Similar Bioavailability of Single-Dose Oral and Intravenous Mesna in the Blood and Urine of Healthy Human Subjects

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ABSTRACT

Although mesna has been used for more than a decade to reduce the incidence of hemorrhagic cystitis induced by ifosfamide and cyclophosphamide, the disposition of i.v. and oral mesna has not been adequately described. To obtain accurate bioavailability data for the design of mesna regimens, we developed procedures to preserve and measure mesna and dimesna in the blood and urine and studied 25 volunteer subjects who received single doses of i.v. mesna and four different formulations of oral mesna in a five-way randomized crossover study. The dose-adjusted area under the blood concentration-time curve showed no difference in bioavailability for i.v. and oral mesna; however, the maximum mesna concentration after oral doses was 16% of that estimated for i.v. doses. The short initial half-life of i.v. mesna indicated that mesna was rapidly cleared; however, the blood concentrations of mesna uniformly exceeded those of dimesna after oral as well as i.v. doses, which suggested that reduced mesna and oxidized mesna disulfide are in equilibrium. The ratio of mesna:dimesna was higher in protein-free plasma than it was in the urine, which suggested that most urinary mesna is produced by glomerular filtration of mesna rather than by renal tubular reduction of dimesna. The sum of mesna and dimesna excretion after the i.v. doses (73% of the dose) and the four oral formulations (68–73%) showed no difference in urinary bioavailability, consistent with the blood data. However, the urinary bioavailability of the therapeutically active free-thiol mesna was greater after i.v. doses (40% of the dose) than it was after oral doses (31–33%). The ratio of oral:i.v. mesna excretion ranged from 0.52–1.23 (mean, 0.82) among the 24 subjects. Urinary mesna concentrations exceeded 50 μM in all subjects for up to 12 h after oral doses as compared to 4 h after i.v. doses. About 90% of this mesna was excreted by hour 2 after i.v. doses and by hour 9 after oral doses. The mean maximum concentration of mesna in blood and excretion into urine were both 2.6 h after dosing. The oral formulations thus showed sustained urinary excretion, and their urinary bioavailability approached that of i.v. mesna.

INTRODUCTION

Mesna (sodium 2-mercaptoethanesulfonate, HS-CH₂-CH₂-SO₃⁻, Na⁺) reduces the incidence of hemorrhagic cystitis in patients given oxazaphosphorines (1, 2). In the absence of mesna, hemorrhagic cystitis is the most frequent adverse effect of ifosfamide and is dose-limiting. In a placebo-controlled study of patients receiving 2.0 g/m² ifosfamide daily for 5 days, i.v. mesna reduced the incidence of moderate (>50 RBCs/high-power field) and visible hematuria from 32.6 to 6.7% (3). Mesna may influence the incidence of renal toxicity (4), which can be dose-limiting in patients who are given large cumulative ifosfamide doses (5) or high-dose ifosfamide and platinum therapy (6). Mesna is used by at least half of bone marrow transplant groups to reduce the incidence of cystitis induced by high-dose cyclophosphamide in regimens for bone marrow ablation (7, 8). Oral mesna is also a potential anticarcinogenic agent (9) to reduce the incidence of bladder cancer in patients given cyclophosphamide for both malignant (10) and nonmalignant disease (11).

In the current model of mesna metabolism and action (1, 12), mesna is irreversibly oxidized in the blood to a disulfide, dimesna, which does not interact with anticancer agents. During passage through the kidneys, dimesna is taken up by the renal tubules and is partially reduced back to mesna, which is excreted into the urine. Mesna exerts its protective effect in the urine by neutralizing acrolein, a urototoxic metabolite of both ifosfamide and cyclophosphamide (13, 14).

Mesna is typically administered as three i.v. doses (each equal to 20% of the ifosfamide dose) at 0, 4, and 8 h after ifosfamide administration. Because of the rapid excretion of i.v. mesna, patients who receive prolonged infusions of ifosfamide or higher doses are sometimes given more than three i.v. mesna doses or a continuous infusion of mesna for up to 24 h after the last dose of ifosfamide. Oral administration of mesna could simplify ifosfamide therapy by eliminating the need for multiple i.v. infusions. Oral mesna may also have value in combination with cyclophosphamide.

The disposition of i.v. and oral mesna in blood and urine has not been fully characterized, and this information would be of value for the design of therapeutic regimens. Bioavailability data for both i.v. and oral mesna in healthy volunteers vary widely because of study designs involving small numbers of

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subjects, inadequate sample collection and preservation of the analytes, and analytical methodology that is nonspecific for mesna or for the inert urinary metabolite, dimesna (15–21). We developed more specific procedures and designed a single-dose randomized five-way crossover study for 25 healthy volunteer subjects who were given an i.v. and four oral formulations of mesna. Here we present clinical data that suggest that dimesna is in equilibrium with mesna in vivo, and that urinary mesna is produced predominantly by glomerular filtration of mesna rather than by renal tubular reduction of dimesna. We also show that the bioavailability of oral mesna approaches that of i.v. mesna.

**SUBJECTS AND METHODS**

**Human Subjects.** Twenty-five healthy adult male volunteers (age, 21–44 years; body surface area, 1.7–2.2 m²; <15% deviation from ideal body weight; serum creatinine, 0.9–1.3 mg/dl) were housed (L.A.B. Pharmacological Research International) for 10 days. One subject withdrew because of ocular inflammation unrelated to the study drug. Written informed consent was obtained before enrollment.

**Drug Administration.** Each subject received by randomized sequence a 600-mg i.v. mesna dose over 15 min (Mesnex injection, 100 mg/ml) and four 1200-mg oral mesna doses (300-mg Eudragit film-coated tablet, 400-mg Pharmacoat film-coated tablet, 600-mg Pharmacoat film-coated tablet, and Mesnex i.v. solution diluted in 240 ml of water). Each dose was separated by a 48-h washout period. The oral formulations were supplied under an Investigational New Drug Application (Canada and the United States) by ASTA Medica (Frankfurt, Germany). The oral doses were twice the size of the i.v. dose because of an earlier reported 50% bioavailability (15). Subjects fasted from 8 h before until 4 h after drug administration and drank 200 ml of water 1 h before each dose, with drug administration (except with the i.v. mesna solution already diluted in water), and at 1, 2, 3, 6, 9, and 12 h after dosing. In an earlier study, food was shown to not affect the excretion of mesna in a crossover study of 12 healthy subjects using the 600-mg mesna tablets.

**Urine Sampling and Processing.** Predose urine samples were obtained to screen for analytical interference. After drug administration, all urine was collected hourly until hour 5 and then at hours 7, 9, 12, 15, 18, 24, and 36. For urine collected up to hour 18, individual voids were refrigerated and then pooled at the end of each collection interval. For the 18–24- and 24–36-h interval, urine voids were processed separately, and the measured amounts of analyte in each void were added for each interval. The volume of each urine specimen was determined gravimetrically. Mesna and dimesna were preserved by the addition of 0.5 ml of 1 n HCl and 0.125 ml of 10% EDTA to 5-ml urine aliquots, followed by storage at a temperature below −70°C until analysis. Creatinine was measured in an unpreserved urine aliquot.

Mesna and dimesna in urine were separated on an anion-exchange column by high-performance liquid chromatography (Hewlett-Packard 1090 system) and quantitated by postcolumn sulfitolysis and reaction with 2-nitro-5-thiosulfobenzoate, an agent that reacts specifically with thiols and disulfides, releasing a chromophore detected at 412 nm (22). Each analytical run was calibrated with seven non-zero standards (range, 25–1200 μM) prepared in pooled preserved urine, and at least three pairs of control samples for each analyte (60, 300, and 900 μM) were evenly spaced throughout the study samples. For results exceeding the highest calibrator, samples were diluted with water and assayed again with a control sample diluted in parallel. Results less than those of the lowest calibrator were reported as zero. The lower limit of quantitation was 50 μM for study samples after adjustment for a 2-fold preanalytical dilution, and the dynamic range extended to 2400 μM.

Before the study, validation experiments verified no interference from endogenous urine components and acceptable performance (<15% change) in mesna and dimesna measurements for accuracy, within and between-run precision, two freeze-thaw cycles, and stability (up to 48 h at room temperature and 1 year at −80°C in preserved urine). Less than 0.4% carryover was detected after injection of the highest mesna calibrator, and no carryover was detected after dimesna injections. Mesna standards contained 0.6% dimesna as an impurity; dimesna standards contained 0.27% mesna.

During the study, mesna and dimesna were measured in 1959 urine aliquots from the 24 subjects in 24 analytical runs within 110 days after the first day of dosing. To assess the stability of the analytes and methodology during the study period, 15% of the samples were randomly selected and assayed a second time in 4 analytical runs after the initial 24 analytical runs; the mean difference between the initial and repeated result was 2.3 ± 7.3% (SD) for mesna and 1.2 ± 5.5% for dimesna. The between-run (n = 28) precision (coefficient of variation <12.3% for all standards and controls) and the linearity of mesna (Pearson coefficient, r > 0.998) and dimesna (r > 0.996) calibration curves were acceptable. No background interference was detected in predose samples.

**Blood Sampling and Processing.** Predose samples were obtained to screen for analytical interference. After oral doses of mesna, blood samples were obtained at 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, and 24 h. After i.v. doses, blood samples were obtained at 5, 10, 20, and 30 min and at 1, 2, 3, 4, 6, 8, and 12 h after the end of the infusion. Mesna and dimesna concentrations were stabilized by the immediate addition of 1 part of a 4°C solution of 5% EDTA and 700 μM dithiothreitol to 2 parts of heparinized blood. After centrifugation, the plasma was added to 0.5 volume of a solution of 1 M perchloric acid and 1% (w/v) EDTA to precipitate plasma proteins. Aliquots of the protein-free plasma were stored at a temperature below −70°C until analysis.

Mesna and dimesna in protein-free plasma were separated by ion-pairing on a reverse-phase column by high-performance liquid chromatography (Beckman System Gold) and then quantitated as for urine by postcolumn sulfitolysis and reaction with 2-nitro-5-thiosulfobenzoate. There were no interfering substances at the retention times for mesna and dimesna, there was no carryover, and the assay showed acceptable performance (<15% change) for accuracy, within and between-run precision.

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3 Study No D-07093-0015 on file with ASTA Medica.
and stability during storage. Each analytical run was calibrated with five non-zero standards (range, 0.3–20 μM), and at least three pairs of control samples for each analyte (0.75, 7.5, and 15 μM) were evenly spaced throughout the study samples. For results exceeding the highest calibrator, samples were diluted with plasma matrix and assayed again with a control sample diluted in parallel. Results less than those of the lowest calibrator were reported as zero. After adjustment for preanalytical dilutions and the addition of preservatives, the lower limit of quantitation was 1.0 μM, and the dynamic range extended to 67.5 μM.

Results were considered to reflect whole blood rather than plasma concentrations because the preservative was added directly to whole blood. If we assume that mesna and dimesna are completely excluded from the RBC volume and occupy only the plasma volume, then plasma concentrations of mesna and dimesna would be expected to be higher than those reported using our dilution factor by an amount proportional to the hematocrit. If so, plasma concentrations for the subject with the highest hematocrit of 46% would be as much as 22% higher than our reported blood concentrations. This difference would not affect our analysis of data for bioavailability. However, we used hematocrit-adjusted values when comparing our blood data with plasma data from other studies.

Validation studies showed that the cold solution of acidified dithiothreitol preserved mesna in fresh whole blood for up to 60 min (<15% decrease in concentration). Moreover, dimesna was not reduced by the acidified dithiothreitol under the conditions of this study. This was verified by the addition of the preservative to 37°C solutions of pure dimesna at concentrations spanning the dynamic range. We detected less than 0.5% reduction of dimesna to mesna after incubation of these samples on ice up to 1 h.

During the study, mesna and dimesna were measured in 1631 blood samples from the 24 subjects in 24 analytical runs within 115 days after the first day of dosing. To assess the stability of the analytes and methodology at the end of the study period, 15% of the samples were randomly selected and subsequently assayed a second time in two additional analytical runs; the mean difference between the initial and repeated results was 8.6 ± 12.1% (SD) for mesna and 6.1 ± 11.9% for dimesna. The between-run (n = 26) precision (coefficient of variation <12.5% for all standards and controls) and the linearity of mesna (Pearson coefficient, r > 0.995) and dimesna (r > 0.996) calibration curves were acceptable. No background interference was detected in predose samples.

**Statistics.** The concentrations of mesna and dimesna were determined (St. Jude Children’s Research Hospital, Memphis, Tennessee) in the study samples with their identity encrypted with regard to drug treatment. Samples from each subject for all five drug treatments were assayed in random order in the same analytical run (one for urine and one for blood). Results from the analytical laboratory were decoded and analyzed (L.A.B. Pharmacological Research International). Three-way ANOVAs (SAS Statistical Package; SAS, Cary, NC) were performed for mesna and dimesna pharmacokinetic parameters with sequence, subject (sequence, nested), period (i.e., days of dosing), and treatment as factors. For blood, analyses were performed for the following parameters: (a) the AUC_{0-4} \(^4\) of the observed data; (b) the AUC_{0-∞}; (c) the maximum concentration; (d) the time of the maximum concentration; (e) the elimination rate constant; (f) the half-life of elimination; and (g) the concentrations at each sampling time point. The preceding parameters were calculated using model-independent pharmacokinetics. The linear trapezoidal method was used to calculate the AUCs. The elimination rate constant and the corresponding half-life were calculated with the log-linear regression method using at least three consecutive data points including the last measurable blood concentration. Analyses were also performed for the following urinary parameters: (a) the urinary concentrations by collection interval; (b) the urinary recovery by collection interval and by cumulative excretion intervals (millimoles and the percentage of the dose); (c) the rate of urinary excretion by collection interval; (d) the maximum excretion rate and the time of the maximum excretion rate; and (e) the minimum postdose urinary concentration (0–24 h). Model-independent pharmacokinetic methods were used for the calculation of the urinary parameters. Volumes of excreted urine were multiplied by the corresponding concentrations of the analytes to obtain the amounts excreted by collection interval and, ultimately, the cumulative urinary excretion parameters. The rate of excretion was obtained by dividing the amount excreted in a given interval by the number of hours in the interval. The interval midpoint times were associated with these rates. Also, blood and urine parameters for M + 2D were derived from data representing the sum of mesna and twice the dimesna value (concentration or amount). Power tests were conducted to determine what percentage of difference between formulations could be detected in the study with an α of 0.05 and a β of 0.20, and the probability of detecting a 20% difference between formulations, for the following parameters: (a) AUC and the maximum plasma concentration; and (b) CUE and the maximum rate of urinary excretion. Ratio analyses and 90% confidence intervals (two one-sided t test method) were also calculated for the measured data and ln-transformed data.

**RESULTS**

**Blood Pharmacokinetic Parameters.** Table 1 shows the pharmacokinetic parameters derived from the measured concentrations of mesna and dimesna in the blood of the 24 subjects. Parameters computed for the M + 2D concentrations are shown as an approximation of the unbound drug in the protein-free plasma.

After a 2-fold dose adjustment, the mean AUC_{mesna} for the 600-mg i.v. dose was similar to those of the 1200-mg oral formulations. The 2-fold dose adjustment was justified because dose proportionality had been shown for urinary and plasma pharmacokinetic parameters in an earlier study of 10 healthy volunteers given single oral doses (600, 1200, and 2400 mg) of mesna and a 600-mg i.v. dose in a crossover design. \(^5\) The mean

\(^4\) The abbreviations used are: AUC, area under the blood concentration-time curve; CUE, cumulative urinary excretion over 36 h; ln, natural log; M + 2D, sum of mesna and twice the dimesna concentration.

\(^5\) Study No. D-07093-0008 on file with ASTA Medica.
Table 1  Blood pharmacokinetic parameters (mean ± SD) for 24 subjects given an i.v. (600-mg dose) and four oral mesna formulations (1200-mg doses) at 48-h intervals

<table>
<thead>
<tr>
<th></th>
<th>i.v.</th>
<th>Oral solution</th>
<th>300-mg tablet</th>
<th>400-mg tablet</th>
<th>600-mg tablet</th>
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<tbody>
<tr>
<td><strong>Mesna</strong></td>
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<tr>
<td>AUC&lt;sub&gt;0-1&lt;/sub&gt; (μM · h)</td>
<td>67.4 ± 14.2</td>
<td>135.7 ± 21.7</td>
<td>139.1 ± 29.2</td>
<td>133.1 ± 30.6</td>
<td>133.3 ± 24.0</td>
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<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (μM)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>138.4 ± 47.6</td>
<td>42.4 ± 12.6</td>
<td>45.0 ± 13.3</td>
<td>40.4 ± 13.9</td>
<td>41.7 ± 15.0</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7 ± 1.0</td>
<td>2.5 ± 0.9</td>
<td>2.5 ± 0.9</td>
<td>2.7 ± 0.8</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>Half-life, initial (min)</td>
<td>10.8 ± 1.9</td>
<td>107.6 ± 22.3</td>
<td>101.7 ± 39.3</td>
<td>105.1 ± 62.4</td>
<td>97.1 ± 44.9</td>
</tr>
<tr>
<td>Half-life, terminal (min)</td>
<td>107.6 ± 22.3</td>
<td>101.7 ± 39.3</td>
<td>105.1 ± 62.4</td>
<td>97.1 ± 44.9</td>
<td>93.6 ± 41.6</td>
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<td><strong>Dimesna</strong></td>
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<tr>
<td>AUC&lt;sub&gt;0-1&lt;/sub&gt; (μM · h)</td>
<td>23.5 ± 5.0</td>
<td>49.7 ± 8.2</td>
<td>49.3 ± 11.3</td>
<td>49.3 ± 11.3</td>
<td>50.6 ± 9.5</td>
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<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (μM)</td>
<td>24.8 ± 4.2</td>
<td>17.7 ± 4.6</td>
<td>19.2 ± 6.5</td>
<td>17.0 ± 6.1</td>
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<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>0.16 ± 0.06</td>
<td>2.9 ± 0.9</td>
<td>2.8 ± 1.0</td>
<td>2.9 ± 0.8</td>
<td>2.9 ± 0.9</td>
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<tr>
<td>Half-life, initial (min)</td>
<td>35.8 ± 8.7</td>
<td>65.5 ± 20.7</td>
<td>62.2 ± 26.2</td>
<td>63.5 ± 29.3</td>
<td>58.7 ± 19.1</td>
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<tr>
<td>Half-life, terminal (min)</td>
<td>114.4 ± 26.0</td>
<td>235.1 ± 32.9</td>
<td>237.7 ± 22.6</td>
<td>231.7 ± 43.2</td>
<td>234.4 ± 35.7</td>
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<tr>
<td><strong>M + 2D</strong></td>
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<tr>
<td>AUC&lt;sub&gt;0-1&lt;/sub&gt; (μM · h)</td>
<td>138.4 ± 53.8</td>
<td>76.7 ± 19.8</td>
<td>82.0 ± 25.8</td>
<td>73.5 ± 24.0</td>
<td>75.7 ± 22.1</td>
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<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (μM)</td>
<td>16.4 ± 3.8</td>
<td>2.8 ± 0.9</td>
<td>2.6 ± 0.9</td>
<td>2.8 ± 0.9</td>
<td>2.7 ± 0.9</td>
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<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>52.9 ± 14.3</td>
<td>81.1 ± 25.6</td>
<td>64.5 ± 20.3</td>
<td>77.3 ± 41.2</td>
<td>70.7 ± 24.9</td>
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<tr>
<td>Half-life, initial (min)</td>
<td>52.9 ± 14.3</td>
<td>81.1 ± 25.6</td>
<td>64.5 ± 20.3</td>
<td>77.3 ± 41.2</td>
<td>70.7 ± 24.9</td>
</tr>
<tr>
<td>Half-life, terminal (min)</td>
<td>52.9 ± 14.3</td>
<td>81.1 ± 25.6</td>
<td>64.5 ± 20.3</td>
<td>77.3 ± 41.2</td>
<td>70.7 ± 24.9</td>
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</table>

<sup>a</sup> Maximum concentration.
<sup>b</sup> Time of the maximum concentration.
<sup>c</sup> C<sub>max</sub> of the i.v. dose was determined from data at 5 min after the end of the infusion.

AUC<sub>dimesna</sub> and consequently the AUC<sub>M + 2D</sub> were slightly lower for the i.v. (dose-adjusted) formulation than they were for the oral formulations. The coefficients of variation for the AUC<sub>mesna</sub> ranged from 16–23% (AUC<sub>M + 2D</sub> 10–19%) for the oral formulations. Bioequivalence among the oral formulations was indicated by 90% confidence interval limits for the AUC (and the maximum concentrations) that were within 80–120% between formulations for the observed and ln-transformed data. The mean ratio of the AUC<sub>0-0.5</sub>:AUC<sub>0-∞</sub> for the five formulations ranged from 0.91–0.93 for mesna and from 0.94–0.96 for dimesna, indicating that the sampling schedule adequately described the plasma profiles.

The maximum concentrations for mesna and dimesna after oral doses were about 15 and 73%, respectively, of those measured 5 min after the end of the i.v. infusion (dose-adjusted). There were no differences between the three tablet formulations and the oral mesna solution for the maximum concentrations (mean, 42–45 μM) or the time to attain the maximum concentrations (mean, 2.5–2.7 h; range, 1–4.5 h; n = 96), which indicated that the tablet coatings did not delay absorption. Mean maximum for dimesna concentrations occurred about 10 min later than they did for mesna after both i.v. and oral doses. The initial half-life for mesna was only 11 min, but the terminal half-life of 108 min was consistent with the persistent excretion of mesna for several hours after an i.v. dose; the terminal half-life for oral doses was similarly long.

For the oral formulations, the mean AUC<sub>mesna</sub> for the second and subsequent doses was higher than that of the first dose by about 12% (mean maximum concentrations, 30%). The mesna half-life is too short for this increase to be explained by carryover across 48-h dose intervals. Saturation of high-affinity endogenous free thiol sites might explain why more mesna (but not dimesna) was detected after the first mesna dose.

**Urinary Concentrations of Mesna and Dimesna.** Fig. 1 shows the range (n = 24 subjects) of measured urinary mesna concentrations for urine collection intervals after the 600-mg i.v. dose and a representative oral 1200-mg dose (three 400-mg tablets). After the i.v. dose, mesna was detectable in all specimens through hour 4. Values fell below the limit of quantitation in an increasing number of subjects after 4 h until the 24–36-h interval, within which no specimens had reportable mesna concentrations (>50 μM). After 1200-mg oral doses, mesna was detectable in all specimens through hour 12, and more than half of the subjects had a reportable mesna concentration in at least one urine void during the 24–36-h interval. The mesna concentrations in the 18–24-h interval, which would represent the daily nadir, were similar for the four oral formulations (medians, 0.11–0.15 mM; no significant differences).

The reduced hydration schedule after hour 3 contributed to the persistence of high urinary mesna concentrations and a slight increase at hour 5 after oral dosing. The median urine output of 228 mL/h during the first 4 h decreased to 95 mL/h during hours 4–9 and decreased to 75 mL/h during hours 9–18. In several instances, subjects did not completely empty their bladders at the specified times, leading to extreme values in urine output (range, 5–520 mL/h) that added variability to the concentration data. Concentrations varied widely among the subjects, varying more than 10-fold within most collection intervals.

The concentration of mesna was higher than that of dimesna in 83% of the 1115 urine specimens that had reportable concentrations (>50 μM) for both analytes. Nearly all of the urine samples in which the dimesna concentration exceeded the mesna concentration were obtained during the 2- and 3-h collection intervals. A slight bias toward higher recovery of mesna in the free thiol form after i.v. doses is possible because most of the i.v. dose was excreted during the earlier 1-h collection intervals, which permitted less oxidation of mesna to dimesna within the bladder.

**Urinary Excretion Rates.** To adjust for varied urine output and the length of the collection interval, data were expressed as the amount of analyte in millimoles excreted per hour. After i.v. doses, the rate of mesna excretion (Fig. 2)
declined rapidly in the first hour and declined by 36-fold to 0.03 mmol/h by hour 4. After oral doses, the rate of mesna excretion increased to a maximum at 2.56 h (mean) after each of the three tablet formulations (2.77 h for the oral solution) and did not decline to 0.03 mmol/h until the 10–12-h interval. The mean rates of dimesna excretion (Fig. 2) paralleled (time of maximum rate, 2.81–2.85 h for the three tablet formulations and 3.04 h for the oral solution) those of mesna but were lower.

**Cumulative Excretion.** Fig. 3 shows the mean cumulative excretion for the i.v. and four oral formulations. More mesna was excreted after the i.v. doses (40% of the dose by hour 36) than after the oral doses (31–33%). Also, mesna was excreted more rapidly after i.v. doses (89% of the excreted mesna was recovered by 2 h) than after oral doses (90–92% by hour 9). By contrast, less dimesna was excreted after i.v. doses (34% of the dose) than after oral doses (38–40%). Consequently, the sum of mesna and dimesna excreted for the oral formulations (68–73% of the dose) was similar to that of the i.v. route (73%). The mean (n = 20) amount of mesna (but not dimesna) excreted after oral doses on days 3, 5, 7, and 9 was 9.6–14.5% higher than that of day 1 despite the 48-h washout period, consistent with the period effect observed for blood AUC data.

The distribution of the CUE data for mesna excretion after oral doses (Fig. 4) was very narrow, which resulted in bioequivalence criteria being met for all oral treatments. The coefficients of variation for the CUE data (Fig. 4) were similar for the i.v. (13%) and oral formulations (range, 11–17%). In accord with the blood data, bioequivalence among the oral formulations was indicated by 90% confidence interval limits for the CUE (and the maximum rate of excretion) that were within 80–120% between formulations for the observed and ln-transformed data.
Mesna – 400mg, 300mg, 600mg

Fig. 3 Mean CUE for the mesna formulations in 24 subjects.

Dimesna

Fig. 4 Thirty-six-hour CUE of mesna for each subject by mesna formulation. Bar, mean value.

Mesna+Dimesna

Fig. 5 Histogram of relative oral:i.v. bioavailability of urinary free thiol mesna. Bioavailability was determined for each subject as the mean of the ratios of the CUEmesna for each of the four oral formulations to the CUEmesna of the i.v. dose.

Interval Endpoint (hour)

Cumulative Excretion (percentage of mesna dose)

Fig. 3 Mean CUE for the mesna formulations in 24 subjects.

The 36-h creatinine excretion in each subject (determined from the sum of creatinine in each urine aliquot) was similar when measured every other day for the five consecutive doses (median coefficient of variation, 8%; range, 3–18%), supporting the completeness of urine collection. The mean creatinine clearance rate for each subject ranged from 86–125 ml/min/m² and was within the reference range for healthy adults. This narrow range of clearance values suggests that variations in mesna excretion (Fig. 4) were due to factors other than just glomerular filtration.

Ratio of Mesna:Dimesna in Blood and Urine. Considering that non-protein-bound mesna and dimesna in the blood should be excreted by glomerular filtration, we compared the ratio of these analytes in protein-free plasma and urine after oral doses (Fig. 6). The mean ratio of the mesna:dimesna concentration in blood was highest when first sampled at 1 h after oral doses, by which time the mean blood concentrations of mesna (18.1 ± 10.5 μM) and dimesna (5.6 ± 3.2 μM) were about 10% of their peak values (Table 1). The ratio declined until about the time of maximum absorption at 3 h. The mean mesna:dimesna ratios in urine paralleled the blood ratios but were lower (Fig. 6). After i.v. doses (data not shown), the mesna:dimesna ratio in blood declined rapidly during the first hour from 6.3 to 1.4 (n = 24), but there was only a single urine collection during the first hour for comparison. The first-hour ratio in urine was lower after the i.v. doses (2.8 ± 0.4) than after the oral doses (3.2 ± 1.0), which may reflect the rapid decline in blood concentrations during the first hour after i.v. doses.

Adverse Effects. Adverse effects possibly associated with mesna administration included the following: (a) the subject who withdrew from the study because of ocular inflammation also developed loss of appetite followed by nausea and vomiting after discharge; (b) one subject had a rash during a period that included three of his four oral doses; (c) two subjects...
had loose stools after one oral dose; (d) one subject reported dizziness after one oral dose; and (e) one subject reported pain at the site of infusion of an i.v. dose. All adverse events were mild or moderate in intensity and resolved spontaneously.

DISCUSSION

This study shows that the bioavailability of oral and i.v. mesna formulations is similar, and that the concentrations of mesna are higher than those of dimesna in both the blood and urine. Our data differ from those of earlier reports in which have reported much less urinary mesna after oral doses than after i.v. doses. In the only other study to evaluate free thiol excretion, Strofer-Vogel et al. (16) reported that the percentage of an 800-mg i.v. dose excreted as free thiol mesna over 24 h was 32% (range, 15–43%; n = 6), which approaches our mean value (40%). However, after oral doses, they detected only 18% (range, 4–27%) of the drug as free thiol mesna in the urine, leading them to conclude that the relative oral:i.v. excretion of mesna available for uroprotection was 53% ± 20%. Others have recovered a wide range of thiol and disulfide concentrations in the urine of small numbers of subjects under various conditions (16–21). Our bioavailability data for mesna differ from those of earlier studies in part because of procedural differences. A large number of subjects received multiple mesna formulations in our randomized crossover study design. Urine was obtained at more frequent intervals and for a longer period of time after each dose than in other studies, and the low intrasubject coefficient of variation for creatinine excretion supported the completeness of urine collection. We determined the concentrations of mesna and dimesna by a single chromatographic injection. Others estimated dimesna concentrations indirectly from the difference between free thiol and total reducible mesna concentrations and may not have adequately preserved the analytes in blood or urine.

The sustained excretion (Fig. 2) and high bioavailability (Fig. 5) of oral mesna suggest that this route of administration should be at least as uroprotective as i.v. mesna. The 1200-mg oral doses resulted in urinary mesna concentrations exceeding 50 μM in all of our subjects for 12 h, as compared with 4 h after a 600-mg i.v. dose (Fig. 1). The four oral formulations showed equivalent urinary bioavailability based on the cumulative urinary excretion (Fig. 3), the pattern of excretion (Fig. 2), and the intersubject variability (Fig. 4).

Our bioavailability data for mesna differ from those of our dose-adjusted blood (AUCmesna and AUCdimesna) and urine (CUEM + 2D) data support near equivalence in the bioavailability of oral and i.v. mesna. The low coefficients of variation of 10–23% for the AUC data (Table 1) and of 11–17% for the CUE data (Fig. 4) are consistent with the complete absorption of oral mesna (23). The i.v. data showed similarly low coefficients of variation (AUC, 21%; CUE, 13%), which indicates equivalent variability for all of the formulations.

Considering only the therapeutically active free thiol form of mesna, the mean ratio of the amount excreted after oral versus i.v. doses was 0.82, which is consistent with the similar urinary recovery of free thiol mesna after oral and i.v. doses in patients who received combined i.v. and oral mesna therapy (24). However, the ratio of oral:i.v. bioavailability ranged from 0.52–1.23 (Fig. 5), and the excretion of mesna after oral doses was distributed over a longer period of time (Fig. 3). Therefore, higher oral doses may be required to maintain uroprotective mesna concentrations comparable to those after i.v. doses (24).

Our urinary data contrast with those of earlier studies, which have reported much less urinary mesna after oral doses than after i.v. doses. In the only other study to evaluate free thiol mesna in the urine of healthy volunteers by a specific chromatographic procedure, James et al. (15) reported that the percentage of an 800-mg i.v. mesna dose excreted as free thiol mesna over the point of each collection interval. Bar, SD.
The decline in the mesna:dimesna ratio with time in both blood and urine after both oral and i.v. doses might reflect the deplet-
ion of cysteine and glutathione (25). About 70% of the drug
was recovered as mesna and dimesna in the urine (Fig. 3); a
proportion of the remainder may be accounted for by reactions
with cysteine (26) or other endogenous substances.

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