p.o. of about 200 mg/kg vs LD_{50} p.o. > 4500 mg/kg for mesna).

Thus, from the standpoint of selectivity of the uroprotective action and from that of the therapeutic range, the only uroprotective agent suitable for clinical use is mesna.

Uroprotective efficacy in function of the time interval. A regional protection of the kidneys and urinary bladder can only be expected if the toxic oxazaphosphorine metabolites and the uroprotective thiol compound are excreted renally at about the same time. We studied this question by varying the times of administration of mesna and ifosfamide (Table 4). In the model of the experimentally induced bladder damage of the rat the detoxifying uroprotective action of mesna against bladder damage induced by ifosfamide is particularly beneficial when mesna is administered shortly before or shortly after the administration of ifosfamide. If mesna is administered more than one hour after the injection of ifosfamide it can only prevent the occurrence of haemorrhages whilst the severity of the inflammation symptoms—swelling and extravasation of trypan blue—results in the same damage scores as those of the unprotected control rats. In a series of experiments designed for this purpose the optimal time for the intraperitoneal administration of mesna was found to be 20 min, and for oral administration 60 min, before the injection of ifosfamide.

Influence of the water balance. By analogy with the formerly practiced clinical prophylaxis against cystitis, we carried out on the test rats experiments with oral hydration, using 30 ml/kg water 2 hr before the administration of ifosfamide and, in addition, 50 ml/kg isotonic saline at the time of administration. Under the experimental conditions which we used, this had little effect on the bladder damage induced by ifosfamide. The uroprotective effect of mesna, on the other hand, was not attenuated.

Table 4. Time-dependent uroprotective action of mesna (21.5 mg/kg i.v.) *against the urotoxic effect of ifosfamide* (68.1 mg/kg i.v.)

Time of administration of mesna before (−) or after (+) ifosfamide (min)	Score of urinary bladder
Control	2.5
−15	0
0	0
+30	0
+60	0.7
+90	2.5
+120	2.5

Pharmacokinetic investigations

The decisive precondition for a pharmacologically mediated uroprotective efficacy is the renal excretion of thiol compounds and thus the presence of sulfhydryl groups in the urine. In the case of some selected test compounds we therefore carried out quantitative assays of the biological availability of sulfhydryl groups in the urine.

We observed almost no excretion of free sulfhydryl groups after oral or after i.v. administration of carboxymethyl-cysteine (316 mg/kg), i.p. administration of disulfiram (100 mg/kg) or i.v. administration of the disulfiram metabolite DDTC (100 mg/kg).

After i.v. administration of *N*-acetyl-cycteine (316 mg/kg) some excretion of sulfhydryl groups with the urine was demonstrable, but the total excretion in the form of the mercapto compound amounted to only 6% of the administered dose within 6 hr. With oral administration of *N*-acetyl-cysteine (316 mg/kg) to rats, only a negligibly small increase in the concentration of sulfhydryl groups in the urine was observed.

The pharmacokinetic behaviour of sodium-2-mercapto-ethane sulfonate (mesna) is totally different. After i.v. administration of 21.5 mg/kg to the rat, about 40% of the administered dose is excreted with the urine as the sulfhydryl compound within the first hour. Within the first 3 hr about 50% of the dose is excreted as the sulfhydryl compound and a further 30% of the dose as the disulfide. Rapid renal excretion of sulfhydryl groups is also observed after oral administration of mesna to rats. Similar findings have also been made on the dog.

The behaviour of dimesna after intravenous and after oral administration to rats and dogs is very similar to that of mesna. After i.v. administration of 42.4 mg/kg to rats, 28% of the dose was excreted with the urine as the free sulfhydryl compound within 3 hr [19].

According to these findings, the suitability of a thio compound for uroprotection against oxazaphosphorine cytostatics depends decisively on the pharmacokinetic behaviour of the compound, particularly on the excretion of uroprotectively effective sulfhydryl groups with the urine.

DISCUSSION

The urotoxic side effects of oxazaphosphorine cytostatics often constitute a therapy-limiting factor. In a search for agents capable of obviating these side effects we have tested various thio compounds for their suitability as uroprotectors. It was known that local application of sulfhydryl compounds, e.g., instillation of *N*-acetyl-cysteine into the bladder, makes it possible to bind and thus to detoxify the toxic metabolites of cyclophosphamide and ifosfamide. Such a local detoxification, however, is limited to the urinary bladder and does not extend to the kidneys, the renal pelves and the ureters, which are also at risk [20]. Furthermore, the instillation procedure itself is unpleasant for the patient and is burdened with risks. On the other hand, the systemic use of mercapto compounds, e.g., of *N*-acetyl-cysteine, has so far remained unsuccessful clinically because these compounds become distributed throughout the organism and reduce not only the urotoxicity but also the antitumoral efficacy of cytostatics.

Based on these considerations, we formulated a list of pharmacokinetic criteria which should be satisfied by the compound for which we were looking. Firstly, the compound concerned should show little penetration into tissues, in order not to attenuate the therapeutic effect of the cytotoxic agents. Secondly, the compound should be water-soluble and well tolerated. Finally, it should be excreted renally rapidly and completely and should be capable of deactivating in the kidneys and in the efferent urinary tract the simultaneously excreted aggressive metabolites of the oxazaphosphorine cytostatics.

Having devised a suitable experimental model for the investigation of urotoxicity, we proceeded to test a large number of sulfur compounds for their suitability for uroprotection with systemic administration. The decisive assessment criteria, apart from uroprotective efficacy as such, were the pharmacokinetic and metabolic behaviour, and the therapeutic range which depends on the intrinsic toxicity of thio compounds.

Most of the sulfur compounds studied were not capable of effecting a regional detoxification of the urotoxic oxazaphosphorine cytostatics. In the case of systemically effective so-called "protective compounds", e.g., against radiation damage, the preconditions for protective efficacy are a wide distribution and a long dwelling time in the organism. Accordingly, these compounds fail to satisfy the essential precondition for uroprotective efficacy, namely, rapid renal excretion with regional detoxification. The excretion of these thio compounds is so slow that the concentration of uroprotectively effective mercapto groups in the urine remains low and cannot match the rapid build-up of high urinary concentrations of 4-hydroxy-oxazaphosphorine metabolites and of the acrolein which they produce.

These considerations have been confirmed by our broad-based investigations with thio compounds of various chemical classes. As expected, most of the thio compounds, quite suitable for the systemic protective indications mentioned earlier, were unable, even at high doses, to prevent the urotoxic side effects of oxazaphosphorines. In some cases we even observed a marked potentiation of the toxicity of oxazaphosphorines. This applies particularly to disulfiram, to D-penicillamine and to cysteamine hydrochloride.

Some degree of protection of the kidneys and urinary bladder could be achieved only with a few thio compounds, including dimercapto-succinic acid, α-lipoic acid, dimercaprol and cysteamine. These compounds, however, become distributed through out the tissues and cells of the organism, so that their systemic intrinsic effects outweigh their regional uroprotective effects. The uroprotectively effective doses are in the toxic range and in some cases even in the sub-lethal range (see Table 2). The same also applies to *N*-acetyl-cysteine, which has occasionally been recommended for uroprophylaxis. With this compound, sufficient protection of the bladder can only be achieved with the high, sub-toxic dose of 464 mg/kg (i.v.). Quite apart from the very large amounts of *N*-acetyl-cysteine which would be required for effective uroprophylaxis in man, the clinical use of this compound would be burdened with the likelihood of undesirable interactions.

Mesna, on the other hand, differs in its pharmacological and pharmacokinetic properties from all the thio compounds investigated in this study. Mesna is generally incapable of permeating through the cellular membrane. In the blood plasma the thiol compound is very rapidly oxidized to the disulfide [21], which is chemically stable and physiologically inert. The renal excretion of mesna and of the corresponding disulfide is exceptionally rapid. The half-life in the serum and the half-life of elimination ($t_{1/2}$) are 1.4 hr in the rat and 1.5 hr in man [22].

Details on the pharmacokinetics of mesna and on the interactions of mesna, e.g., with the curative efficacy of oxazaphosphorine cytostatics, will be reported in Part 3 of this communication.

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