Potentiation of Ifosfamide Neurotoxicity, Hematotoxicity, and Tubular Nephrotoxicity by Prior cis-Diaminedichloroplatinum(II) Therapy


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ABSTRACT

We investigated the relationship between prior therapy and three distinct forms of toxicity that developed during ifosfamide administration (1.6 g/m²/day for 5 days) in 36 children with malignant solid tumors. Ten therapies that were studied by multiple regression techniques, only the number of doses of cisplatin that patients had received was significantly related to neurotoxicity, hematotoxicity, and tubular nephrotoxicity, with the more severe cases occurring after three or more doses (P < 0.05). Increased urinary concentrations of the renal tubular enzyme N-acetyl-β-D-glucosaminidase, measured before each course of ifosfamide, were predictive of neurotoxicity (P = 0.02) and hematotoxicity (P = 0.01). We suggest that cisplatin-induced renal tubular damage, leading to the impaired clearance of ifosfamide metabolites, may account for this added toxicity.

INTRODUCTION

Ifosfamide is an oxazaphosphorine with activity against many solid tumors (1). Its most significant side effects are hemorrhagic cystitis, nephrotoxicity, hematotoxicity, and neurotoxicity (1-4). Although concomitant administration of the uroprotectant mesna prevents bladder toxicity (5, 6) and ameliorates nephrotoxicity (6), renal impairment remains a potential hazard of ifosfamide therapy (7-10). Myelosuppression limits the dosage of ifosfamide that can be administered with mesna (6).

Patients with impaired renal function are at increased risk of ifosfamide nephrotoxicity (1). Wheeler et al. (11) observed serious ifosfamide nephrotoxicity in patients who had been treated with cisplatin and whose pre-ifosfamide serum creatinine values exceeded 1.5 mg/dl. Niederle et al. (12) reported a death from renal failure after a 5-day course of ifosfamide (40 mg/kg) and cisplatin (20 mg/m²) was administered to a patient who had previously received cisplatin monotherapy. Fossa et al. (8) noted a significant decrease in the creatinine clearance rate and one uremic death among 14 uninephrectomized patients who had received 50 to 60 mg/kg ifosfamide for 5 days. The incidence of severe leukopenia (WBC nadir, 1000 cells/µl; 6 patients) and neurotoxicity (5 patients) was higher than that usually associated with ifosfamide therapy.

We have therefore investigated the influence of prior therapy on the acute nephrotoxicity, hematotoxicity, and neurotoxicity associated with ifosfamide administration. The potential effects of tubular nephrotoxins [cisplatin (13-15), high-dose methotrexate (16), and aminoglycosides (17)] and of the isomeric oxazaphosphorine analogue, cyclophosphamide, were of particular interest. Ifosfamide-induced renal tubular damage (18) was assessed by monitoring the excretion of urinary NAG, a high-molecular-mass enzyme released by tubular cells (17), and of urinary total protein to detect low-molecular-mass serum proteins not reabsorbed by tubular epithelium (19).

MATERIALS AND METHODS

We studied 36 patients in a Phase II trial of ifosfamide (20) whose tumors did not involve the kidneys or ureters. Ifosfamide (1.6 g/m²) was administered i.v. over 15 min. This was followed by three i.v. infusions of mesna (400 mg/m²), each given for 15 min at 15 min, 4 h, and 6 h after ifosfamide. Since the hematotoxicity and nephrotoxicity associated with ifosfamide can differ from the first to subsequent courses, we used only data that were recorded during the initial course. Each course consisted of five daily doses of ifosfamide for a total of 8 g/m². None of the patients received aminoglycosides or other antineoplastic agents during ifosfamide therapy.

Neurotoxicity was graded according to impairment of mental status; cranial nerve, cerebellar, and motor system function; and seizure activity (see Table 1 of Ref. 4).

Daily urine specimens were obtained before and on each of the 5 days of ifosfamide therapy. They were stored at 4°C and centrifuged to remove amorphous salts before analysis. Microhemoglobinuria was evaluated with a urinary test strip (Boehringer-Manheim, Indianapolis, IN). Urinary NAG activity was determined by an automated spectrophotometric procedure (21). Urinary total protein was assayed with Coomassie Brilliant Blue (22). Enzyme and protein measurements were expressed relative to the urinary creatinine concentration to adjust for variations in urine output. For each course, the average NAG and total protein concentrations were calculated for the 5 days of ifosfamide administration. Increases over pretreatment levels (average 5-day concentration minus the pretreatment concentration) were used for statistical analyses.

Serum concentrations of albumin, determined with bromocresol green, and creatinine (23) were measured in specimens obtained before ifosfamide therapy. WBC counts were determined weekly, beginning on the first day of ifosfamide therapy, to determine nadir values.

Stepwise logistic regression and multiple linear regression techniques were used to evaluate prior therapies that may have contributed to ifosfamide toxicity. In some instances, the number of courses of drug received by each patient was entered in regression models; in others, the entry was whether or not the patient had received the therapy. For multiple linear regression, multiple R² and the P value from the overall F test for the model are reported. For logistic regression models, the P value indicates the significance of the improvement in X². The Mann-Whitney test was used for two-group comparisons of mean values. Fisher's exact test (two-tailed) was used for contingency table analysis.

RESULTS

We evaluated the influence of eight drugs, lower abdominal irradiation, and uninephrectomy (Table 1) on ifosfamide-related tubular nephrotoxicity in 36 patients with solid tumors. There were 18 males and 18 females with ages of 3 to 24 yr.
POTENTIATION OF IFOSFAMIDE TOXICITY BY CISPLATIN

Table 1  Treatment received before ifosfamide

<table>
<thead>
<tr>
<th>Prior therapy</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>25 (19)*</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>17 (10)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>22 (11)</td>
</tr>
<tr>
<td>High-dose methotrexate</td>
<td>14 (8)</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>13 (7)</td>
</tr>
<tr>
<td>Etoposide</td>
<td>12 (8)</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>8 (5)</td>
</tr>
<tr>
<td>Teniposide</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Lower abdominal irradiation</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Uninephrectomy</td>
<td>3 (2)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number who had received ≥3 doses of cisplatin.

(median, 11 yr). Prior therapies included three tubular nephrotoxins—cisplatin, high-dose methotrexate, and aminoglycosides. By multiple linear regression analysis, ifosfamide-induced increases of NAG ($R^2 = 0.38, P < 0.001$) and total protein excretion ($R^2 = 0.26, P = 0.002$) were most closely related to the number of previous doses of cisplatin (90 to 100 mg/m$^2$ per dose). Fig. 1 shows the relationship between increase in mean NAG or protein excretion and number of prior doses of cisplatin. Patients who had received at least three doses of cisplatin had significantly higher levels of NAG and total protein excretion than did those who had received fewer doses ($P < 0.01$), indicating that ifosfamide nephrotoxicity is potentiated by cisplatin therapy. The balanced distribution of other therapies among patients who had received three or more cisplatin doses (Table 1) indicates that the influence of cisplatin does not reflect a bias in the data.

Although mesna prevents bladder cystitis (6), clinically inapparent hemoglobinuria can be detected by sensitive methods in some patients receiving ifosfamide (1). We did not observe any appreciable microscopic hematuria (>50 erythrocytes per high-power field) in our patients; however, using a urinary test strip, we found trace to 3+ hemoglobin levels during 16 of the 36 ifosfamide courses. In contrast to the relationship between prior cisplatin therapy and ifosfamide-induced nephrotoxicity, none of the treatments listed in Table 1 showed an influence on the development of microhemoglobinuria, most likely because of the protective effects of mesna.

The contribution of prior therapy to ifosfamide-induced hematotoxicity was evaluated by stepwise multiple regression analysis. WBC nadirs occurred between 6 and 20 days after ifosfamide administration and correlated best with the number of doses of cisplatin ($R^2 = 0.26, P = 0.003$). Fig. 2 shows the distribution of WBC nadirs according to number of previous doses of cisplatin. Patients who had received at least three doses of cisplatin had a significantly lower nadir ($P = 0.004$) than did those who had received fewer doses.

The number of prior doses of cisplatin was likewise the best predictor of neurotoxicity in a stepwise logistic regression model ($P = 0.02$). Further analysis indicated that the frequency of neurotoxicity was significantly greater in patients who had received at least three doses of cisplatin compared with those who had received fewer doses ($7 of 19 versus 1 of 17; P = 0.04$; Fisher’s exact test). Neurotoxicity scores, determined as described in Ref. 4, were also higher in patients who had received cisplatin (Fig. 3). Other factors may have contributed to neurotoxicity, since one neurotoxic patient had not received cisplatin, and two neurotoxic patients who had received cisplatin also had brain tumors (glioblastoma and nasopharyngeal carcinoma) for which they had received radiotherapy.

The predictive value of prior cisplatin therapy was not influenced by age, sex, or race in regression models. The distribution of major categories of solid tumors between patients receiving 0 to 2 or 3 to 8 doses of cisplatin did not show any bias that would appreciably limit our findings (Table 2).

The consistent association between prior cisplatin therapy and potentiation of three distinct ifosfamide toxicities suggested a common mode of pathogenesis. Because renal tubular damage is the principal side effect of cisplatin (13, 15), and cyclophos-
phamide, an isomer of ifosfamide, is extensively reabsorbed (24) and probably metabolized by renal tubular cells, we considered the possibility that prior renal tubular damage influences ifosfamide toxicity. This impression was tested in a logistic regression model that included pre-ifosfamide levels of urinary NAG and total protein; serum creatinine and albumin concentrations were also evaluated because of their reported usefulness as predictors of ifosfamide neurotoxicity (25, 26). The only variable that showed a significant relationship to neurotoxicity \((P = 0.02)\) and severe hematotoxicity \((WBC \text{ nadir}, <1000 \text{ cells/\mu l}; P = 0.01)\) was urinary NAG concentration, indicating that renal tubular damage contributes to ifosfamide toxicity. Moreover, among patients who had received at least three cisplatin doses, the median NAG concentration in the 7 neurotoxic patients \((3.4 \text{ units/mmol creatinine})\) was significantly higher than that in the 12 patients without neurotoxicity \((1.55 \text{ units/mmol creatinine}; P = 0.03)\; \text{one-sided Mann-Whitney test}.\)

**DISCUSSION**

The biochemical basis for ifosfamide-related neurotoxicity, hematotoxicity, and nephrotoxicity is as yet unclear because of the complex metabolism of oxazaphosphorines and differences in host tolerance. Our data indicate that prior therapy with cisplatin enhances the susceptibility of patients to the side effects of ifosfamide. The toxic effects appear to be mediated through cumulative cisplatin-induced renal tubular damage, as indicated by chronically elevated urinary NAG concentrations \((>1.5 \text{ units/mmol creatinine})\). The potentiation of three distinct toxic effects in patients with renal tubular damage suggests a common pathogenetic mechanism: impaired clearance of one or more ifosfamide metabolites.

Cumulative renal tubular damage, leading to impaired glomerular filtration, is a characteristic finding in patients receiving cisplatin (13–15). Renal insufficiency results in increased plasma concentrations and prolonged excretion of cyclophosphamide and its metabolites (27, 28). Dysfunctional tubular cells may secrete or detoxify ifosfamide metabolites less efficiently. Since mesna disulfide becomes active only after its plasma concentrations and prolonged excretion of cyclophosphamide and its metabolites, damaged tubules may yield less of this uroprotectant. Thus, cisplatin-induced renal tubular damage could lead to alterations in the clearance of ifosfamide metabolites through several distinctive mechanisms.

The prior administration of three or more doses of cisplatin \((90 \text{ to } 100 \text{ mg/m}^2 \text{ per dose})\) appeared sufficient to potentiate ifosfamide toxicity in many of our patients. This finding is consistent with the observed impairment of methotrexate clearance \((30)\) and potentiation of methotrexate nephrotoxicity \((16)\) in patients who had received a cumulative cisplatin dosage of \(>360 \text{ mg/m}^2\). Manke et al. (31) reported that three courses of combination chemotherapy, which included a 5-day course of ifosfamide \((2 \text{ g/m}^2)\) and cisplatin \((75 \text{ mg/m}^2)\) on Day 1, resulted in reduced creatinine clearance rates in most of their patients.

Preclinical (32) and clinical (1, 12) data indicate the therapeutic value of combined cisplatin-ifosfamide therapy. Ifosfamide is also useful in the treatment of patients who have relapsed after multiple courses of cisplatin \((1, 11, 20)\). However, continued use of these agents in multidrug regimens should be undertaken with certain considerations in mind. Not all patients who receive cisplatin develop renal impairment (15). The low excretion of urinary NAG that was observed in some of our cisplatin-treated subjects (Fig. 1) may reflect resistance to the nephrotoxic effects of either prior cisplatin therapy or subsequent treatment with ifosfamide. The majority of patients who developed neurotoxicity, severe leukopenia, or acute tubular damage could be identified by persistently elevated urinary concentrations of NAG despite serum creatinine concentrations that remained within an acceptable range for ifosfamide treatment. Detection of subclinical tubular damage by monitoring NAG concentrations may provide clinically useful information (33).

Prior cisplatin therapy can potentiate ifosfamide-induced acute tubular cell necrosis and increase the incidence of neurotoxicity and severe hematotoxicity. Our findings merit consideration in the design of multidrug regimens that include these agents. Moreover, the relationship between renal tubular damage and multiple ifosfamide toxicities should stimulate research on the metabolism and clearance of oxazaphosphorines. For example, differences in the metabolism of ifosfamide and its isomer, cyclophosphamide (34), suggested to us that delayed clearance of the metabolite chloroacetate and may underlie the increased frequency of neurotoxicity in cisplatin-treated patients (35).

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