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Excretion of Sodium 2-Mercaptoethanesulphonate (MESNA) in the Urine of Volunteers after Oral Dosing

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Abstract—*Sodium 2-mercaptoethanesulphonate (MESNA) is a uroprotective agent generally given i.v. to prevent haemorrhagic cystitis during oxazaphosphorine cancer chemotherapy. Oral administration of the drug is described since this might be an important route during long-term oxazaphosphorine treatment. MESNA is absorbed from the GI tract and excreted in the urine (about 41.5% of the dose), peak excretion being 2-3 hr after administration. A proportion of the excreted dose is as free thiols (about 24.2%) and the remainder is as disulphides. MESNA is shown to enhance excretion of cysteine in urine.*

INTRODUCTION

MESNA is a recently introduced thiol drug which prevents the haemorrhagic cystitis associated with very high-dose oxazaphosphorine [e.g. cyclophosphamide (CP)] cancer chemotherapy. MESNA is given by repeated i.v. bolus injection and is quickly excreted in the urine. The mechanism of action of MESNA depends upon chemical reaction in the urine between toxic oxazaphosphorine metabolites and the thiol group of MESNA, resulting in the formation of relatively stable non-toxic compounds [1]. Some metabolites of the oxazaphosphorines are bladder carcinogens and MESNA is known to reduce the incidence of bladder tumours in rats given cyclophosphamide [2]. Chronic low-dose cyclophosphamide therapy is used in patients with severe systemic lupus erythematosus and other autoimmune diseases, since CP is a powerful immunosuppressant. CP at low doses is not associated with severe haemorrhagic cystitis and so regular i.v. administration of MESNA is not necessary. However, the carcinogenic metabolites of CP are still present in urine and so might eventually result in bladder tumours [3]. Administration of MESNA orally might therefore prevent the formation of bladder tumours in these patients. For this reason we have studied the excretion of orally administered MESNA in man and present data which suggest that giving MESNA by mouth might be a useful route of administration to be used for prophylaxis against oxazaphosphorine-associated toxicity to the bladder.

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MATERIALS AND METHODS

Chemicals

Sodium 2-mercaptoethanesulphonate (MESNA) was a gift from Boehringer Ingelheim, Hospital Division, Bracknell, Berks, U.K. All other chemicals were of general laboratory reagent grade.

Volunteers

Ethics committee approval was obtained before commencing this study.

Three male volunteers, A (71 kg, 25 yr), B (59 kg, 24 yr) and C (60 kg, 27 yr), were given 400 mg of MESNA orally dissolved in approximately 50 ml of undiluted orange squash, immediately followed by water (200 ml orally). Urine samples were collected at 15-min intervals after administration, the volumes measured and their free thiol content determined (see below). A control urine sample was taken shortly (less than 1 min) after administration. To facilitate regular urine collections the volunteers were given water (200 ml) orally at 30-min intervals for 150 min before and for 240 min after the MESNA administration.

Measurement of urinary free thiols

Thiols were measured by the Ellmans free thiol assay [4]. To an aliquot (0.1 ml) of urine, or diluted urine, was added water (0.9 ml), phosphate buffer (4.5 ml; 0.25 M, pH 7.4) and Ellmans reagent (0.5 ml). The mixture was allowed to stand at room temperature for at least 10 min before reading the $A_{412\,nm}$.

Measurement of urinary disulphides

Disulphides were reduced to thiols using sodium borohydride as follows: to an aliquot (3 ml) or urine was added water (1 ml) and sodium borohydride [1.5 ml; 4% w/v (aq.)]. The mixture was then incubated at 50°C for 30 min, then acetic acid [1.5 ml; 9% v/v (aq.)] was added, and when effervescence had subsided an aliquot (1 ml) of this was analysed for free thiols (see above).

RESULTS

Free thiols were excreted in the urine after MESNA oral dosing. About 98.8 ± 35.9 mg (mean ± S.D., $n = 5$) thiol equivalent (about 0.6 ± 0.22 mmol MESNA) of the MESNA dose appeared in urine within 4.5 hr. This is approximately 24.2% of the thiol dose. The first (major) peak in urinary excretion of thiols was between 1 and 2.5 hr after administration (Fig. 1) and represented about 10% of the dose.

Control urine contained very low levels of free thiols (control urine thiol level = 1.43 ± 0.57 μg/ml, $n = 6$) and thiol disulphides (control urine disulphide + thiol level = 11.2 ± 10.2 μg/ml, $n = 6$). MESNA readily forms a disulphide (2,2-dithio-bis-mercaptoethanesulphonate) and mixed disulphides with endogenous thiol compounds (e.g. cysteine); for this reason total thiol levels were measured after borohydride reduction of the urine samples. Approximately 166.2 ± 71.3 mg ($n = 5$) thiol equivalent as disulphides + free thiols was excreted within 4.5 hr, this being about 41.5% of the administered thiol dose (Table 1). The peak excretion was between 2 and 3 hr (Fig. 1). The thiols excreted as disulphide compounds are not likely to be important in the prevention of bladder tumours associated with carcinogenic oxazaphosphorine metabolites, or in the prophylaxis of haemorrhagic cystitis.

Table 1. Excretion of thiols (-SH) and thiols + disulphides (S-S) in volunteers given 400 mg MESNA orally

Volunteer	% dose excreted -SH	-SH + S-S
A	36.5	72.3
B	17.0	38.0
	30.5	37.6
C	15.8	26.0
	21.4	34.0

Excretion data are shown for duplicate studies for volunteers B and C.

In order to investigate the nature of the thiols excreted a specific high-pressure liquid chromatography (hplc) technique [5] was applied to the urine samples at the time of peak thiol excretion was found to comprise MESNA plus cysteine (Fig. 2). MESNA therefore results in enhanced excretion of cysteine in the urine.

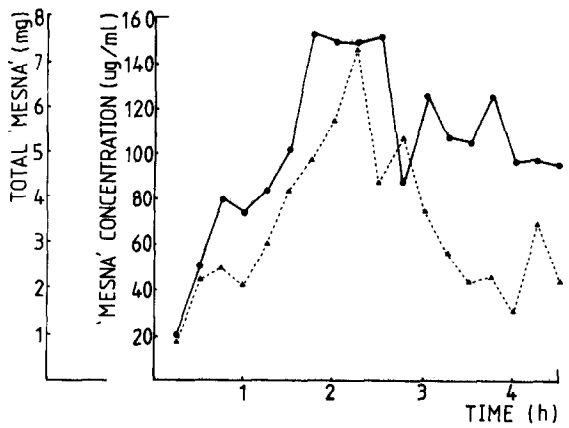

Fig. 1. Excretion of thiols, expressed as MESNA equivalents ('MESNA') in urine of volunteers (mean, n = 5) given 400 mg MESNA orally. (●——●) urinary total 'MESNA' and (▲- - - -▲) urinary 'MESNA' concentration.

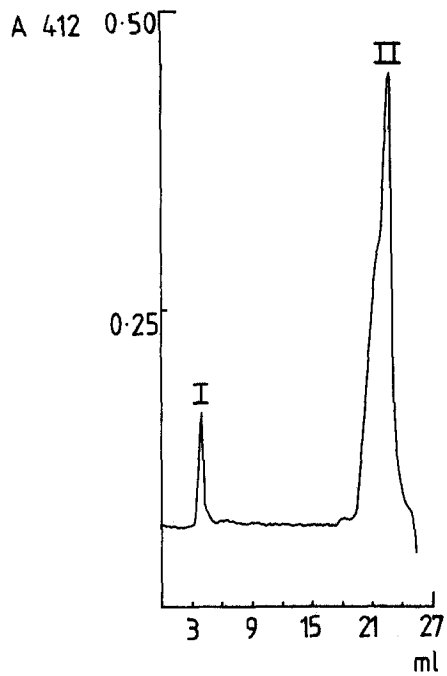

Fig. 2. Hplc trace of urine (1:5 v/v dilution with water) from a volunteer (B) given MESNA (400 mg) orally. Peak I = cysteine and peak II = MESNA. Cysteine is not detectable in diluted (1:5 v/v) control urine samples employing the same hplc conditions as used above.

DISCUSSION

Administration of MESNA orally results in its absorption (either as free MESNA or as DIMESNA) from the gastrointestinal tract, followed by excretion of urine. The peak urinary excretion of free thiols following the MESNA dose comprised MESNA plus cysteine. From the point of view of bladder protection, the finding that cysteine is a component of the free thiols excreted in urine following a MESNA dose is probably of little importance, since several thiols, including cysteine, might react with oxazaphosphorine metabolites, so reducing their toxicity. It is therefore likely that the presence of free thiols in any form is important in bladder protection.

Free thiol exretion into the urine was in the form of two distinct peaks. This may reflect differential absorption of MESNA and DIMESNA from the gastrointestinal tract, possibly resulting in two peaks of DIMESNA (MESNA is very rapidly oxidised in the bloodstream) in plasma. On excretion via the kidney DIMENSA is reduced, and so two peaks of free thiol appear in the urine.

The finding that MESNA enhances the excretion of cysteine in urine could be explained by several possible mechanisms: (1) MESNA might interfere with the mechanism of tubular excretion and/or re-uptake of cysteine in the kidney. This might be due to structural analogies between MESNA and cysteine; (2) MESNA might displace cysteine from cystine in plasma, the cysteine then being available for excretion; or (3) MESNA might interact with cystine in the urine in the bladder, so increasing the cysteine content of the urine. We are presently investigating these possibilities.

In conclusion, MESNA is absorbed from the gastrointestinal tract and excreted into the urine. Therefore oral administration of MESNA might find application in the prophylaxis of the putative bladder damage associated with oxazaphosphorine metabolites. In order to effect protection throughout chronic low-dose cyclophosphamide several oral MESNA doses would be required each day to maintain sufficiently high urinary thiol levels to protect against toxic damage to the bladder.

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