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 intraternal metabolism may contribute to nephrotoxicity.

IF 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 prodrug 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 chloroaacetalddeylene, 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 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 ± 6 mg) 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, storage, and metabolism of IF and metabolites was calculated as the product of urine flow rate and extraction ratio. Total renal clearance encompassing filtration, secretion, reabsorption, and metabolism of IF and metabolites 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...
Conditions is therefore uncertain (12). Further study is needed to define metabolic pathways between the first and last experimental collection intervals (IPM) was approximately 0.005, 0.004, and 0.01% of infused IF, the 30-min experimental period. Urinary recovery of N2D, N3D, and m aflatoxins they appeared in both perfusate and urine (table 2). Approximately 3.5 × 10^8 rabbit renal proximal tubule cells can experience a substantial loss of cytochrome P450 activity in cultures, IF in concentrations of 75–400 μmol/L and exposure times of 30, 60, and 120 min. Assumptions were made that this activity is not recoverable and that it is due to a decrease in the number of active sites or to an inactivation of the enzyme.

Although kidney function was not affected in the present study, the results 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.

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### References