RENAL CLEARANCE OF IFOSFAMIDE

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ABSTRACT:
Nephrotoxicity is an important clinical side effect of the chemotherapeutic agent ifosfamide. This medication is activated by the hepatic cytochrome P450 system with potentially toxic metabolites produced through both ring hydroxylation and chloroethyl side chain oxidation pathways. Using an isolated perfused rat kidney preparation, we examined the possibility that renal metabolism of ifosfamide also occurs. Renal function before and after addition of ifosfamide to perfusate was not significantly different. After addition of ifosfamide to the perfusate, the metabolites N2-dechloroethylifosfamide, N3-dechloroethylifosfamide, and isophosphoramidemustard were recovered from urine and renal venous effluent. These results provide the first demonstration of ifosfamide metabolism by the kidney and suggest the possibility that intrarenal metabolism may contribute to nephrotoxicity.

Ifosfamide is being used increasingly for treatment of pediatric malignancies. Despite concurrent use of the uroprotectant medication mesna, many IF-treated children have subclinical nephrotoxicity, and approximately 5% develop a persistent Fanconi syndrome (1, 2). IF is a produg that must be biotransformed by the hepatic cytochrome P450 system before it can exert its therapeutic or toxic effects (1, 3). Ring hydroxylation produces 4-hydroxyIF, which is then converted into the active alkylating agent isophosphoramidemustard (IPM) and acrolein, a putative cause of hemorrhagic cystitis and in vitro nephrotoxin (1, 3–5). IF also undergoes considerable chloroethyl side-chain oxidation yielding N2-dechloroethylIF (N2D) and N3-dechloroethylIF (N3D) as well as chloroacetaldelyde, a chemical that is potentially responsible for IF’s neurotoxic and nephrotoxic side effects (3–6).

Based on clinical studies, Boddy et al. (7) have proposed that, although hepatic metabolism has some influence on IF nephrotoxicity, renal metabolism may also be involved. They noted that P450 enzymes involved in IF metabolism are polymorphically expressed in the kidney and suggested that differential expression of these enzymes contribute to kidney damage independent of systemic metabolism (7). The purpose of this study was to examine the possibility that the kidney can activate IF by using the isolated perfused rat kidney.

Materials and Methods
Perfusion experiments have been previously described (6). In brief, female Lewis rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 300–350 g were anesthetized with intraperitoneal Inactin (100 mg/kg body wt). After the right renal artery, inferior vena cava and right ureter were cannulated, the kidney was removed and placed in a heated chamber (38°C). Kidneys were perfused at a mean pressure of 100 mm Hg distal to the tip of a 21-gauge stainless steel arterial cannula. The perfusate consisted of 6.6 g/dl dialyzed Pentex fraction V with 1081 mg/dl urea, 7 mM glucose, 6 mM lactate, and amino acids and was continuously gassed with a mixture of 95% O₂ -5% CO₂.

Kidneys were perfused for 90 min. Seven consecutive 10-min urine collection intervals were performed beginning 20 min after renal artery cannulation. Arterial and venous perfusate samples were taken at the midpoint of each collection interval. Each experiment had a control period (the first three collection intervals from 20–50 min of perfusion) when IF was absent from the perfusate, an equilibration period (the fourth collection interval from 50–60 min of perfusion) during which 20 mg (77 μmol) IF (Bristol-Myers Squibb Co., Princeton, NJ) was added to the perfusate, and an experimental period (the final three collection intervals from 60–90 min of perfusion) when IF was present in the perfusate.

Polyfructosan was measured by the Anthrone method (8). A commercial kit was used to determine glucose (Boehringer-Mannheim Diagnostics, Indianapolis, IN). Sodium was measured by flame photometry. IF, N2D, N3D, and IPM were determined using a previously described capillary gas chromatography/chemical ionization mass spectrometry procedure (9). Detection limits were IF 0.04 μM, N2D 0.03 μM, N3D 0.03 μM, and IPM 0.05 μM. We did not attempt to trap the labile compound, 4-hydroxyIF, during specimen collection, and this metabolite was therefore not measured. Glomerular filtration rate and fractional reabsorptions of sodium and glucose were calculated using standard formulas. Total renal clearance encompassing filtration, secretion, reabsorption, storage, and metabolism of IF and metabolites was calculated as the product of perfusion flow rate and extraction ratio. Urinary clearance encompassing filtration, secretion, reabsorption and metabolism of IF was determined by dividing the product of urine concentration and flow rate by arterial concentration. Clearance by kidney storage and metabolism was calculated by subtracting urinary clearance from total renal clearance.

Samples from each collection interval were analyzed individually. Data from each control and experimental period were averaged (or summed in the case of urinary IF and metabolite excretion rates) and this average (or sum) represented the result of that experiment. Displayed results are mean ± SE of four perfusion experiments. A two-tailed Student’s t test for paired samples was used to compare kidney function during control and experimental periods and to compare arterial and venous perfusate concentrations during experimental periods, with p < 0.05 considered significant.

Results and Discussion
Perfused kidneys demonstrated normal function when compared with published data from other investigators (10, 11). There were no significant differences in kidney function between control and experimental perfusion periods (table 1). Neither IF nor metabolites were
detected in perfuse or urine during control periods; subsequently, they appeared in both perfuse and urine (table 2). Approximately 3.5 μmol or 5% of infused IF was recovered unchanged in urine during the 30-min experimental period. Urinary recovery of N2D, N3D, and IPM was approximately 0.005, 0.004, and 0.01% of infused IF, respectively. Comparison of urinary excretion rates of IF and metabolites between the first and last experimental collection intervals showed no significant differences in IF (−3.1 ± 8.4 nmol/min) or N3D (−0.01 ± 0.02 nmol/min) excretion and significant increases in N2D (0.07 ± 0.02 nmol/min, p < 0.05 vs. 0) and IPM (0.05 ± 0.01 nmol/min, p < 0.05 vs. 0) excretion.

Urinary clearance accounted for only 7 ± 1% of IF removal from perfuse and was 48 ± 3% of glomerular filtration rate. Assuming IF is freely filtered and not significantly protein bound (3), the latter finding indicates tubular reabsorption of this medication. Nonurinary clearance was responsible for most IF removal implying substantial storage or metabolism by renal tissue. In contrast, mean values for total and nonurinary clearance of N2D and N3D were negative and significantly less than zero for IPM. These results are consistent with metabolite production by the kidney and their release into the systemic circulation.

The above results provide the first demonstration of renal IF metabolism through both ring hydroxylation and side-chain oxidation pathways. The contribution of this biotransformation to IF nephrotoxicity is not clear. Although kidney function was not affected in the present study, the amount of metabolites excreted was small, and the duration of IF exposure was brief compared with clinical practice. In renal tubule cell cultures, IF in concentrations of 75–400 μmol/L and exposure times of 1–24 hr modestly suppresses DNA synthesis but does not impair cell viability or transport function (4, 5). These findings suggest that renal IF metabolism does not play a major role in kidney damage. However, renal cells can experience a substantial loss of cytochrome P450 activity in culture and the ability of this cell culture system to reflect in vivo conditions is therefore uncertain (12). Further study is needed to define the mechanisms responsible for IF nephrotoxicity.

### References