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Excretion of Disodium *Bis*-2-Mercaptoethanesulphonate (Dimesna) in the Urine of Volunteers after Oral Dosing

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Abstract—*Dimesna was given to volunteers* (n = 6) *and levels of free thiols, mesna, cysteine and disulphides measured in urine. Mesna is excreted in the urine following oral dimesna administration. Peak urinary free thiol levels occur between* 10 *and* 20 hr. *Cysteine and mixed disulphides are also excreted. Mesna might be useful in prolonged bladder protection during oxazaphosphorine cancer chemotherapy.*

INTRODUCTION

DIMESNA (disodium *bis*-2-mercaptoethanesulphonate) is a dimer comprising two molecules of mesna (sodium 2-mercaptoethanesulphonate) linked by a disulphide bond (Fig. 1) and has potential use in the prophylactic management of oxazaphosphorine-induced bladder toxicity. Presently mesna is employed in this context [1], being absorbed quickly following oral administration and eliminated in the urine within some 3 hr: therefore its usefulness in long-term oral prophylaxis is limited. On reaching the circulatory system mesna is very rapidly oxidized to dimesna and is reduced prior to its excretion in urine. Since mesna has a single ionized sulphonate group while dimesna has two, we would expect, purely on polarity grounds, dimesna to be absorbed more slowly than mesna following oral administration. Dimesna might therefore be more useful for long-term bladder protection.

The presence of mesna in the urinary bladder prevents haemorrhagic cystitis due to toxic oxazaphosphorine metabolites (e.g. acrolein) because it reacts chemically with the metabolites to form relatively stable non-toxic thioethers [1].

We have studied urinary levels of bladder-protective thiols after administration of dimesna to healthy volunteers by mouth. The experiments were designed to assess whether dimesna might be a more useful means of providing bladder-protective levels of thiols for long-term prophylaxis during oxazaphosphorine cancer chemotherapy.

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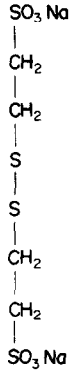

Fig. 1. The molecular structure of dimesna (disodium bis-2-*mercaptoethanesulphonate).*

MATERIALS AND METHODS

Chemicals

Disodium *bis*-2-mercaptoethanesulphonate (dimesna)was prepared for us by Asta Werke, Bielefeld, F.R.G. All other chemicals were of laboratory reagent grade.

Volunteers

Ethics Committee approval was obtained before commencing this study.

Six volunteers, three male, A (74 kg, 26 yr), C (64 kg, 26 yr) and E (60 kg, 29 yr) and three female, B (66 kg, 24 yr), D (62 kg, 20 yr) and F (57 kg, 23 yr) were given dimesna (6 mg/kg) orally in approx. 200 ml orange juice.

Initial studies (volunteers A and B) involved timed collection of urine samples at 15 min intervals for 5 hr, then urine was collected as produced between 5 and 24 hr. In all studies a control sample

was obtained before the dimesna was given. To promote micturition, in experiments where 15 min urine samples were collected, volunteers were given 200 ml water every 30 min for 150 min before, and 240 min after. Results from the initial study showed that excretion of mesna in urine began after 5 hr and therefore timed samples were collected in volunteers C, D, E and F at hourly intervals for 7 hr and then urine collected as produced between 7 and 24 hr. Water was not given in these cases.

Measurement of urinary free thiols

The level of free thiols in the samples was measured using Ellman's assay [2, 3]. To urine (0.1 ml) was added water (0.9 ml), phosphate buffer [pH 7.4, 0.25 M; (4.5 ml)] and Ellman's reagent (0.5 ml). The mixture was allowed to stand for 10 min and then $A_{412\,nm}$ was read.

Measurement of urinary disulphides

A modification of a previously described [3] method was used, in which disulphides were reduced to free thiols using sodium borohydride. To urine (3 ml) was added water (1 ml) and sodium borohydride [4% w/v (aq); (1.5 ml)]. The mixture was incubated at 50° C for 30 min and then acetic acid [9% v/v (aq) (1.5 ml)] added. The samples were allowed to stand overnight to permit completion of hydrogen evolution and then an aliquot (1 ml) of the mixture was subjected to Ellman's assay (see above).

HPLC thiol analysis

Selected samples were further studied by HPLC [4] in order to identify specifically the free thiols present in the urine. The solvent system was phosphate buffer (pH 7.4; 0.25 M)–methanol (95 : 5 v/v) containing the ion pair agent (TBAP). Flow rate = 1 ml/min. Detection was by post column Ellman's reaction; Ellman's reagent flow rate = 0.5 ml/min. Samples of borohydride reduced urine were analysed.

RESULTS

Free thiols and disulphides were excreted in the urine following an oral dimesna dose (6 mg/kg). The percentage of the dose excreted was 19.5 ± 10.9 (mean ± standard deviation, $n = 3$) in female volunteers and 17.5 ± 6.4 (mean ± standard deviation, $n = 3$) in male volunteers. The individual volunteers' results are shown in Table 1. The mean free thiol excretion (irrespective of sex) was 18.5 ± 8.1 (mean ± standard deviation, $n = 6$). The major peak of free thiol excretion in urine was quantitatively variable, but always occurred between 10 and 20 hr after the oral dimesna dose (Fig. 2). Endogenous thiol levels in urine prior to dimensa administration were very low 9.0 ± 6.5 mcg/ml (mean ± standard deviation, $n = 6$).

Table 1. Percentage of dimesna dose excreted as free thiols (e.g. free thiol plus disulphides); calculated as mesna equivalents. –SH = free thiols; S–S = disulphides

Volunteer	% dose excreted SH	–SH + S–S
A	15.7	62.3
B	6.9	25.4
C	24.6	66.9
D	25.1	57.1
E	12.1	52.6
F	26.3	52.3
Total ± SD	18.5 ± 8.1	52.8 ± 14.6

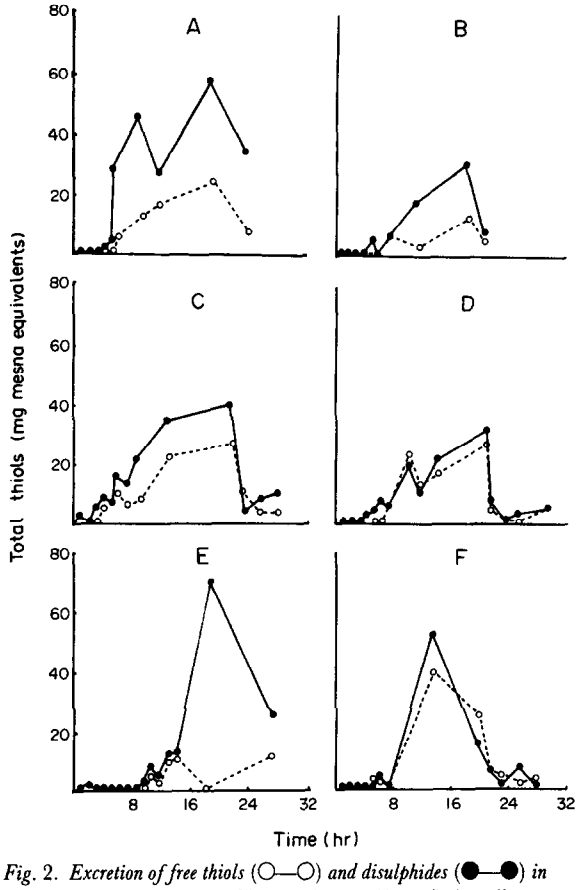

Fig. 2. Excretion of free thiols (○—○) and disulphides (●—●) in urine of volunteers (A–F) given dimesna (6 mg/kg) orally.

Total thiol excretion in female volunteers was 44.9 ± 17.1% (mean ± standard deviation, $n = 3$) of the dimesna dose; the corrsponding value in male volunteers was 60.6 ± 7.3% (mean ± standard deviation, $n = 3$) (Table 1). The overall total thiol excretion was 52.8 ± 14.6% (mean ± standard deviation, $n = 6$) of the dimesna dose. In this case the major thiol peak occurred between 9 and 21 hr following administration of dimesna.

Mesna readily forms a disulphide (dimesna) and mixed disulphides with other thiol compounds (e.g.

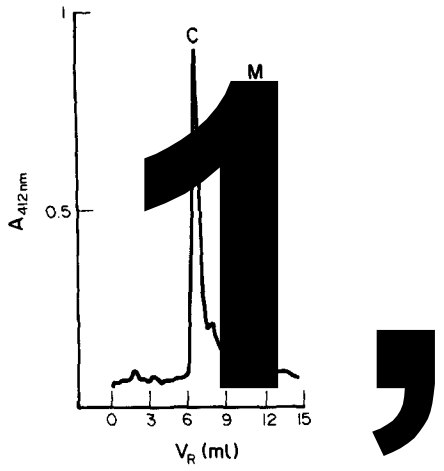

Fig. 3. HPLC trace showing the presence of both cysteine (C) and mesna (M) in urine of a volunteer (D).

cysteine). Indeed very soon after administration of mesna these disulphides are formed in the circulatory system. On excretion via the kidney a proportion of the disulphides is cleaved to liberate free thiols which are eliminated in the urine. Administration of dimesna therefore results in elimination of mesna in urine by the same biochemical mechanism. Borohydride reduction of these disulphides results in liberation of their constituent free thiols. HPLC analysis both before and after borohydride reduction gives information about free thiol excretion and the constituents of the disulphides. The HPLC procedure was carried out on the peak urine samples only. The major HPLC peak in these samples co-chromatographed with authentic mesna. In some cases another HPLC peak was also observed which co-chromatographed with cysteine.

In all cases control urine samples contained low levels (about 9.0 mcg/ml) of free thiols as determined by Ellman's assay; HPLC analysis showed that cysteine (V_0 = 6.3 ml) and homocysteine (V_0 = 6.6 ml) were undetectable in these samples, but that a small amount of an unidentified thiol (V_0 = 5.9 ml) was present. HPLC analysis of urine samples coresponding to the peak free thiol excretion prior to borohydride treatment, demonstrated a large peak of mesna and a peak which co-chromatographed with authentic cysteine (Fig. 3).

Borohydride treatment followed by HPLC analysis demonstrated a massive enhancement of the mesna peak and in addition an enhancement of the cysteine peak in four cases. This suggests that dimesna was excreted in all cases and that cysteine–mesna disulphide or cysteine was/were excreted in four of the six volunteers.

DISCUSSION

Oral administration of dimesna results in excretion of mesna in the urine; it is therefore likely that protection of the bladder against the toxic effects of oxazaphosphorine cytostatics would be achieved by giving dimesna by mouth. Cysteine and a number of thiol disulphides are also excreted. This is very similar to the situation during mesna administration [3]; cysteine is probably displaced from cystine and excreted in the urine [4], while disulphides are formed between two mesna molecules or mesna and endogenous thiols (e.g. cysteine). The thiol disulphides when reduced and analysed by HPLC were found to comprise mesna and cysteine only and are therefore probably dimesna and/or mesna–cysteine disulphide. Dimesna is reduced to mesna in the kidney [5] and the mesna is then excreted in the urine.

Some 18% of the dimesna dose was eliminated in the urine as free thiols: this value appeared to be unaffected by the sex of the subject. When mesna is given orally the corresponding value is about 24%. Therefore bladder-protective doses of oral dimesna would be expected to be of the same order as those of mesna.

The excretion peak of thiols in the urine when dimesna is given orally is very much later than that observed when mesna is given [3]; peak thiol excretion after oral mesna is at 2.5 hr while that after oral dimesna is at 10–20 hr. This may be useful for providing prolonged bladder protection during oxazaphosphorine cancer chemotherapy.

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