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Evaluation of the nephrotoxicity of iproplatin (CHIP) in comparison to cisplatin by the measurement of urinary enzymes*

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Summary. Levels of three enzymes, leucine aminopeptidase (LAP), N-acetyl-β-D-glucosaminidase (NAG), and β-glucuronidase (BGA) were measured in the urine of patients receiving hematologically toxic doses of iproplatin (a) with or (b) without pretreatment hydration. The maximum post-treatment increases in the levels of each of the enzymes were compared between these two groups of patients. In addition, the maximum increases in urinary enzyme levels in iproplatin-treated patients were compared with those in patients treated with 40 mg/m^2 cisplatin, a known nephrotoxic agent.

Increases in LAP levels after cisplatin treatment in the periods studied are significantly higher than those after iproplatin treatment ($P < 0.05$). No differences were found in the increases in BGA and NAG levels between iproplatin treatment and cisplatin treatment. No differences were found in the increases in levels of any of the enzymes between patients receiving iproplatin with pretreatment hydration and no prior hydration.

Introduction

Cisplatin (*cis*-dichlorodiammineplatinum II) is an important antineoplastic agent of proven effectiveness against a number of solid tumors, both as a single agent and when used in combination. However, the use of this agent is limited by nephrotoxicity [6, 9, 13, 16, 17], which can be at least partly irreversible [4]. As a result, there has been considerable interest in developing platinum analogs that retain the antitumor activity of cisplatin without the attendant renal toxicity [2, 7]. Iproplatin (*cis*-dichloro-*trans*-dihydroxy-bis-isopropylamine Pt IV, CHIP, JM9) a quadrivalent Pt complex, is one such second-generation Pt complex. It is more soluble in water than cisplatin (water solubilities: 44.1 m*M* and 8.9 m*M* for iproplatin and cisplatin, respectively) and has little nephrotoxicity in experimental animals [11] while retaining a high degree of experimental antitumor activity [11]. Iproplatin is currently being evaluated in phase II studies. Its dose-limiting toxicity in phase I evaluation was myelosuppression, seen as both thrombocytopenia and leukopenia [3]. There was no evidence of renal toxicity as determined by BUN, serum creatinine, and creatinine clearance [3]. Although BUN, serum creatinine, and creatinine clearance have traditionally been used for the measurement of renal damage, some recent studies have indicated that elevation in the levels of certain urinary enzymes may be a more sensitive indicator of renal tubular damage after cisplatin [5, 8, 10]. After studying patients treated with cisplatin, Jones et al. [8] suggested that measurement of urinary leucine aminopeptidase (LAP) and N-acetyl-β-D-glucosaminidase (NAG) might be a more sensitive way of comparing the nephrotoxic potential of the second-generation platinum analogs. In this paper we compare the activities of three urinary enzymes, LAP, NAG, and β-glucuronidase (BGA), in patients receiving iproplatin at the three highest doses examined in the phase I trial (180–350 mg/m^2) with those in patients receiving cisplatin at a low dose (40 mg/m^2) with pre- and post-treatment hydration and diuresis.

Materials and methods

Sample collection. Urine was collected from a group of patients receiving iproplatin (180–350 mg/m^2) in a 2-h IV infusion following pretreatment hydration (2 l 0.5*N* saline, $n=8$) and a group receiving the same dose of iproplatin with no pretreatment hydration ($n=17$). Urine was collected prior to treatment and as voided up to 24 h. Urine passed between 24 and 48 h was pooled. Each sample of urine was divided into a series of aliquots and kept frozen until the time of analysis.

Cisplatin was administered as a single agent in a 4-h IV infusion at a dose of 40 mg/m^2, preceded and followed by a 2-h IV infusion of 1 l 5% dextrose in 0.3 *N* NaCl. Mannitol (36 g) and furosemide (40 mg) were given IV before cisplatin administration. Because patients left the hospital 1–8 h after the end of the cisplatin infusion, urine samples ($n=24$) were obtained before treatment and at 1–8 h after the end of treatment, except in one patient for whom samples were obtained up to approximately 14 h after the end of infusion. Samples were divided and frozen soon after collection.

Enzyme assays. LAP was assayed as described by Appel et al [1]. The assay is based on the hydrolysis of leucine *p*-nitroanilide into leucine and *p*-nitroaniline. *p*-Nitroaniline is converted into a colored complex in a subsequent reaction

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with N-naphthylethylene diammine dichloride and measured spectrophotometrically at 546 nm. The assay was carried out as follows: Urine (0.1 ml), H_2O (0.4 ml), and L-leucine-*p*-nitroanilide (0.8 m*M*, 1.0 ml) in Tris–HCl buffer (33 m*M*, pH 7.2) were incubated for 2 h at 30 °C. The reaction was stopped by the addition of 0.5 ml 20% perchloric acid. Sodium nitrite (0.2%, 2 ml) was added at 4 °C, followed by ammonium sulfamate (0.5%, 2 ml) to destroy excess nitrite. Naphthylethylene diammine (0.05%, 4 ml) was added and the tubes set in the dark at 37 °C for 30 min before reading at 546 nm in a spectrophotometer (Spectronic 20, Bausch and Lomb). For each sample, blanks consisted of the urine and leucine *p*-nitroanilide to which 20% perchloric acid was added at time zero. Osmolalities were measured for each urine sample, and LAP activity was expressed as micromoles of *p*-nitroaniline liberated in the 2-h incubation per milliosmole of solute present in the urine.

NAG was assayed as described by Jones et al. [8]. This assay is based on the hydrolysis of 4-methyl umbelliferyl N-acetyl-β-glucosaminide to 4-methyl umbelliferone and N-acetyl-β-glucosamine, and measurement of the liberated 4-methyl umbelliferone was carried out fluorimetrically. The reaction mixture consisted of 25 µl urine, 100 µl methyl umbelliferyl N-acetyl-β-D glucosaminide (2.6 m*M*) prepared in 0.05 *M* sodium citrate buffer (pH 5.0), 100 µl 0.1% bovine serum albumin in 0.05 *M* sodium citrate buffer (pH 5.0), and 775 µl sodium citrate buffer (pH 5.0). After a 30-min incubation at 37 °C the reaction was stopped with 3 ml 0.2 *M* sodium glycinate buffer (pH 10.6), and the liberated 4-methyl umbelliferone was measured in a ratio fluorometer (Farrand) at λ_{EXC} 360 nm and λ_{FL} 450 nm. Blanks consisted of the reaction mixture with urine added after the 30-min incubation, immediately before the addition of sodium glycinate buffer. Standards were made up of 4-methyl umbelliferone (0.025–0.1 µg/ml) in sodium glycinate buffer (pH 10.3). The NAG activity was expressed as micromoles of 4-methyl umbelliferone liberated in a 30-min incubation per milliosmole of solute present in the urine.

β-Glucuronidase (BGA) was measured by the hydrolysis of phenolphthalein glucuronic acid with the release of phenophthalein, which was measured spectrophotometrically at basic pH [10]. For this assay urine (300 µl) was added to phenophthalein glucuronic acid (0.01 *M*, 200 µl), 0.5 *M* sodium acetate buffer (pH 4.9, 100 µl), and water (400 µl), and incubated for 4 h at 37 °C. The reaction was stopped by the addition of 3 ml glycine-sodium hydroxide buffer (pH 10.5). The absorbance was measured at 550 nm in a spectrophotometer (Bausch and Lomb Spectronic 20). Blanks consisted of the complete reaction mixture with the omission of the urine. BGA activity was expressed as micromoles of phenophthalein liberated in 4 h per milliosmole of solute present in the urine.

The Wilcoxon and Mann-Whitney tests were used for the measurement of statistical differences between groups. Computations were carried out using the program of the statistical package for social sciences (SPSS) [12] on a Univac 90/80 computer.

Results

The urinary enzyme activities were compared in three groups: (a) patients receiving iproplatin with no pretreatment hydration; (b) patients receiving iproplatin with prehydration; and (c) patients receiving cisplatin.

In most patients treated with iproplatin at doses > 180 mg/m^2, elevations in the levels of the enzymes were found in at least some of the post-treatment samples, without any particular pattern in the time of the occurrence of the maximum. The medians (and ranges in parentheses) of the levels of the three urinary enzymes prior to the drug treatment and the maximum post-treatment levels (regardless of the time these were seen) are presented in Table 1. Pre- and post-treatment levels are significantly different for all the enzymes after all the treatments, except in the case of NAG in patients receiving iproplatin with prehydration ($P = 0.069$) and for BGA in patiens treated with cisplatin ($P = 0.091$).

In Fig. 1 the increases in LAP levels in the first 4 h after cisplatin treatment are shown with the corresponding increases seen after iproplatin (at the highest dose of 350 mg/m^2) in the same time period. There is a marked difference between the increases in the levels of the enzyme associated with the two drugs in this early phase. The maximum increases in urinary LAP after iproplatin and cisplatin in individual patients are shown in Fig. 2. The overall median of the maximum increase in LAP and the range for iproplatin (with or without pretreatment hydration) at doses of 180–350 mg/m^2 are shown in Table 2, to-

Table 1. The medians (and ranges) of the three urinary enzymes before and after the drug treatment

Treatment	Enzyme activity [a]					
	Pretreatment level			Maximum post-treatment level [b]		
	LAP	NAG	BGA	LAP	NAG	BGA
Iproplatin (no prehydration)	0.072 (0.00–1.36)	0.270 (0.001–2.385)	0.192 (0.09–1.48)	0.180 (0.026–2.9)	0.319 (0.011–2.786)	0.300 (0.076–3.206)
Iproplatin (prehydration)	0.059 (0.000–2.230)	0.166 (0.049–0.289)	0.270 (0.045–0.40)	0.418 (0.117–3.17)	0.400 (0.103–2.071)	0.380 (0.177–0.982)
Cisplatin	0.240 (0.000–3.04)	0.250 (0.054–0.803)	0.155 (0.066–1.17)	2.850 (0.007–16.120)	0.351 (0.087–3.745)	0.310 (0.073–0.98)

[a] Enzyme activities reported are units. The unit used for each enzyme is defined in *Materials and methods*

[b] Regardless of the time the maximum was observed. Maximum activities only for iproplatin; for cisplatin the values given are the only ones available 1–8 h after treatment

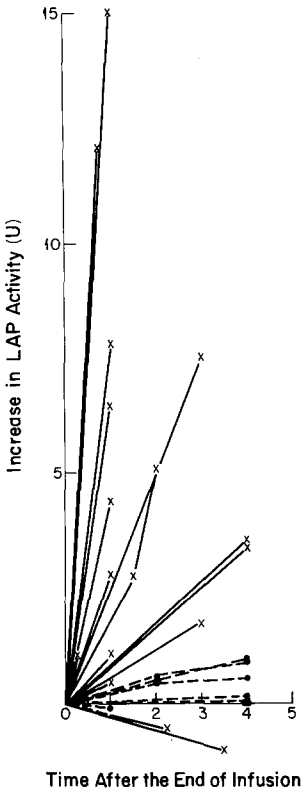

Fig. 1. Increases in urinary LAP levels in the first 4 h after iproplatin (-●-) (350 mg/m^2) or cisplatin (-x-) (40 mg/m^2) administration

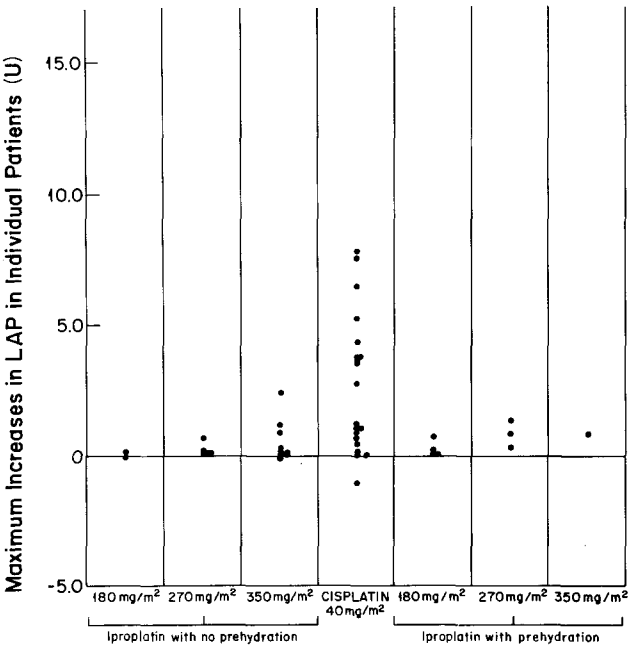

Fig. 2. Maximum increases in urinary LAP levels in individual patients after iproplatin or cisplatin administration

Table 2. Observed maximum[a] increases in the urinary enzymes after the administration of drug

Drug	Median (range)[b] of maximum increase		
	LAP	NAG	BGA
With no prehydration	0.13 (−0.140–2.41)	0.184 (−0.163–1.49)	0.10 (−0.13–1.74)
With prehydration	0.37 (0.060–1.41)	0.335 (−0.066–1.797)	0.26 (−0.17–0.65)
Cisplatin	2.79 (−1.036–14.97)	0.163 (−0.066–3.014)	0.08 (−0.91–0.837)

[a] Only for iproplatin; for cisplatin the values given are the only ones available 1–8 h after treatment
[b] Values presented are increases in units of enzyme activity. For definition of units for each enzyme see *Materials and methods*

gether with the range for cisplatin. A statistical evaluation of the data using the Mann-Whitney test, comparing the maximum increases in the levels of LAP after iproplatin (with or without prehydration) with that after cisplatin, showed that the differences were significant ($P=0.047$ and 0.003, respectively). A similar statistical evaluation carried out between iproplatin with and without prehydration indicated that the differences between these two treatments were not significant.

The maximum increases in urinary NAG and BGA in individual patients after iproplatin and cisplatin are shown in Fig. 3 and 4. In neither case was there a difference between the effect of the two drugs, nor was there a significant difference between iproplatin treatment with or without pretreatment hydration ($P=0.05$ taken as level of significance; Mann-Whitney). Table 2 shows the medians of the maximum increase in NAG and BGA after iproplatin and cisplatin treatments.

Discussion

The enzymes BGA [10], LAP [8], NAG [5, 8], and alanine aminopeptidase [5] were measured in the urine of patients receiving cisplatin, and the general conclusion inferred from these studies is that urinary enzyme levels may be more sensitive indicators of the nephrotoxicity of cisplatin than the creatinine clearance [10] and the levels of BUN and serum creatinine [5, 8]. In this study we have measured LAP, NAG, and BGA. All these enzymes have high molecular weights and are not filtered through the glomerulus in normal conditions. They are localized in the proximal renal tubule [17]. They are present at low levels in normal urine, and their presence is believed to be due to the turnover of the proximal renal tubular epithelium [14, 15]. Under the influence of nephrotoxins affecting the proximal renal tubule or the glomerulus the levels of these enzymes rise markedly [14, 15]. From the studies of Jones et al. [8] it

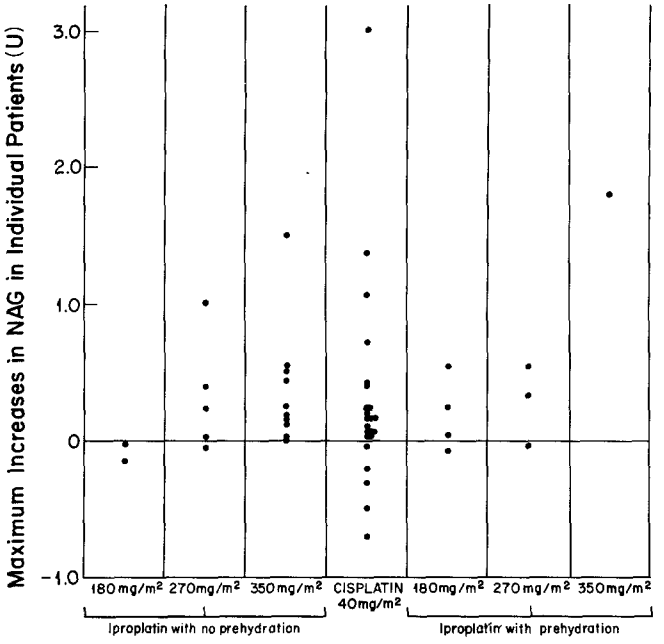

Fig. 3. Maximum increases in urinary NAG levels in individual patients after iproplatin or cisplatin administration

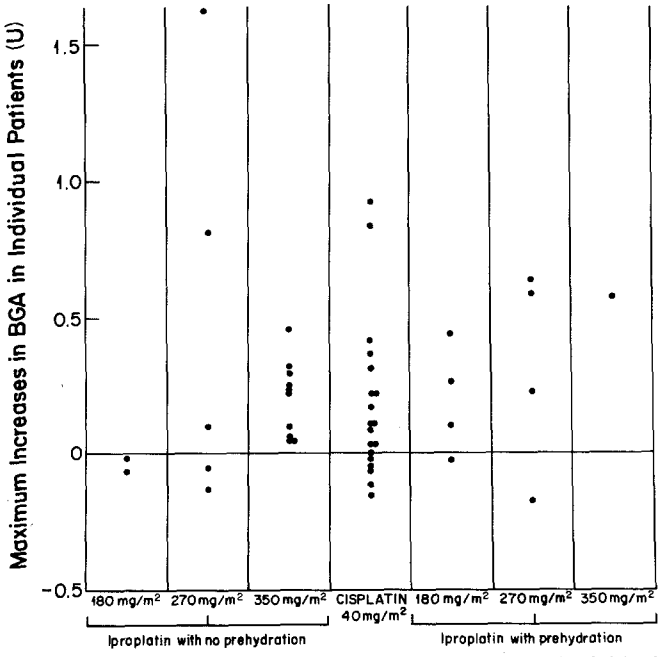

Fig. 4. Maximum increases in urinary BGA levels in individual patients after iproplatin or cisplatin administration

appears that the peak levels of NAG and LAP occur in the first 48 h after cisplatin administration, possibly between 6 and 48 h. In the study of Kuhn et al. [10], 16 h samples were studied for the measurement of BGA. Our major aim in this study was to compare the nephrotoxic potential of iproplatin administered with or without pretreatment hydration with that of cisplatin administered at a low dose with pre- and post-treatment hydration and diuresis. Assessment of renal function by BUN and creatinine clearance measurement indicated no changes between pretreatment levels and those after treatment in any of the patients, including those who had received cisplatin. Since we took samples only for 1–8 h after cisplatin treatment it seems likely, from what has been said above, that we may not be measuring the peak levels of the urinary enzymes in patients receiving cisplatin. Even so, when a comparison is made between the maximum increase in enzyme levels over the first 48 h after iproplatin treatment and that after cisplatin, striking differences are seen in the levels of LAP (Fig. 1 and 2). Although significant increases were seen in NAG after cisplatin and after iproplatin when it was given without pretreatment hydration, no differences were found between iproplatin and cisplatin treatments. The urinary levels of BGA did not show any significant increase after cisplatin treatment in the samples measured. These results may indicate that LAP is a more sensitive early indicator of renal damage than the other two enzymes, at least during the immediate post-treatment period, which is the only period about which conclusions can be drawn on the basis of the present study. Since we are comparing the maximum increases observed in a 48-h period after CHIP treatment at doses up to the maximum tolerated dose with the increase in a 1- to 8-h period after a low dose of cisplatin (40 mg/m^2), the finding of such marked differences in the increases in LAP indicates a much lower nephrotoxic potential for iproplatin, at least under the conditions of the present study. Although the maximum increases in all the enzymes after iproplatin (except NAG after iproplatin with pretreatment hydration) were statistically significant the increases were transient, occurring at one or two sampling times after iproplatin treatment, and levels equal to or lower than those before treatment were immediately recovered. Possibly this is a reflection of the fact that many post-treatment values were compared with one pretreatment value. Jones et al. [8] found persistent high excretion values for the enzymes 6 weeks after a dose of cisplatin. This finding suggests that transient increases in the enzymes such as those seen after iproplatin in this study may not represent true structural damage to the tubules. Since iproplatin is currently undergoing phase II clinical evaluation at several centers in the United States of America and Europe, we expect to learn more about its toxicities in future years. From the data reported in this paper we conclude that iproplatin is not significantly nephrotoxic even at its maximum tolerated dose (270–350 mg/m^2), whether administered with or without pretreatment hydration, which is consistent with the findings recorded in the phase I study of this drug [3].

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