Pharmacokinetics and Metabolism of Sodium 2-Mercaptoethanesulfonate in the Rat

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ABSTRACT

The synthetic low-molecular-weight thiol, 2-mercaptoethanesulfonate (mesna), exerts effective protection against oxazaphosphorine-induced urothelial toxicity by binding the renally excreted and concentrated toxic metabolite(s). In this study, the pharmacokinetics and metabolism of mesna and its disulfide form (dimesna) have been investigated in the intact rat and in several in vitro systems, including isolated perfused organs, freshly isolated cells, and subcellular fractions; the mechanism of reduction of dimesna to form the pharmacologically active thiol mesna has been further studied with purified enzyme preparations. The results may be summarized as follows: (a) After p.o. administration, mesna and dimesna are both absorbed from the intestine, and dimesna undergoes reduction to mesna during intestinal absorption; (b) when present in plasma, mesna is rapidly oxidized to dimesna by a metal-dependent reaction; (c) mesna and dimesna pass unchanged through the hepatic vasculature, are not taken up into liver cells, and are not excreted in bile; (d) in the kidney, dimesna is filtered through the glomeruli and subsequently reabsorbed, whereupon reduction to the pharmacologically active thiol form occurs in the renal tubular epithelium, and the thiol is then reexcreted into the tubular lumen; (e) reduction of dimesna to mesna occurs in intestinal and renal epithelial cells by a mechanism involving the cytosolic enzymes thiol transferase and glutathione reductase. Thus, the formation of the pharmacologically active thiol form from dimesna is associated with the consumption of equimolar concentrations of reduced glutathione.

INTRODUCTION

Oxazaphosphorine cytostatics (cyclophosphamide, ifosfamide, etc.) have proved to be valuable tools in the chemotherapeutic treatment of malignant disease. The antitumor effect is dose dependent; however, the administration of adequate doses of oxazaphosphorine drugs is often limited by the occurrence of toxic effects in the urinary tract, especially hemorrhagic cystitis (2). The urotoxic compound is not cyclophosphamide or ifosfamide itself, but its activated metabolite(s), particularly acrolein, which are excreted in urine and may reach considerable concentrations exerting toxic effects on the mucosal lining of the urinary passages, especially the bladder (2, 6, 7).

A series of compounds, many of them with thiol groups, have been tested for ability to bind and detoxify the urototoxic metabolites of oxazaphosphorine cytostatics (3). Thus far, the most promising results have been obtained with sodium mercaptoethanesulfonate (HS—CH₂—CH₂—SO₃Na) (mesna; Uromitexan); mesna is rapidly and completely excreted by the kidneys, giving rise to high concentrations of reactive thiol groups in the urine. In a series of experimental and clinical studies, the properties of this compound have been investigated, and it has been shown not to interfere with the antitumor effect of cytostatic agents, not to be toxic in any species investigated, and to be biologically inert (4, 22).

After systemic administration of mesna, the thiol rapidly disappears from plasma and is replaced by the disulfide form (5, 18). In order for the drug to exert its uroprotective action, however, it must exist in thiol form in the urine. In the present study, we have therefore investigated the pharmacokinetics and metabolism of mesna and dimesna² at various levels of biological organization in the rat. Our studies have included experiments with the intact rat as well as with isolated perfused organs, isolated cells, subcellular fractions, and purified enzyme preparations. The results clearly demonstrate that the excretion of mesna in urine is preceded by reuptake of dimesna from the glomerular filtrate, reduction of the disulfide in the tubular epithelium by a mechanism involving the cytosolic enzymes thiol transferase and GSH reductase, and reexcretion of the active thiol into the urine.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 200 to 300 g, were kept in stainless steel cages and fed tap water and pelleted rat food ad libitum.

For studies on the course of elimination of mesna or dimesna in the urine, animals (n = 20) were kept in metabolism cages. Individual urines were intercepted in an ice bath, pooled at hourly intervals, and acidified with HCI to pH 4.5 to avoid autocatalysis of thiols.

Kidney perfusion was performed as described previously (18). Renal tubular epithelial cells were isolated according to the method of Jones et al. (12). Subcellular fractions were prepared from a kidney cortex homogenate; the plasma membrane fraction was isolated as described by Coleman et al. (8), whereas the cytosolic fraction was recovered as the 105,000 × g supernatant after sedimentation of the microsomal fraction. Intestinal mucosal cells were isolated according to the method of Dawson and Bridges (9), and hepatocytes were isolated as described by Moldéus et al. (14).

An isolated intestinal segment was prepared from the proximal part of the jejunum by gently dissecting free a 10-cm segment, rinsing it with cold 0.9% NaCl solution, inverting it, and tying off the ends after filling the lumen (serosal side) with Krebs-Henseleit bicarbonate buffer, pH 7.4. The segment was placed in a beaker containing the same buffer, supplemented with 1 mM dimesna, and was kept at 37° and continu-
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ously gassed with carbogen (95% O$_2$-5% CO$_2$). Samples were drawn from the surrounding medium and from the interior of the segment via a polyethylene tube inserted at one end of the closed loop and were assayed for free thiols.

Mesna was analyzed as total thiol according to the method of Saville (21) or by the procedure of Ellman (10) as modified by Stekar (23), in cellular systems after subtraction of GSH as measured by high-performance liquid chromatography (20). Dimesna was assayed as reducible disulfides after treatment with sodium borohydride according to the method of Stekar (23).

The measurement of free thiols and reducible disulfides is not specific for mesna or dimesna but includes the negligibly low base-line values of physiological thiol compounds, i.e., cysteine, cystine, GSH, etc. Blood plasma of untreated animals ($n = 50$) contains 204 ± 84 (S.D.) nmol of disulfides per ml (measured as thiols after reduction).

Free thiols were not detectable in our animals.

Cellular uptake of radiolabeled dimesna was determined by incubation of isolated cells from kidney, liver, or intestinal mucosa with $[^{14}C]$-dimesna; separation of the cells from the medium by rapid Millipore (100 mesh) filtration; and counting of cellular radioactivity in a Beckman LS 100 liquid scintillation counter.

Metabolic incubations were routinely performed in rotating, round-bottomed glass flasks at 37° under continuous gassing with carbogen or 100% N$_2$. The incubation volume was 10 ml at the start of the experiments. Incubation medium was a modified Krebs-Henseleit bicarbonate buffer, pH 7.4, supplemented with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Mesna, dimesna, and $[^{14}C]$-dimesna (1.73 μCi/mg) were supplied by Asta-Werke AG, Degussa Pharma Gruppe, Bielefeld, Federal Republic of Germany. Anthglutin [1-γ-L-glutamyl-2-(2-carboxyphenyl)hydrazine] was a generous gift from Dr. M. Tanaka, Sankyo Laboratories, Tokyo, Japan. Thiol transferase, purified from rat liver, was kindly supplied by Dr. V. Yahiel, Department of Biochemistry, Arrenius Laboratory, University of Stockholm, Stockholm, Sweden. GSH reductase (from yeast) was obtained from Boehringer-Mannheim GmbH, Mannheim, Federal Republic of Germany. All other reagents were of at least reagent grade and were purchased from local commercial sources.

RESULTS

After i.v. injection of mesna (100 mg/kg) into rats, there was an increase in the concentration of free thiols in plasma, which disappeared within minutes; a concomitant increase in the level of reducible disulfides was observed during a much longer time period. The plasma disappearance rate of the sum of administered thiols and immediately oxidized disulfides after i.v. injection of mesna was almost identical to the rate of plasma elimination of disulfides after administration of dimesna (Chart 1, top).

After i.p. administration of mesna or dimesna, there were no apparent differences in the low plasma concentration of free thiols and the high concentration of reducible disulfides. The rate of absorption from the peritoneal cavity appeared to be equally fast for both compounds, and peak plasma concentrations were reached within the first 10 min after administration. Furthermore, the slope of the disappearance curve for plasma reducible disulfides, after i.p. administration of either mesna or dimesna, was similar to the slope of the plasma disappearance curve observed after i.v. administration (Chart 1, middle).

Also, after p.o. administration, both mesna and dimesna were readily absorbed, as demonstrated by significant elevation of disulfide concentration in plasma; free thiols were hardly or not at all observed. Mesna seemed to be absorbed somewhat better (or earlier) during the intestinal passage than dimesna was. Due to a prolonged absorption phase, the plasma concentration of reducible disulfides increased only slowly and reached a plateau maximum between 1 and 3 hr after administration (Chart 1, bottom).

Independently of the route of administration of mesna or dimesna, the renal elimination rate corresponded quite well to the disappearance of reducible disulfides from peripheral plasma. The metabolic output is very low in untreated animals. We measured a spontaneous urinary excretion rate ($n = 50$) of 0.58 ± 0.44 μmol/kg/hr for free thiols and 2.46 ± 1.25 μmol/kg/hr for thiols after reduction of disulfides. These values would correspond to an excretion rate for mesna of 0.1 or 0.4 mg/kg/hr, respectively. Renal excretion of mesna or dimesna started immediately after i.v. injection, whereas it was slightly delayed after i.p. administration, and it showed a plateau maximum from 1 to 3 hr after p.o. administration. In all instances, the dose of mesna or dimesna administered was excreted almost completely within 6 hr in the rat (Chart 2). Moreover, in all experiments, significant amounts (40 to 70%) of the total dose of mesna or dimesna given appeared as free thiol in the
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Absorption from the Gastrointestinal Tract

Absorbed from the gastrointestinal tract after p.o. administration to rats in vivo. To further substantiate this finding, an isolated, inverted intestinal segment was incubated with 1 mm [14C]dimesna in vitro, and the amount of radioactivity was measured in the medium and in the lumen contents. After 60 min of incubation, the concentration of radiolabel in the lumen contents was almost twice that originally present in the surrounding medium (191 ± 28%). It is of interest to note that, in spite of the fact that there were no measurable free thiols in the medium during the entire experiment, the lumen contents of the closed segment contained a slowly increasing amount of thiol groups, amounting to 30 to 60% of the accumulated radioactivity. During control incubations in the absence of dimesna, no luminal thiol accumulation occurred.

Uptake of dimesna was also observed in experiments with isolated cells from small intestine and kidney but not with hepatocytes (Chart 4). In order to investigate whether this uptake was coupled to membrane-bound γ-glutamyltransferase activity, which is abundant in intestine and kidney but very low in liver in the rat, we performed a series of experiments with the potent γ-glutamyltransferase inhibitor, anthglutin (13), added to the cellular incubation at a final concentration of 2 mM. This resulted in almost complete inhibition of γ-glutamyltransferase activity without measurable effect on the rate of dimesna uptake in any of the cell types investigated.

There was no apparent formation of mesna from dimesna during incubation of the disulfide in buffer, plasma, or urine. In contrast, incubation of dimesna with cells, isolated from either small intestine or kidney, was associated with reduction of the disulfide and accumulation of increasing concentrations of free thiol in the medium (Chart 5). On the other hand, incubation of dimesna with isolated hepatocytes did not result in any increased level of mesna in the medium, suggesting that the reduction of dimesna to mesna is mediated by an intracellular mechanism and occurs only in cells which can take up the disulfide. This hypothesis received further support from the results of experiments illustrated in Chart 6. Incubation of dimesna with the dialyzed cytosolic fraction of a kidney ho-
of perfusion experiments were performed. When the isolated rat liver was perfused with [14C]dimesna, there was no detectable extraction of radiolabel during passage through the hepatic vascular bed, no excretion of dimesna or mesna in bile, and no accumulation of thiol or radiolabel in the liver tissue (Chart 7). Thus, it appears that the liver is totally inactive in the whole-body handling of mesna and dimesna in the rat. When similar experiments were performed with the isolated perfused rat kidney, [14C]dimesna gradually disappeared from the recirculating perfusate but never appeared in urine when the perfusate concentration of dimesna was kept below 2 mM (Chart 7). On the other hand, whereas reactive mesna was excreted in the urine at high concentrations, it could be detected only in trace amounts in the perfusate leaving the kidneys. Compared to clearance data for exogenously added creatinine (1.6 ± 0.2

Chart 4. Uptake of [14C]dimesna (MSSM) into isolated cells. Incubations were performed with 2 × 10^6 cells/ml at 37° under continuous oxygenation in Krebs-Henseleit bicarbonate buffer, pH 7.4, supplemented with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and 1 mM dimesna. △, kidney cells; ◇, intestinal cells; ○, hepatocytes. Points, means of 4 separate incubations; bars, S.D.

Chart 5. Reduction of dimesna during incubation at 37° under a N₂ atmosphere. Initial dimesna concentration was 1 mM. Cell concentration was 2 × 10^6 cells/ml. ○, hepatocytes; ◇, intestinal cells; △, kidney cells; ×, Krebs-Henseleit bicarbonate buffer, pH 7.4; ●, urine. Points, means of 6 separate incubations; bars, S.D.

mogenate in the presence of added NADPH and GSH resulted in rapid conversion of the disulfide to the thiol (Chart 6A); other renal subcellular fractions tested were inactive in this respect. Moreover, when the dialyzed cytosolic fraction was replaced by purified preparations of thiol transferase and GSH reductase, the reduction of dimesna to mesna was shown to proceed at a similar rate (Chart 6B). The involvement of thiol transferase in the reduction of dimesna was further substantiated by similar experiments with the cytosolic fraction of a liver homogenate, which was also found to catalyze the GSH- and NADPH-dependent reduction of dimesna to mesna. Thus, it appears that the difference in capacity to reduce dimesna to mesna, observed with isolated cells from the various tissues, was related to differences in the ability to take up the disulfide rather than to lack of the enzymatic mechanism involved in dimesna reduction.

To further substantiate the findings reported above, a series

Chart 6. Reduction of dimesna to mesna catalyzed by renal cytosolic fraction or purified thiol transferase and GSH reductase. Incubation was performed at 37° under a N₂ atmosphere. A, 1 mM dimesna incubated with dialyzed rat kidney cytosolic fraction (50 µg protein per ml). ○, no addition; ●, plus GSH (0.5 mM); ●, plus GSH (0.5 mM) and NADPH (1 mM). B, ○, 1 mM dimesna incubated with purified preparations of thiol transferase (5 µg/ml); ●, plus GSH (0.5 mM); ●, plus GSH (0.5 mM) and NADPH (1 mM); ●, plus GSH (0.5 mM) and NADPH (1 mM); ●, plus GSH (0.5 mM), NADPH (1 mM), and purified GSH reductase (10 µg/ml). Points, means of 3 separate incubations; bars, S.D.

Chart 7. Extraction of [14C]dimesna during perfusion of isolated rat liver and kidney. Perfusate concentration, 0.5 mM. ○, liver perfusate; ●, bile; △, kidney perfusate; ●, urine. Points, means of 3 separate experiments; bars, S.D.
ml/min), the extraction of dimesna from the perfusate during kidney passage was somewhat lower (1.0 ± 0.1 ml/min). This observation indicates that, in addition to glomerular filtration, dimesna undergoes reabsorption from the tubular fluid, a process which is compatible with the aforementioned intracellular reduction mechanism. Furthermore, it seems that after reabsorption most of the thiol formed is reexcreted back into the tubular lumen, whereas any disulfide exceeding the cellular reduction capacity is transported across the basal part of the tubular plasma membrane to reenter the peritubular vessels.

**DISCUSSION**

The absorption, disposition, and pharmacological activity of mesna and dimesna have been studied previously in a series of experimental and clinical investigations (2–7, 19, 22). In the present study, the pharmacokinetics and metabolism of mesna and dimesna have been further investigated by the use of isolated organs, cells, subcellular fractions, and purified enzymes in an attempt to elucidate the mechanisms involved in the handling of this compound in the organism.

The observation that mesna is rapidly oxidized to dimesna in peripheral blood after i.v. administration was confirmed in vitro by incubating mesna with murine and human blood or plasma. The inhibitory effect of EDTA suggests that the oxidation of mesna is catalyzed by metal ions; the mechanism of this reaction remains, however, to be elucidated. Anyhow, due to this reaction, mesna is mainly present in disulfide form in circulating blood, irrespective of whether it is administered as a thiol or as a disulfide, and will reach the various organs in this form.

Among the cell types studied, only intestinal and renal cells were able to take up radiolabeled dimesna. The ability to take up other low-molecular-weight disulfides and thiols is variably developed among different cell types; e.g., cystine is rapidly taken up by kidney cells (16) but hardly at all by hepatocytes (24), whereas both cystine and cysteine can penetrate into intestinal mucosal cells, and there is evidence for an active cellular uptake of GSH as well as glutathione disulfide in the kidney (1, 17). It has been suggested that in the kidney one possible mechanism for cystine uptake is dependent on the function of the γ-glutamyl cycle (11, 15). In the present study, however, no support for a quantitatively important involvement of a similar mechanism for disulfide uptake was obtained with either renal or intestinal cells, since the efficient γ-glutamyl-transferase inhibitor, antihgulatin, had no apparent effect on cellular uptake of dimesna.

The renal reduction of dimesna is a prerequisite for its uroprotective action to occur. Although the cystolic fraction from all 3 tissues tested could catalyze the conversion of disulfide to thiol, the inability of hepatocytes to take up the disulfide appears to restrict dimesna reduction in vivo to the kidney and the intestine.

The stimulatory effect of GSH and NADPH on dimesna reduction in the dialyzed, cystolic fraction and the results obtained with purified enzymes strongly suggest that reduction of dimesna (MSSM) to mesna (MSH) is achieved by a reaction sequence analogous to that previously suggested for reduction of cystine in the kidney (16), in which GSSG is glutathione disulfide.

\[
\text{MSSM} + \text{GSH} \rightarrow \text{MSH} + \text{GS-SM} \quad (A)
\]

\[
\text{GS-SM} + \text{GSH} \rightarrow \text{MSH} + \text{GSSG} \quad (B)
\]

\[
\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADP}^+ \quad (C)
\]

The first reaction may occur spontaneously but may also be catalyzed by cytosolic thiol transferase. Reaction B is also catalyzed by thiol transferase, and Reaction C, which restores the level of GSH, is catalyzed by GSH reductase. Thus, the combined functions of thiol transferase and GSH reductase seem to constitute a potent mechanism for the reduction of dimesna, as previously shown for cystine (16). The possible existence of other pathways for disulfide reduction remains, however, to be investigated.

As judged from its molecular weight and water solubility, dimesna is freely filtrable across the glomerular capillary membrane. However, compared to the clearance of creatinine, dimesna excretion is somewhat less efficient, suggesting retransfer of a fraction of filtered dimesna from the tubular fluid to the peritubular capillaries. However, the capacity for reuptake of filtered dimesna is probably limited, since at perfusate concentrations of dimesna above 1 mM a fraction of the disulfide remained in urine, whereas at lower perfusate concentrations the thiol form was recovered in urine. Although the renal reductive capacity must be limited, we observed no saturation of renal thiol excretion, even after very high doses of dimesna. A surplus of intracellular disulfide may be either reexcreted to the tubular lumen or transported across the cell to enter the capillary bloodstream via the basal part of the cellular plasma membrane. The latter transport path is favored by the clearance data as well as by the difficulty in imagining a brush-border transport mechanism, which actively carries the same molecule in both directions through the cellular plasma membrane. As for the intracellularly formed thiol, however, it seems that it is almost exclusively transported across the brush border back to the tubular fluid. In the urine, mesna is neither taken up nor oxidized but remains and exerts its detoxifying activity.

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