Mesna Excretion and Ifosfamide Nephrotoxicity in Children


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ABSTRACT

To characterize the excretion of 2-mercaptoethanesulfonate sodium (mesna) administered by intermittent infusion, urinary concentrations of mesna and its corresponding inactive disulfide were measured during 50 courses of ifosfamide (1.6 g/m² for 5 days) and mesna (400 mg/m² at 0.25, 4, and 6 h after each ifosfamide dose) administered i.v. to 19 patients. Some patients had previously received nephrotoxic therapy that might influence the excretion of mesna and its associated uroprotective effects. The median urinary free thiol concentration increased to 3.5 nM by 1 h after mesna infusion, declining to background levels by 4 h. The rate of mesna excretion correlated with the creatinine clearance rate in a subset of six patients. The proportion of mesna recovered in urine within 4 h after infusion was lower (P < 0.05) in children who had evidence of preexisting renal tubular damage. Ifosfamide-induced tubular proteinuria was associated with lower urinary mesna recovery. Low urinary mesna concentrations indicated potentially subtherapeutic renal tubular levels. However, ifosfamide nephrotoxicity was subclinical and is not necessarily linked to differences in mesna excretion.

INTRODUCTION

Mesna, a free thiol, has been shown to be an effective uroprotective agent when administered with the oxazaphorine ifosfamide (1). After i.v. infusion, mesna is oxidized in the blood to mesna, an inactive disulfide (2). Approximately 10% of drug is serum protein bound (3). Animal studies indicate that mesna and dimesna are cleared by renal filtration (4); hepatic uptake is negligible (3, 4). Dimesna in the glomerular filtrate can be reabsorbed by proximal renal tubular epithelium (5), enzymatically reduced to mesna, and secreted back into the tubular lumen (4), where it can neutralize the toxic metabolites of ifosfamide.

Many cancer patients who are treated with ifosfamide and mesna have sustained renal tubular damage from earlier nephrotoxic therapy (6). Such renal damage might influence the excretion of mesna and its associated uroprotective effect. We undertook this study to characterize the excretion of mesna administered by intermittent infusion and the effect of preexisting renal tubular damage.

PATIENTS AND METHODS

Patients. We studied 19 children and adolescents (10 males and 9 females) with cancer who had been enrolled in a phase II trial of ifosfamide (7) and had consented to urine collection during courses of ifosfamide and mesna (Table 1). Multiple courses were studied in some children to identify any course-to-course variation in drug elimination. Some patients had previously received other nephrotoxic therapy; six children had received >300 mg/m² cisplatin, which can potentiate the nephrotoxic effects of ifosfamide (6).

Drug Administration. Ifosfamide (1.6 g/m²) was given i.v. over 15 min, followed by three 15-min i.v. infusions of mesna (400 mg/m²) at 15 min, 4 h, and 6 h. The drugs were given daily for 5 days in each course.

Urine Specimens. We collected urine specimens that were voided during the 4 h between the first and second doses of mesna, and during the 2 h between the second and third doses. The stability of mesna was increased by adding 0.1 ml of 6 N HCl/12 ml of urine to an aliquot of each specimen. We determined the ratio of the amount of creatinine over the collection time interval for each urine specimen to identify potentially mislabeled collection times. If the creatinine-time ratio for a specimen differed by more than 2-fold from rates for other specimens obtained from the same patient on the same day, that specimen was excluded from analyses that required properly timed collections.

Mesna and Dimesna. Mesna concentrations were determined with Ellman's reagent (8-10). The free thiol concentration was determined using the extinction coefficient at 409 nm for 2-nitro-5-thiobenzoic acid with correction for the absorbance of a reagent blank. To determine dimesna concentrations, the sum of reducible disulfides and thiols was measured (11, 12), and the concentration of mesna was subtracted. Both procedures were adapted to a Gilford 203 analyzer (Oberlin, OH) for semiautomated analysis. The between-run coefficients of variation for 1 mm aqueous controls of mesna and dimesna were 4.0 and 4.3%, respectively. A urine specimen was obtained before each course to correct for endogenous concentrations of thiols and disulfides.

The proportion of mesna that could be recovered in the urine as free thiol within 4 h after infusion was calculated as the sum of the moles of mesna present in all urine specimens voided between the first and second doses of this agent. Similarly, the proportion of infused mesna recovered in urine in either the free thiol (mesna) or disulfide form (dimesna) was calculated by summing the amount of mesna and twice the amount of dimesna present in all urine specimens voided between the first and second doses of mesna. These values, defined as the 4-h urinary recovery of mesna and of mesna-dimesna, respectively, were expressed as percentages of the i.v. dose.

The rate of urinary excretion of mesna was estimated by monitoring the proportion of the dose that was recovered in the urine during each timed urine collection. The half-life and corresponding elimination rate constant [ln(2)/half-life] were estimated from least-squares lines fitted to semilog plots of the quantity of residual nonexcreted mesna over time. Elimination rate constants were estimated in instances when three or more timed urine specimens were collected between the first and second doses of mesna; values on 3 or more days of a course of ifosfamide and mesna therapy were averaged to obtain a value associated with the course. These estimates were possible in six patients who had received >300 mg/m² cisplatin, which can potentiate the nephrotoxic effects of ifosfamide (6).

Tubular Nephrotoxicity. To assess the effect of cumulative renal tubular damage from prior therapy, we divided the patients into two groups based on the concentration of the renal tubular enzyme NAG (13) in urine specimens obtained before the patients began ifosfamide therapy. Patients with a urinary NAG concentration exceeding 1.5 units/mmol creatinine were considered to have preexisting renal tubular damage (14). To evaluate the acute nephrotoxic effect of the subsequent ifosfamide treatment, concentrations of total urinary protein and NAG were determined in urine specimens obtained daily during each course of ifosfamide therapy. NAG and protein measurements were expressed relative to the urinary creatinine concentration to compensate for potential variation in urinary protein concentration.
MESNA AND IFOSFAMIDE NEPHROTOXICITY

Table 1 Clinical features of the patients

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<th>No.</th>
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<th>Diagnosis</th>
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* A, aminoglycoside; CDDP, cis-diaminedichloroplatinum(II); CY, cyclophosphamide; IFN, a-interferon; HDM, high-dose methotrexate; R, lower abdominal radiation; U, uninephrectomy. Numbers in parentheses, number of courses of CDDP given.

variations in urine output. The average values were used as a measure of the acute nephrotoxic effect of a given course of ifosfamide on the renal tubules (6, 15).

Statistics. The Mann-Whitney rank sum test was used for two-group comparisons of mesna recovery, mesna-dimesna recovery, and concentrations of urinary protein and NAG. The paired t test was used to test intrapatient differences in urinary recoveries between day 1 and days 2 to 5 of a particular course. Fisher's exact test (two-sided) was used to compare the frequencies of neurotoxicity and severe hematotoxicity.

RESULTS

Concentrations of mesna, dimesna, and creatinine were determined in 974 urine specimens collected during 50 courses of ifosfamide and mesna given to 19 patients. Fig. 1 shows median values with interquartile ranges (25th to 75th percentile) of urinary mesna concentrations in specimens voided during 30-min intervals after drug infusion. The median values increased during the first hour to 3 mM, declining within 4 h to a level that approached that for endogenous free thiols. Mesna-dimesna concentrations paralleled those shown for mesna concentrations in Fig. 1.

The capacity of the renal tubules to generate mesna from dimesna might vary with the concentration of plasma dimesna to which the kidneys are exposed. Presuming that urinary mesna-dimesna concentrations roughly reflect plasma dimesna concentrations, we determined the median and interquartile range for the ratio of mesna to mesna-dimesna concentrations in urine specimens collected within the first 4 h after mesna administration (Fig. 2). Approximately 30 to 45% of urinary drug was present in the free thiol form. The relative mesna concentration was highest in the mesna-dimesna range of 4 to 10 mM. At concentrations greater than 10 mM, the proportion of urinary dimesna that had been reduced to mesna was lower, suggesting saturation of the capacity of the kidney to metabolize dimesna.

Amounts of mesna and mesna-dimesna recovered in the urine within 4 h after infusion could be calculated for at least 3 of the 5 days of ifosfamide and mesna therapy for each of the 50 courses. Values obtained on later days did not differ significantly from those for the first day (paired t test); therefore, an average value was used for each course. For the 50 courses, between 27 and 60% of the infused mesna was recovered as free thiol in urine during the first 4 h. The 4-h urinary recovery of mesna-dimesna ranged from 59 to 100%. In the five patients studied over at least four courses (Table 1), there was no apparent trend in urinary recoveries from course to course. The average urinary recovery for the first course given to each patient was used for subsequent comparisons.

The rate of renal excretion of mesna-dimesna, calculated for the six children who were frequent voiders, correlated with the creatinine clearance rate (Fig. 3). The corresponding half-lives for renal excretion ranged from 42 min (elimination constant, 0.99/h) to 143 min (0.29/h). We then examined in 18 patients the predictive value of creatinine clearance rates (computed during the 6 h after ifosfamide infusion; range, 45 to 179 ml/min/1.73 m²) for 4-h urinary recoveries of mesna and mesna-dimesna. The median recoveries of mesna and mesna-dimesna in the nine children with creatinine clearance rates less than 95 ml/min/1.73 m² were lower than those in the nine children with greater clearance rates; however, these rates were not
predictive of drug recoveries in individual patients.

Six patients (patients 3, 4, 7, 12, 15, and 19 in Table 1) were considered to have preexisting renal tubular damage based on urinary NAG levels of >1.5 units/mmol creatinine. Four of these had received >300 mg/m² cisplatin. The 4-h recoveries of both mesna (P < 0.05) and mesna-dimesna (P < 0.01) were somewhat lower for these patients with elevated preifosfamide NAG levels (Fig. 4). There was no statistical difference between the two groups in the ratio of mesna to mesna-dimesna recoveries (data not shown), suggesting no difference in renal capacity to reduce dimesna to mesna.

When divided into two groups based on the midpoint of urinary mesna-dimesna recovery values in this study, patients with recoveries <85% showed significantly greater excretion of both total urinary protein (P < 0.005) and NAG (P < 0.05) than patients with recoveries >85% (Fig. 5). The acute tubular nephrotoxicity represented by this proteinuria was reversible, and there was no evidence of persistent clinical nephrotoxicity based on serum creatinine values 3 weeks posttherapy. An evaluation of ifosfamide-associated hematotoxicity (WBC nadir <0.5 cells x 10⁹/liter) and neurotoxicity [evaluated according to Pratt et al. (16)] in these two groups showed that of the seven patients with mesna-dimesna recoveries <85%, two developed symptomatic ifosfamide neurotoxicity, and two of three patients with evaluable WBC counts developed a low WBC nadir.

DISCUSSION

Urinary mesna concentrations were nearly undetectable by 3 h after infusion (Fig. 1), indicating a concurrent decline in renal intratubular concentrations of mesna. Thus, although mesna can accumulate within the bladder, the renal tubules may lack protective concentrations when administered at 4-h intervals. Infusion of mesna (17) induces detectable cysteinuria and the excretion of mixed disulfides such as mesna-cysteine disulfide (18); a conjugate of mesna to 4-hydroxyifosfamide has also been detected at a concentration of <10 µM (19). We assumed that the effect of these sulphydryls on our measurements was small in comparison to the millimolar concentrations of mesna and dimesna being studied.

Since dimesna becomes active only after its conversion to a free thiol, we considered the possibility that damaged renal...
tubules might yield less mesna. This appears unlikely because there was no difference in the ratio of urinary mesna to mesna-dimesna for patients with elevated versus normal preifosfamide NAG values. Moreover, the capacity of the kidneys to metabolize dimesna appears to become saturated only when the kidney is exposed to the relatively high mesna-dimesna concentrations (Fig. 2) that occur during and shortly after infusion. A proportion of infused mesna can also be presumed to pass unchanged from the blood into the urine.

The rate of renal elimination of mesna in the children we studied (Fig. 3) was similar to that reported by Pohl et al. (2) in adults who were given 60 mg/kg i.v. (half-life, 1.08 h) and by James et al. (20) in volunteers given 800 mg i.v. (half-life, 1.17 h). We determined the rate of elimination by monitoring the appearance of drug in the urine, whereas these investigators monitored the rate of disappearance of dimesna from the plasma. The similar clearance rates obtained by both procedures support renal filtration as the principal mode of drug elimination (21).

We observed 4-h urinary drug recoveries that were somewhat higher than the 4.5-h urinary recoveries reported for volunteers given mesna orally, presumably because intestinal absorption of the drug is slower and incomplete (17). Mean urinary recoveries of mesna and mesna-dimesna during a 3-day period in volunteers given multiple infusions at 4-h intervals were 32 and 93%, respectively (22). In rats, 6-h urinary free thiol recoveries were somewhat higher (47 to 72%) than in the children we studied, but the sum of free thiols and reducible disulfides (81 to 91%) was similar (23).

The increased ifosfamide-induced proteinuria associated with low urinary mesna-dimesna recovery (Fig. 5) is probably attributable more to preexisting renal dysfunction than to reduced uroprotection by mesna. Differences in disposition of mesna were relatively small (Fig. 4). Most of the patients with high NAG values and low mesna recovery had received cisplatin. This renal tubular toxin can induce persistently elevated NAG values (14). Cisplatin-damaged renal tubular cells might also be more susceptible to the nephrotoxic effect of ifosfamide.

The identity of the specific ifosfamide metabolites responsible for nephrotoxicity remains uncertain. Acrolein, released from urinary 4-hydroxyifosfamide, may be the primary toxin (24, 25). Chloroacetaldehyde, when present in high concentration in the urine (26), probably contributes as well. Mesna appears to prevent ifosfamide-induced hemorrhagic cystitis by chemical neutralization of acrolein (27) and by stabilization of 4-hydroxyifosfamide (28), the metabolite that releases acrolein. The mechanism by which mesna ameliorates ifosfamide nephrotoxicity may differ from that for hemorrhagic cystitis because of the potential for renal tubular reabsorption of mesna, dimesna, and toxic ifosfamide metabolites. Mesna (19, 29), cysteine (29), and glutathione (30, 31) could neutralize intracellular ifosfamide metabolites.

A relatively low dosage of mesna appears to be sufficient for uroprotection during fractionated ifosfamide therapy. Although the daily dosage of mesna was only 75% (w/w) of that for ifosfamide, we did not observe ifosfamide-induced hemorrhagic cystitis in these patients or in a larger group receiving the same schedule of therapy (32), consistent with findings by others using this schedule of mesna (21, 33). Moreover, acute (15) and course-to-course (34) nephrotoxicity has been subclinical in our current therapeutic protocols. In patients given higher ifosfamide dosages, mesna has been administered at more frequent intervals (35, 36). Maintaining a constant intratubular mesna level by continuous rather than intermittent infusion may provide more effective uroprotection (35, 37, 38), although this remains unproved (21). Oral preparations of mesna (17, 20, 39, 40) and dimesna (10), producing peak urinary thiol levels at 2 to 3 and 10 to 20 h after ingestion, respectively, may be more practical for outpatient therapy. It is probably wise to use the smallest effective dosage of mesna in view of reports that large mesna dosages can interfere with the therapeutic efficacy of activated oxazaphosphorines in animals (29).

ACKNOWLEDGMENTS

We thank Linda Dunawilez (Rhodes College), Jackie Li, and Elizabeth Pell (Department of Pathology and Laboratory Medicine) for technical assistance, and Cristy Wright for editorial review.

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