**Featured Article**

**Crossover Randomized Comparison of Intravenous versus Intravenous/Oral Mesna in Soft Tissue Sarcoma Treated with High-Dose Ifosfamide**

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**Abstract**

**Purpose:** We conducted our study to determine the pharmacokinetics (PK) and clinical efficacy of oral mesna in patients receiving ifosfamide for soft tissue sarcoma.

**Experimental Design:** Seventeen patients were enrolled in a randomized prospective Phase I/II study. Seventeen patients were exposed to study medication. Ifosfamide was given at a dose of 2 g/m²/day for 5 days on a 21-day cycle. Before the first cycle, all patients were randomized onto a crossover design and received either the approved i.v. or i.v./oral mesna regimen, with crossover for the second cycle of chemotherapy. The i.v. mesna regimen consisted of dosings (20% ifosfamide dose) at 0, 4, and 8 h. The i.v./oral arm consisted of an i.v. mesna dosing (20% ifosfamide dose) at 0 h, followed by oral tablet dosing (40% ifosfamide dose) at 2 and 6 h. In-patient clinical monitoring and phlebotomy and urine sampling for mesna, dimesna, and ifosfamide PK were performed on all chemotherapy days.

**Results:** Thirteen patients were evaluable for PK and 17 for efficacy and toxicity. No significant differences were detected in the plasma PK of the concomitantly infused ifosfamide. Rates of hemorrhagic cystitis were similar across mesna schedules.

Four of 10 evaluable patients demonstrated objective response.

**Conclusion:** On the basis of our study, an i.v./oral mesna regimen is at least as uroprotective as the approved i.v. regimen. The i.v./oral regimen will improve patient tolerance and convenience, allow for a reduction in elective hospitalizations for ifosfamide chemotherapy, reduce the potential morbidity associated with inpatient administration of chemotherapy, and likely result in decreased costs of care.

**Introduction**

First synthesized in 1965, ifosfamide [3-(2-chloroethyl)-2-[(2chloroethyl)-amino]tetrahydro-2H-1,3,2-oxazaphosphorin-2-oxide] is a member of the oxazaphosphorine family of alkylating agents (1, 2) and has a broad spectrum of activity. Early clinical studies of ifosfamide demonstrated its encouraging efficacy in a variety of tumor types, however, additional investigation was hampered by unacceptably high rates of dose-limiting urothelial toxicity (3–5).

The pharmacology of ifosfamide has been studied extensively (6–10). Ifosfamide differs from another oxazaphosphorine, cyclophosphamide, by the position of its two chloroethyl groups on the central ring (Fig. 1). This is responsible for the comparatively greater water solubility, antitumor activity, and toxicity profile of ifosfamide. Like cyclophosphamide, ifosfamide is administered as a prodrug, which requires activation by the hepatic cytochrome P450 mixed oxidase system 3A4 (Refs. 11 and 12; Fig. 2). The initial metabolic step is hydroxylation, producing an active alkylator, 4-hydroxyifosfamide, that is in turn transformed into active electrophiles that mediate most of the toxic effects of ifosfamide (13).

Administration of ifosfamide for five or fewer daily doses of <1.2 g/m² modestly reduces the rates of hemorrhagic cystitis; however, significant numbers of patients continue to experience this complication, and without the efficacy benefit of dose escalation (14, 15). Several approaches have been studied in an attempt to control ifosfamide-induced urothelial toxicity and permit the clinical investigation of higher doses of this agent. Direct intravesicular injection of a variety of anti-inflammatory agents has been attempted but is logistically difficult and does not address the effects of acrolein on the pyelocalyceal system and ureters. Vigorous hydration, which both lowers the concentration of acrolein and accelerates their transit time, continues to be a valuable tool. However, as a direct result of the development of the uroprotective agent mesna (sodium-2-mercaptopethane sulfonate; Refs.
Comparison of Intravenous versus Intravenous/O oral Mesna

Intravenous administration

The study of ifosfamide, in particular at active high doses, could then be broadly pursued. Mesna is hydrophilic, preventing its passage out of the vascular bed into cells. This results in efficient renal clearance and avoids any adverse impact on the cytotoxic effects of ifosfamide. After i.v. administration, mesna is rapidly oxidized in the plasma to dimesna (disodium 2,2′-dithiodiethylnesulfonate), which is the predominant circulating form (Fig. 2). After glomerular filtration, dimesna undergoes reabsorption in the proximal tubules. Before secretion in the distal tubules, one-third of the dimesna is rapidly converted back to the active thiol mesna by glutathione reductase in the cytoplasm of distal tubular epithelial cells. Mesna then readily detoxifies urinary 4-hydroxyifosfamide metabolites and acrolein (6, 9, 24, 25). The time to maximal urinary excretion is ~60 min after i.v. administration and, on average, ~150 min after oral administration (9, 25, 26). The minimum uroprotective concentration of mesna is ~1.7 μmol/liter or 100 μg/ml, which is achieved far more quickly with an i.v. dose (5.85 ± 2.16 h) compared with an oral dose (13.11 ± 6.11 h; Ref. 26).

Patients and Methods

Eligibility. This was a prospective, randomized Phase I/II multi-institutional crossover study comparing the PK and clinical efficacy of i.v., versus i.v. followed by oral mesna in tablet form in patients diagnosed with soft tissue sarcoma treated with ifosfamide.

Oral administration of mesna solution significantly increases the rates of gastrointestinal distress, likely because of its poor palatability (11, 22). We, therefore, conducted a prospective, randomized Phase I/II study comparing the PK and clinical efficacy of i.v., versus i.v. followed by oral mesna in tablet form in patients diagnosed with soft tissue sarcoma treated with ifosfamide.

Fig. 1 Structure of ifosfamide and cyclophosphamide. (Reprinted with permission from Ref. 5.)
ted to an inpatient treatment unit for both cycles of chemotherapy. PK, toxicity, and clinical efficacy data were collected for the first two cycles of ifosfamide. After two cycles, based on either radiographic or other measure of antitumor efficacy, patients could receive additional cycles of ifosfamide or initiate alternative and clinically appropriate sarcoma therapy. Patients who experienced emesis within 1 h of receiving oral mesna on days 1 or 5 were removed from the protocol and subsequently received clinically appropriate therapy (ifosfamide with i.v. mesna support) off-study.

**PK Data Collection and Processing.** To avoid contamination of study samples, blood and urine samples for ifosfamide and mesna were performed in separate locations within the processing area. Blood samples for mesna were collected into 5-ml graduated syringes and transferred immediately into prechilled sample tubes containing a 2c solution of 700 µmol/liter DTT in 5% EDTA and placed on an ice bath. Within 30 min of obtaining blood samples, each sample tube was centrifuged at 10°C and 3cc of plasma was transferred into a prechilled sample tube containing 1M perchloric acid and 1% EDTA. The remaining plasma from the initial sample tube was stored at −65 to −85°C, pending final analysis. Blood samples for ifosfamide were collected into 10-ml graduated syringes, and 2 cc were transferred into a sample tube containing 3 ml of sodium heparin. These tubes were then centrifuged within 60 min of collection at ambient temperature for 10 min. Plasma obtained was then stored at −65 to −85°C, pending final analysis. Urine samples were stored at 4–10°C during the collection period. Samples were transferred into 500-ml containers containing 8.25 ml of 6 M HCl and 12.5 ml of 10% (w/v) EDTA solution and were quantified. Two 2-ml aliquots of urine were transferred into sample tubes and stored at −65 to −85°C, pending final analysis.

On days 1 through 4, blood samples for mesna and ifosfamide were obtained before the administration of the ifosfamide and first dose of mesna. On day 5, blood samples for patients on the i.v. mesna arm were performed at time 0 (start of ifosfamide and first dose of mesna), 15 and 30 min, 1 h, 2 h, 4 h, 4 h 15 min, 4 h 30 min, 5 h, 6 h, 8 h, 8 h 15 min, 8 h 30 min, 9 h, 10 h, 12 h, 14 h, 16 h, 20 h, 24 h, and 28 h. Blood samples for patients on the i.v./oral arm were performed at time 0 (start of ifosfamide and first dose of mesna), 15 and 30 min and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 20, 24, and 30 h. Individual urine samples

### Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Gender</th>
<th>Diagnosis</th>
<th>Metastatic (site)</th>
<th>Prior chemotherapy</th>
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<td>1</td>
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<td>F</td>
<td>MFH</td>
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<td>2</td>
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<td>A + I + DTIC + VP16 + CP + CX + V</td>
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<td>A + I</td>
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<td>11</td>
<td>30</td>
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<td>A + I</td>
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<td>73</td>
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<tr>
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<td>25</td>
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<td>Synovial sarcoma</td>
<td>Y (lungs)</td>
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</tbody>
</table>

* F, female; M, male; MFH, malignant fibrous histiocytoma; NOS, not otherwise subclassifiable; ST, soft tissue; P, pancreas; A, doxorubicin; I, ifosfamide; DTIC, dacarbazine; CP, cisplatin; CX, cytoxan; V, vincristine; Y, yes; ASPS, alveolar soft part sarcoma.*
were collected over 12 2-h periods on days 1 and 5 on both arms, starting 2 h before the first dose of mesna and ifosfamide.

On days 3 and 4, a single 2-h sample was obtained on all patients starting 2 h before the first dose of mesna and ifosfamide.

Plasma PK parameters for mesna and ifosfamide were:
- maximum concentrations (C_max) of mesna and dimesna after each mesna administration (i.v. or oral) on day 5
- C_max of ifosfamide at the end of infusion
- time to reach maximum plasma concentration (t_max) after each oral mesna administration on day 5
- area under the concentration-time curves (AUC_0-last and AUC_0-24 h) for i.v. and oral mesna on day 5 over all three mesna administrations
- terminal half-lives (t_1/2) after the final mesna dose on day 5
- predose plasma levels on days 2–5.

Urine PK parameters were:
- cumulative urinary excretion at times 0–12 h and 0–24 h as amounts and as a fraction of the daily mesna dose
- maximum urinary excretion rates (R_max) after oral and i.v. mesna doses; time to reach maximum excretion rates (t_max); nadir excretion rates (R_min) at 22–24 h; and predose urine levels on days 2–5.

The sample size was determined on the basis of PK considerations. The study was not powered to detect differences in hematuria.

**Results**

Seventeen patients have been enrolled, 16 patients were exposed to study medication, with characteristics summarized in Table 1.

Three of the 16 patients (3, 5, and 12) did not complete the protocol. Patient 12 had extensive retroperitoneal sarcoma with hepatic involvement and experienced significant azotemia (serum creatinine, 3.8 mg/dl) associated with renal tubular acidosis (serum bicarbonate, 9 mg/dl) on day 3 of cycle 1. These adverse events were attributed to ifosfamide. Patient 5 experienced emesis shortly after receiving the first oral mesna dose on the first day of cycle 1. The patient subsequently admitted to consuming a heavy and fatty meal before initiating chemotherapy. Patient 3 experienced dyspnea because of tumor progression and was removed from protocol after completing one cycle of treatment.

Results of the day 5 PK analysis of the standard i.v. and i.v./oral mesna regimens are summarized in Table 2. The patient analysis of ifosfamide is detailed in Tables 3 and 4.
observed in the i.v./oral regimen, with excretion rates at the end of the 24-h period above that seen in the standard i.v. regimen. No significant differences in the plasma PK of the concomitantly infused ifosfamide were detected between the two mesna schedules. One patient who showed visible blood in the first cycle with mesna/i.v./i.v. dosing had microhematuria only in the subsequent cycle with mesna i.v./oral/oral dosing. Overall, equal rates of microhematuria were observed with both dosing regimens (3 of 16 for i.v./i.v./i.v. dosing and 4 of 16 for i.v./oral/oral dosing). Of the 16 patients evaluable for safety, there were no serious adverse events attributed to oral or i.v. mesna. There was more nausea in the i.v./oral/oral arm with 14 of 16 patients experiencing this effect versus 9 of 16 in the i.v./i.v./i.v. arm. Seven patients in the oral schedule had emesis versus five patients on the i.v. schedule. Of 10 patients evaluable for tumor response, four demonstrated objective improvement, consisting of three partial responses (defined as a 50% reduction in clinically evident disease by cross-sectional imaging or physical examination) and one cytoreductive response that did not meet criteria for a partial response.

Discussion

Ifosfamide is an alkylating chemotherapeutic agent with significant activity in a variety of malignancies. Among its toxicities, irritation of uroepithelium by excreted 4-hydroxyifosfamide and spontaneously formed acrolein was dose limiting, before the development of mesna. The efficacy of this thiol in preventing ifosfamide-induced urotoxicity is well documented, and both have demonstrated excellent and equivalent clinical results. The use of a tablet formulation of oral mesna will eliminate the issue of palatability seen with the oral administration of mesna solution. This, in turn, will improve patient compliance and may result in lower complication rates from outpatient therapy with ifosfamide. In addition, the i.v./oral regimen will improve patient convenience, decrease care costs associated with prolonged patient monitoring in infusion centers, and allow for a reduction in elective hospitalizations. The tablet formulation of oral mesna with the schedule described in this study has been approved by the U.S. Food and Drug Administration.

References


Table 4  PK parameters of ifosfamide in plasma

<table>
<thead>
<tr>
<th>Ratio i.v.:oral:oral vs. i.v.:i.v.:i.v. (ANOVA)</th>
<th>Cmax (μmol/l)</th>
<th>tmax (h)</th>
<th>AUC0–12 (μmol/h/liter)</th>
<th>AUC0–24 (μmol/h/liter)</th>
<th>AUC (μmol/h/liter)</th>
<th>CL (liter/h/kg)</th>
<th>Vss (liter/kg)</th>
<th>MRTss, 2h-infusion (h)</th>
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<tbody>
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<td>Estimate</td>
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<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.98</td>
<td>1.02</td>
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<tr>
<td>90% CI</td>
<td>0.91–1.10</td>
<td>0.95–1.05</td>
<td>0.92–1.09</td>
<td>0.93–1.08</td>
<td>0.92–1.07</td>
<td>0.90–1.07</td>
<td>0.98–1.06</td>
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</tr>
</tbody>
</table>
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