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Phase I study of methylacetylenic putrescine, an inhibitor of polyamine biosynthesis*

Michael A. Cornbleet[1], Andrew Kingsnorth[2,], Guy P. Tell[3], Klaus D. Haegele[3], Anne-Marie Joder-Ohlenbusch[3], and John F. Smyth[1]**

Departments of [1] Clinical Oncology and [2] Surgery, University of Edinburgh, Scotland, and [3] Merrell Dow Research Institute, Strasbourg, France

Summary. In a phase I clinical trial, nine patients with advanced malignancies not amenable to alternative therapy received α-methyl-δ-acetylenic putrescine (MAP), an enzyme-activated, irreversible inhibitor of ornithine decarboxylase (ODC). MAP was given orally in increasing doses to successive groups of three patients as follows: 375 mg, 750 mg and 1500 mg/day, given as three equally divided doses for 4 weeks. Doses of 375 and 750 mg/day were well tolerated, with no detectable toxicity. Of three patients receiving 1500 mg/day, two experienced moderate to severe myelosuppression; one of these also became anuric, requiring the discontinuation of therapy after 9 days. Both effects were reversible after treatment was stopped. No objective responses were observed, with five patients having stable disease and four, progressive disease during the study period. In the seven patients in whom it could be calculated, the plasma elimination half-life $t_{1/2}$ of MAP measured on the last day of treatment was between 3.9 and 9.2 h in six patients (mean, 5.6 h) and 26.1 h in the seventh. Mean steady-state trough concentrations of MAP were 2.3 μmol after the 375 mg/day dose, 7.1 μmol after 750 mg/day and 16.6 μmol after dosing with 1500 mg/day for 4 weeks, the levels after each treatment schedule being sufficient to inhibit ODC as demonstrated by increases in the urinary excretion of decarboxylated S-adenosylmethionine (dc-SAM). MAP treatment was associated with mean maximal increases in the urinary excretion of dc-SAM of 2.6-, 9.3- and 17.9-fold after 375, 750 and 1500 mg/day for 4 weeks, respectively, but no consistent changes in the urinary excretion of the polyamines, putrescine, spermidine or spermine were observed. Thus, the 24-h urinary excretion of dc-SAM may be used as a conveniently accessible marker of ODC inhibition in cancer patients.

Introduction

The rate-limiting step in the biosynthesis of the polyamines is catalysed by the enzyme ornithine decarboxylase (ODC). Inhibition of this enzyme by α-difluoromethylornithine (DFMO, eflornithine), an irreversible, enzyme-activated inhibitor, has been associated with anti-tumour activity both in vitro and in vivo in laboratory animals [7, 11]. [2-(R)-5-(R)]-α-Methyl-δ-acetylenic putrescine (MAP), a more recently developed ODC inhibitor [3], has been shown to reduce tumour polyamine content and growth in animals bearing L1210 leukaemia, EMT6 mammary sarcoma and Lewis lung carcinoma [2]. MAP has little toxicity in laboratory animals (data on file, Merrell Dow Research Institute, Strasbourg, France); the LD_{50} in mice and rats is >1 g/kg when it is given orally, i.p. or i.v. After its oral administration to rats for 13 weeks, the primary toxic effects were moderate thrombocytopenia at doses of 360 mg/kg per day and decreased body weight gain. In cynomolgus monkeys, oral doses up to 80 mg/kg per day for 12 weeks were well tolerated, but decreased platelet, WBC and RBC counts were found at doses of 240 mg/kg per day.

To determine the effects and tolerability of MAP in man, three groups of patients with advanced, refractory cancer were recruited to receive three dose levels in an open manner. Plasma MAP concentrations and the urinary excretion of MAP and decarboxylated S-adenosylmethionine (dc-SAM) were determined at each dose level. dc-SAM acts as the aminopropyl-group donor in the biosynthesis of the polyamines spermidine and spermine (Fig. 1). Inhibition of ODC results in the accumulation of dc-SAM as a consequence of (a) the reduced availability of putrescine and spermidine as aminopropyl-group acceptors and (b) increased SAM-decarboxylase activity [9]. The urinary excretion of dc-SAM was shown to increase in rats after treatment with MAP and may serve as a means of indirectly assessing ODC inhibition [17]. We recently reported that the i.v. administration of large doses (10–90 g/day) of the ODC inhibitor DFMO produced marked increases in the urinary excretion of dc-SAM without consistently affecting urinary polyamines [5].

Patients and methods

Nine patients with histologically confirmed, advanced and/or refractory malignant tumours (Table 1) not considered amenable to alternative therapy were admitted to the study after their written informed consent was obtained. Groups of three patients each received oral doses of 375, 750 or 1500 mg/day given in three equally divided doses (7 a.m., 3 p.m. and 11 p.m.) for 28 days. Pretreatment

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** *Present address:* Department of Surgery, University of Liverpool

Offprint requests to: John F. Smyth, University Department of Clinical Oncology, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, Scotland, UK

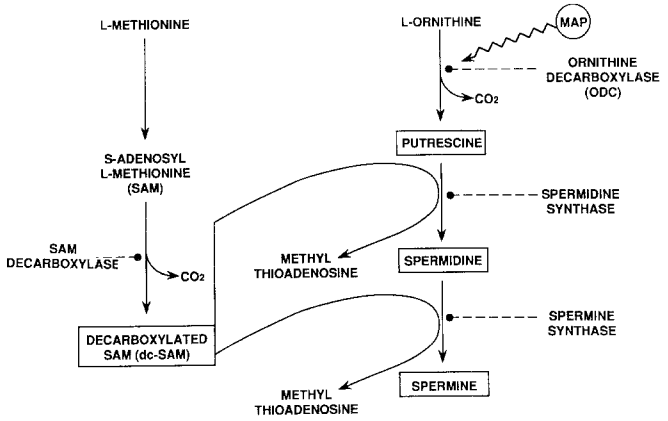

Fig. 1. Biosynthesis of polyamines. One biochemical consequence of ODC inhibition by MAP is the accumulation of dc-SAM

evaluation included: history and physical examination (with particular reference to appetite, bowel habits and hearing by audiometry), performance status, measurement of evaluable lesions, full blood count (including differential white cell count), sedimentation rate, plasma urea and electrolytes, calcium, glucose, urate, liver function tests, urinary glucose, protein and sediment. Blood and urine evaluation was repeated once or twice (highest dose level) a week until 2 weeks after the end of the study period. Prior to commencing MAP therapy and every 2 weeks while on study, 24-h urine samples were collected for the determination of concentrations of MAP, polyamines and dc-SAM. MAP was also determined in plasma obtained 3 h after the first oral dose and, on the last study day, prior to the final dose (approximately 8 h after the last evening dose) and every 2 h after the final dose for 8 h. Plasma and urine samples were stored at $-18°$ C until analysed.

The method for the determination of putrescine, cadaverine, spermidine, spermine, N_1-monoacetylspermidine and N_8-monoacetylspermidine in urine samples was based on gradient elution high-performance liquid chromatography (HPLC) and post-column derivatisation with o-phthaldialdehyde-mercaptoethanol. The fluorescent derivatives formed were detected spectrophotometrically [14]. For quantitation, the internal standard 1,7-diaminoheptane was added to the samples prior to their being processed. Analysis of the parent drug (data on file, Merrell Dow Research Institute, Strasbourg, France) was carried out analogously, but it required a lesser degree of sensitivity as its concentrations were higher than those observed for the polyamines. For the determination of dc-SAM a highly sensitive method was used [18]. In brief, the reaction of dc-SAM with chloroacetaldehyde leads to the formation of a highly fluorescent tricyclic derivative [16]. After a cation-exchange column pre-purification step, the urinary extracts were derivatised with 2-chloroacetaldehyde and analysed by reversed-phase HPLC with fluorometric detection. Decarboxy-S-adenosylmethionine was used as an internal standard for quantitation. This method allows the determination of pmol/ml quantities of dc-SAM.

Results

At doses of 375 and 750 mg/day, MAP was well tolerated, with no clinically evident toxicity. In particular, there was no evidence of myelosuppression or ototoxicity on post-treatment audiograms. At 1500 mg/day, one of the three patients developed moderate neutropenia (WBC nadir, 1.5×10^9/l) and severe thrombocytopenia (platelet nadir, 10×10^9/l) but no anaemia. This patient (no. 7, Table 1) underwent only 9 days of treatment with MAP due to the onset of acute renal failure; prior treatment with cis-platin-containing combination chemotherapy for extensive

Table 1. Patient characteristics

Patient number	Age	Sex	PS	Body area (m^2)	Diagnosis	Prior therapy	Dose (mg/day)	Duration of MAP therapy	Outcome
1	79	M	1	1.75	Renal cancer	None	375	28 days	PD
2	37	F	0	1.57	Colonic cancer + liver 2″	Colectomy, 5FU, BCNU	375	28 days	Stable
3	52	F	1	1.52	Renal cancer	Embolisation, medroxy-progesterone	375	28 days	Stable
4	55	F	2	1.60	Metastatic leiomyosarcoma	Local excision	750	28 days	Stable
5	66	M	2	1.85	Rectal cancer + liver 2″	None	750	28 days	PD
6	60	F	2	1.70	Breast cancer	Multiple chemo-, hormono- and radiotherapy	750	28 days	PD
7	63	F	1	1.70	Ovarian cancer	Cisplatin	1500	9 days[a]	Stable
8	73	F	1	1.48	Colonic cancer + liver 2″	Colectomy	1500	28 days	PD
9	31	M	2	1.80	Recurrent fibrosarcoma	Ifosfamide/Adriamycin, platinum/etoposide	1500	28 days	Stable

[a] Acute renal failure requiring cessation of therapy

PS, performance status (Eastern Cooperative Oncology Group); PD, progressive disease; liver 2″, secondary liver invasion; 5FU, 5-fluorouracil; BCNU, 1,3-bis-2-chlorethylnitrosourea

carcinoma of the ovary (stage III) had previously caused impaired renal function (creatinine clearance, 36 ml/min). Following the discontinuation of MAP therapy, the renal function recovered with conservative management and without recourse to dialysis, but the patient died 6 weeks later of progressive cancer. The maximal reduction in platelet count in this patient was reached 16 days after treatment was discontinued. Subsequently, recovery was prompt, normal counts being reached after a further 11 days. Another patient in this group (no. 9) presented with a gradual decrease in platelet count during the study (platelet, 70×10^9/l on day 28 of MAP administration), with progressive recovery being recorded after drug discontinuation. WBC and RBC counts were not affected.

No other abnormal clinical or laboratory finding was observed at any dose during the study (data on file, Merrell Dow Research Institute, Strasbourg, France). No objective therapeutic response [10] was observed in the nine patients studied; the disease remained stable in five, and in four it progressed during the study period.

Plasma concentrations of MAP achieved at the three dose levels studied are given in Table 2. At 3 h after the first dose of MAP, mean plasma levels were 3.13, 5.64 and 8.77 nmol/ml for the 125-, 250- and 500-mg doses, respectively. After 4 weeks of treatment, mean plasma trough levels prior to the last dose were 2.26, 7.1 and 16.6 mmol/ml, respectively, Following the completion of the 28-day treatment period, the mean (±SD) terminal plasma elimination half-life ($t_{½}$) of MAP was 5.6 (±1.9 h), excluding patient 1, who had a $t_{½}$ of 26.1 h. This higher $t_{½}$ value was possibly related to moderate renal impairment in the 79-year-old patient with renal cancer (blood creatinine and uric acid values were in the range of 0.14–0.16 mmol/l and 0.50–0.71 mmol/l, respectively, during the study). The mean 24-h urinary excretion of the parent drug during the last day of treatment measured 1.24, 1.95 and 3.17 mmol/24 h for the 375-, 750- and 1500-mg dose regimens, respectively (Table 2). These values correspond to a 65.9%, 51.8% and 42.1% recovery of parent drug at the respective daily doses.

The urinary excretion of dc-SAM prior to and during the treatment period and for 2 weeks after treatment is shown in Fig. 2. All doses studied resulted in an increase in the 24-h excretion of dc-SAM, amounting to a mean maximal increase of 2.6-fold (range, 2.0–4.1) after 375 mg/day, 9.3-fold (range, 4.7–14.9) after 750 mg/day and 17.9-fold (range, 15.1–20.7) after 1500 mg/day.

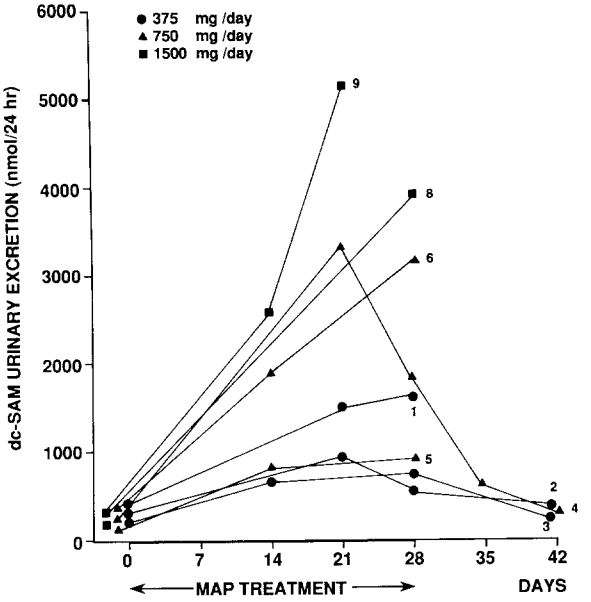

Fig. 2. Urinary excretion of dc-SAM in eight patients treated with MAP; numbers to the right of the curves refer to individual patients

No consistent changes in urinary spermidine and spermine excretion were identified (Fig. 3). Because of interference in the measurement of N_1- and N_8-monoacetylspermidine by high urinary concentrations of MAP, these two products could only be determined before, and after the cessation of, MAP therapy. After MAP administration, the 24-h urinary excretion of putrescine increased by more than 100% in four cases (patients 3, 5, 6 and 9) and showed little modification in four (patients 1, 2, 4 and 8). Interpretation was confounded by the large intra-individual variability observed in the control values of urinary putrescine excretion (Fig. 3).

Discussion

Although the precise function of polyamines in mammalian cells is still not well understood, it is well established that they are essential for cell growth and proliferation [11]. Therefore, the introduction of inhibitors of polyamine biosynthesis has offered a novel approach to cancer chemotherapy [7, 15]. In fact, even before its mechanism of ac-

Table 2. Plasma levels (nmol/ml) and urinary excretion (mmol/24 h) of MAP

Patient number	MAP daily dose (mg)	MAP concentration 3 h after first dose		Trough steady-state concentration (prior to final dose)		$t_{1/2}$ after final dose	Urinary MAP (day 28)	
1	375	2.94	3.13±1.95	4.01	2.26±1.53	26.1	1.34	1.24±0.11
2	375	5.17		1.55		5.1	1.25	
3	375	1.28		1.21		n/a	1.13	
4	750	6.38	5.64±0.78	11.51	7.10±3.82	6.3	2.70	1.95±0.65
5	750	4.83		4.91		4.1	1.52	
6	750	5.72		4.87		3.9	1.64	
7	1500	11.66	8.77±2.55	n/a	16.6	n/a	n/a	3.17
8	1500	7.79		26.47		9.2	3.34	
9	1500	6.85		6.72		5.2	2.99	

Individual values are followed by mean ± SD; n/a, not available

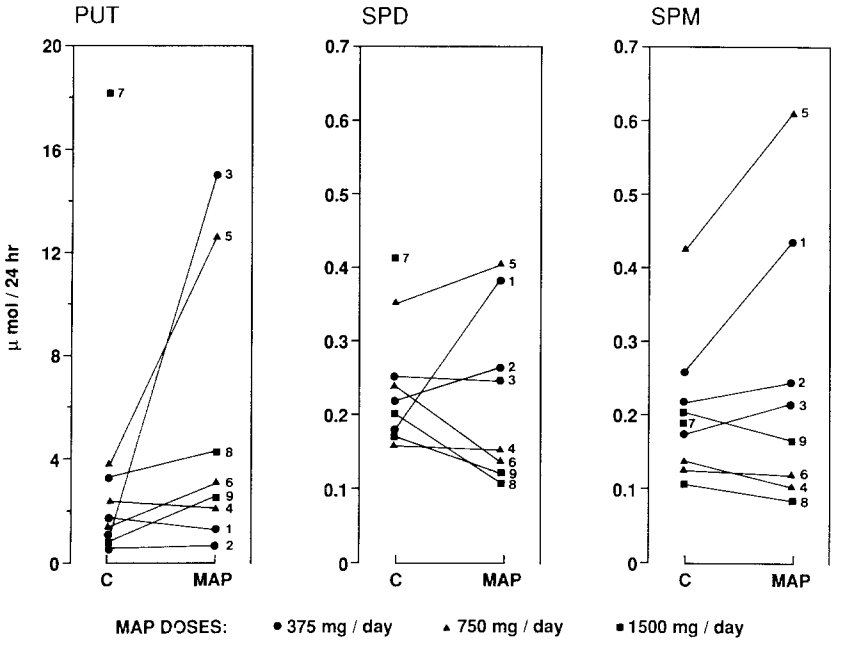

Fig. 3. Urinary excretion of putrescine (PUT), spermidine (SPD) and spermine (SPM), including control values before treatment (C) and values on the last day of MAP treatment (MAP)

tion was understood, the polyamine anti-metabolite methylglyoxal-bis (guanylhydrazone) (MGBG), an inhibitor of S-adenosylmethionine decarboxylase [19], was used in the treatment of acute leukaemia and produced remission and prolonged survival [4]. However, MGBG has little or no activity against solid tumours such as advanced breast, renal and colonic cancer, squamous carcinoma of the oesophagus or head and neck cancers. In addition, significant toxicity (including gastrointestinal toxicity, bone marrow suppression, hypoglycaemia, and cardio-, nephro- and hepatotoxicity) has been reported for MGBG [18], some of these effects being possibly related to the inhibition of diamine oxidase, mitochondrial damage and the inhibition of oxidative phosphorylation [6]. This toxicity precluded its continued use as other, less toxic but active agents became available.

DFMO, an enzyme-activated, irreversible inhibitor of ODC, has been used successfully in the treatment of parasitic diseases [13] but has given disappointing results in the treatment of various malignant conditions in man [15]. Although the toleration of DFMO has been acceptable, thrombocytopenia, leukopenia, anaemia, hearing loss and diarrhoea have been reported. These adverse effects were reversible after the discontinuation of DFMO (1, 8, 15].

MAP has been shown to have anti-proliferative properties in several animal tumour models. It was more active than DFMO in EMT6 sarcoma in mice [2] and in two murine lymphocytic leukaemia cell lines [12]. Another potential advantage of MAP over DFMO may be its longer plasma $t_{½}$: in normal volunteers, MAP had a terminal $t_{½}$ of 5.7 ± 1.4 h (mean $\pm$SD) compared with 3.3 ± 0.3 h for DFMO (data on file, Merrell Dow Research Institute, Strasbourg, France).

The present clinical study indicates that MAP may have anti-proliferative activity in man, as reflected by the significant myelosuppression observed in two patients at the highest dose given (1500 mg/day). Impaired renal function in one patient was the major toxic effect encountered. No ototoxicity was reported. The mean terminal plasma $t_{½}$ of MAP was in the same range as that observed in normal volunteers and was consistent with administration of the drug every 8 h. The increase in 24-h urinary dc-SAM excretion observed at the clinically tolerated dose of 750 mg/day provides indirect evidence of significant ODC inhibition at this dose level. Overall, the results of this study indicate that 750 mg/day MAP can be given with relative safety for at least 4 weeks and suggest that this is an appropriate dose for further exploration of the efficacy of MAP in refractory malignant disease.

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