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Assessment of renal toxicity by urinary enzymes in patients receiving chemotherapy with 8-methyl-8-acetylenic-putrescine

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Summary. Renal toxicity was assessed in 19 patients receiving methyl acetylenic putrescine (MAP), an irreversible inhibitor of ornithine decarboxylase. Patients received 250 mg t. d. s. for up to 13 weeks. This dose effectively inhibited the target enzyme, as shown by elevations in decarboxylated S-adenosyl methionine levels. No significant nephrotoxicity was observed in these patients as determined by plasma urea, creatinine and creatinine clearance measurements, although minor elevations of the urinary enzymes lactate dehydrogenase, *N*-acetyl-β-glucosaminidase, alkaline phosphatase and alanine aminopeptidase were observed. As this could represent sub-clinical renal damage, caution should be excercised when using MAP in combination with other cytotoxic drugs.

Introduction

Cell growth or multiplication is associated with an increase in activity of an enzyme, ornithine decarboxylase, resulting in polyamine formation [11]. 8-Methyl-8-acetylenic putrescine (MAP) is an enzyme-activated, irreversible inhibitor of ornithine decarboxylase [5]. In phase I clinical trials MAP was found to be generally well tolerated, but dose-limiting toxicity was observed at 1,500 mg/day, with renal failure observed in one patient at this dose [4]. MAP is currently being assessed in a broad phase II clinical trial. In this study detailed renal function was measured in a sub-group of patients.

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Patients and methods

MAP (MDL 72.175) was provided by Merrell Dow Research Institute, Strasbourg, France. Patients usually received one capsule (250 mg) three times daily. A total of 19 patients with solid (excluding renal) tumours were studied; their mean age was 57 years (range, 36–70 years). Urine samples were analysed weekly (mean, 6 samples/patient). Renal toxicity was assessed by measuring plasma urea, creatinine and creatinine clearance. β2 microglobulin was estimated using the Phadebas Kit (Pharmacia Ltd).

The urine samples were dialysed overnight against 50 × vol. 10 m*M* TRIS-HCl buffer (pH 7.8) containing 12.3 m*M* sodium azide. Urinary protein [1] creatinine [7], and lactate dehydrogenase (LD) [12] were estimated using previously described techniques. In addition, the urinary brush-border enzymes alkaline phosphatase (ALP) and alanine aminopeptidase (AAP) were measured using the techniques of Bretaudiere and Spillman [2] and Jury and Scholz [9], respectively. The lysosomal enzyme *N*-acetyl-β-D-glucosaminidase (NAG) was measured according to Maruhn [10].

Results

Urinary protein and enzyme excretion of these patients is summarized in Table 1 with the reference range obtained from random samples from healthy adults. Plasma urea, plasma creatinine and creatinine clearance data are also summarized.

Statistical analysis of pre- and post-treatment plasma urea, creatinine and creatinine clearance values using Student's *t*-test, Wilcoxon's matched pairs and the Mann-Whitney procedure showed no statistically significant changes. Urinary β_2-microglobulin excretion was also measured at intervals, but the mean and range of values found did not change significantly during the period of treatment.

Pre-treatment, four patients showed elevated urinary concentrations of protein; six elevated LD activity; four, elevated ALP activity; eight elevated NAG activity; and six, elevated AAP activity. When the results from paired pre-treatment samples and those immediately post-treatment were compared, the urinary protein concentration was found to have increased in 6 patients; the LD output, in

Table 1. Plasma creatinine and urea, creatinine clearance, urinary protein, enzyme and β_2-microglobulin excretion of patients with non-renal tumours receiving MAP

	Units	ULN	Pretreatment	Post-treatment:		
				Week 1	Week 3	Week 6
Patients (*n*)			19	19	16	8
Plasma:						
Urea	mmol/l	7.5	4.7± 1.4	4.9± 1.3	5.2± 1.2	3.9± 1.0
Creatinine	μmol/l	120	90 ± 20	88 ± 9	92 ±17	88 ±11
Creatinine clearance	ml/min	120	77 ± 38	75 ±30	82 ±34	79 ±30
Urine:						
Protein	mg/g Cr	173	151 ±128	127 ±87	87 ±41	94 ±44
AAP	units/g Cr	8.0	10.7± 13.7	15.6±21.6	9.9± 6.3	11.6± 6.6
ALP	units/g Cr	2.5	2.2± 1.8	2.1± 1.5	2.7± 2.1	2.9± 1.8
LD	units/g Cr	14.0	14.0± 14.4	21.6±29.9	16.5±14.6	25.2±20.2
NAG	units/g Cr	5.0	6.3± 8.1	5.6± 6.0	16.5±14.6	6.4± 2.9
β_2-microglobulin	mg/g Cr	0.5	0.9± 0.7	1.2± 0.6	1.1± 1.1	

AAP, alanine aminopeptidase; ALP, alkaline phosphatase; LD, lactate dehydrogenase; NAG, *N*-acetyl-β-D-glucosaminidase; ULN, upper limit of normal reference range; Nd, not done

15; the ALP output, in 11; the NAG output, in 18; and the AAP output in 12 patients. However, the majority of these increases remained within the normal range.

Overall, no significant differences were detected between treatment groups (*t*-test; Wilcoxon test). Statistical analysis by the paired *t*- and Wilcoxon test showed that there were no significant differences ($P > 0.05$) between the pre-treatment and the first post-treatment values of any analyte. The Mann-Whitney test was also used to analyse these values as well as to compare successive post-treatment values; again, no significant differences were observed. For no analyte was there a consistent increase in urinary excretion during treatment.

Discussion

The current study was concerned with assessment of the renal toxicity of the ornithine decarboxylase inhibitor MAP. At the dose level used in this trial, MAP has been shown to inhibit effectively the target enzyme in these patients, as shown by increases in the levels of decarboxylated S-adenosyl methionine [3]. Renal toxicity was assessed by measurement of urinary enzymes, which have proved to be more sensitive indicators of damage than measurements of plasma creatinine or creatinine clearance [6, 8]. Although increased urinary enzymes were observed in some of the 19 patients studied, these abnormalities were present pre-treatment and related to the disease rather than the therapy.

It would seem unlikely that MAP could be used alone as a cytostatic agent because of its minimal cytotoxicity. However, in view of its action in inhibiting polyamine synthesis, it would appear to be suitable for use in combination with other cytotoxic drugs. However, minor elevations in some urinary enzymes were observed that could be indicative of sub-clinical renal damage. Caution should therefore be exercised if MAP is used in combination with other cytotoxic drugs that are either nephrotoxic or renally excreted.

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