Cancer Chemother Pharmacol (1989) 23: 308–310

Cancer Chemotherapy and Pharmacology

A phase I study of meta-azidopyrimethamine ethanesulphonate (MZPES) – a new dihydrofolate reductase inhibitor*

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Summary. A total of 68 patients were treated in a phase I study of meta-azidopyrimethamine ethanesulphonate (MZPES) – a novel lipophilic dihydrofolate reductase (DHFR) antagonist. The dose was increased from 5.4 mg/m^2 to 460 mg/m^2 given as a 1-h infusion, with 460 mg/m^2, 600 mg/m^2 and 800 mg/m^2 given as a 24-h infusion. The dose-limiting toxicity was nausea and vomiting, which was marked at doses above 360 mg/m^2 by 1-h infusion and 600 mg/m^2 by 24-h infusion. Above 250 mg/m^2 patients also described subjective neurological symptoms, although no objective signs were apparent. Myelosuppression was not consistent at any dose level. No objective responses were seen. In view of the lack of anti-folate activity at toxic levels, no phase II trials are currently proposed; toxicological and in vitro studies will continue.

Introduction

The diaminopyrimidine class of anti-folates do not bear a close structural similarity to folic acid and are known as the non-classical anti-folates. These were first synthesised in the 1950s [7], and although the initial compounds in this group, metoprin and etoprin, showed clinical activity against leukaemia, they proved to be too toxic for clinical use. The classical anti-folates are structurally similar to folic acid and were first used in cancer chemotherapy in 1948 by Farber et al. [4], who showed aminopterin to be active in acute leukaemias. The less toxic, classical anti-folate, methotrexate, has since become a standard agent in the treatment of malignancy.

Although methotrexate is the standard anti-folate drug, it has several potential disadvantages: it exhibits little activity against most solid tumours, shows minimal penetration of the blood-brain barrier at lower doses and has a wide range of toxicities; moreover, drug resistance can develop. This drug resistance can be due to, among several mechanisms, reduced active transport of the drug into cells [8] or increased cellular synthesis of dihydrofolate reductase (DHFR). Lipid-soluble anti-folate drugs should be able to overcome transport-mediated drug resistance by passive diffusion into cells and have the advantage of crossing the blood-brain barrier. However, previous lipid-soluble anti-folates have prolonged biological half-lives (metoprin = 10 days) contributing to their unacceptable toxicity [1].

Meta-azidopyrimethamine ethanesulphonate (MZPES) (Fig. 1) is a non-classical anti-folate, related to metoprin, which was developed to combine the potential advantages of lipid solubility with a relatively short half-life and thus the possibility of reduced toxicity [6]. Its short half-life is promoted by the presence of the azido group, which has the potential to be biologically transformed to the more polar and less active amine.

Pre-clinical studies. MZPES, in the form of the free base MZP, has shown in vivo activity against a range of murine tumour lines (see Table 1) as well as cell line L5178Y, which shows resistance to methotrexate due to impaired transport-mediated uptake. Pharmacokinetic studies in mice show a mean elimination half-life of 4 h at a dose of 10 mg/kg, with oral bioavailability in excess of 95% [9]. Toxicity studies in Charles River CD1 mice showed an LD_{10} of 18 mg/kg and an LD_{50} of 44 mg/kg when the drug was given as an i.v. bolus and an LD_{10} of 41 mg/kg and an LD_{50} of 43 mg/kg by the i.p. route. Clinical signs of toxicity in mice comprised decreased motor activity, muscle tremor and clonic convulsions. Deaths were preceded either by no clinical signs or by convulsions. No haematological toxicity or histological changes in any organ was observed at any dose level.

Patients and methods

Treatment given. The starting dose for this study was 1/10th the LD_{10} in mice, which is equivalent to 5.4 mg/m^2. Treatment was given on a single-dose, intermittent schedule, this being the simplest for assessment of pharmacokinetics and acute toxicity. Dose increments were based on a modified Fibonnacci search, with a minimum of three patients and four courses evaluable at each dose level. A maximum of one dose escalation was allowed in each patient. MZPES was diluted in 500 ml 5% glucose and was given as 1-h infusion at doses of 5.4, 11, 18, 27, 38, 50, 67, 83, 105, 125, 150, 180, 210, 250, 300, 360, 400 and 460 mg/m^2. Doses of 460, 600 and 800 mg/m^2 were also delivered by 24-h infusion. Table 2 shows the number of patients and courses for each treatment level. Pharmacokinetic studies were carried out at all dose levels except the first. In all, 68 patients were treated with a total of 133 courses, with treatment repeated every 21 days when possible.

* This paper is the second in a series on medicinal azides

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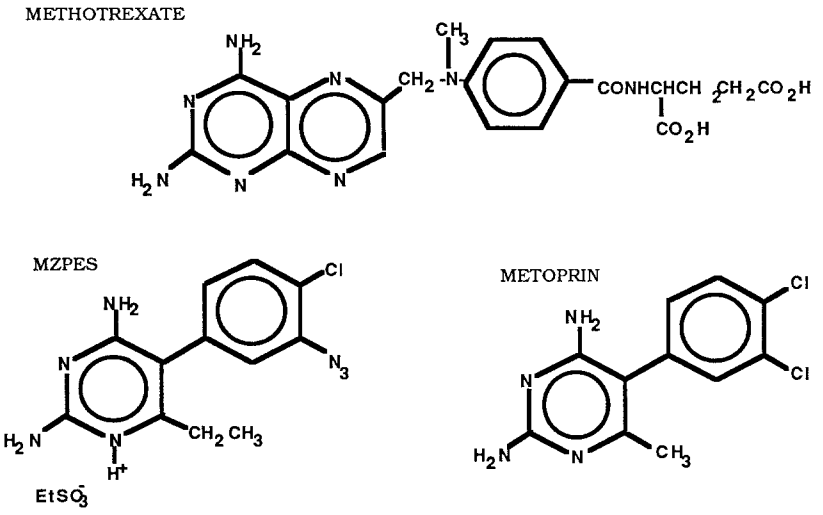

Fig. 1. Dihydrofolate reductase inhibitors

Patient characteristics. All patients had advanced malignancy unresponsive to standard treatment. Tumour types were as follows: gastrointestinal (stomach, colon, pancreas), 21: adenocarcinoma of unknown site, 7; sarcoma, 6; bronchial 6; head and neck, 4; renal, 4; melanoma, 3; cervical, 3; others, 14. A total of 41 patients had received previous chemotherapy, of which 12 had shown a response, and 12 patients had undergone previous treatment with methotrexate. All patients had WHO performance scores of grade 2 or better, with expected survival of more than 2 months. All patients gave written informed consent in a form determined by the participating institution. The drug was supplied centrally for each treatment course, with patients registered on entry to the study.

Results

Toxicity

Patients had full-blood count and hepatic and renal function assessed weekly after treatment whenever possible. Leukopenia and thrombocytopenia occurred sporadically at doses as low as 25 mg/m^2 but were not consistent at any dose level and were never worse than WHO grade 2 (see (Table 3). For patients not previously exposed to chemotherapy, nausea and vomiting were first noted at 83 mg/m^2. At higher doses nausea and vomiting became more severe, being marked in the majority of patients at 400 and 460 mg/m^2 despite combination anti-emetics (high-dose metoclopramide, dexamethasone and lorazepam). At doses of 250 mg/m^2 and above, patients experienced subjective neurological toxicity comprising malaise, unsteadiness, dizziness and incoordination, although no objective neurological signs were apparent. After a 24-h infusion at a dose of 800 mg/m^2, toxicitiy was similar to that experienced at 460 mg/m^2 over 1 h. One patient had a grand mal convulsion immediately following 210 mg/m^2;

Table 1. In vivo activity of MZP against mouse tumours

Tumour type	Optimal T/C (%)[a]	Activity of MZP[b]
P388 leukaemia	151	+
L1210 leukaemia	158	+ +
B16 melanoma	157	+ +
TLX5 lymphoma	135	+
M5076 reticulum cell sarcoma	174	+ +
Lewis lung carcinoma	< 140	−
$CD87_{F1}$ mammary	24	−
Colon 38	> 42	−

[a] Compounds tested according to National Cancer Institute protocols [5]

[b] For P338 leukaemia and TXL5 lymphoma, + refers to T/C > 120. For L1210 leukaemia, B16 melanoma and M5076 reticulum cell sarcoma, + + refers to T/C > 150. For Lewis lung carcinoma, $CD8_{F1}$ mammary carcinoma and colon 38 tumours, − indicates that the compound was inactive

Table 2. Number of patients, evaluable courses and first courses for each dose level

Dose level (mg/m^2)	Number of patients	Number of evaluable courses	Number of first courses
5.4	4	7	4
11	4	7	4
18	6	8	3
27	5	6	2
38	3	7	3
50	4	7	3
67	5	7	4
83	6	11	5
105	7	9	5
125	4	6	2
150	3	6	2
180	5	6	3
210	6	10	6
250	3	3	3
300	4	5	3
360	10	13	8
400	5	6	3
460	2	2	1
460 (24-h infusion)	1	1	0
600 (24-h infusion)	3	3	3
800 (24-h infusion)	2	3	1
Total	92	133	68

Table 3. Incidence of toxicity (number of courses affected/number assessed)[a]

Dose level (mg/m^2)	Leukopenia ($2.5-3.5 \times 10^9/l$)	Thrombocytopenia ($50-150 \times 10^9/l$)	Severe vomiting[b] (WHO grade 3/4)
≤150	2/61	2/61	2/73
180/210/250	3/15	1/15	2/17
300/360	3/16	3/16	4/18
400/460	0/7	1/7	5/8
24-h infusion (460/600/800)	0/6	1/6	1/7

[a] Not all toxicities were assessed for all courses
[b] Despite combination anti-emetics (high-dose metoclopramide + dexamethasone + lorazepam)

an EEG and computerised axial tomographic (CAT) scanning suggested a vascular cause for this convulsion. EEGs recorded during treatment with 210 and 250 mg/m^2 in other patients showed no abnormality. One patient became suddenly unconscious 20 h after receiving 400 mg/m^2 and died without regaining consciousness; a post-mortem examination was refused, but clinical examination suggested cerebral haemorrhage as a cause for the coma. No hepatic, renal, mucosal or cutaneous toxicity was observed, and there was no evidence of cumulative toxicity.

Clinical results

In all, 31 patients had disease evaluable for response by standard UICC (Union International Contra le Cancrum: International Union Against Cancer) criteria. None of the patients showed a clinical response to treatment, but three had static disease beyound 12 weeks.

Pharmacokinetic studies

Pharmacokinetic studies indicated that the drug obeys two-compartment, non-dose-dependent kinetics, with a mean elimination half-life of 33.52 h [10].

Discussion

MZPES is a novel lipophilic, anti-folate drug that has shown good anti-tumour activity in in vitro studies. Toxicity studies in mice failed to give a good indication of toxic levels in humans, and 20 dose increments were required to reach the maximum tolerated dose. The failure of pre-clinical studies to give a good indication of toxic levels in humans may have occurred because toxicity is related to peak drug levels and the experimental animals were treated by i.v. bolus rather than infusion. Alternatively, metabolic pathways may differ between the experimental animals and humans. This study again raises doubts as to the suitability of the modified Fibonnacci regime for determining the dose level for phase II studies of non-myelotoxic agents. Other groups involved in phase I studies have considered this topic in more detail and have suggested that dose escalation regimes based on the AUC would more quickly determine the maximum tolerated dose [2, 3].

The dose-limiting toxicity in the present study comprised marked nausea and vomiting and subjective neurological disturbance, with a maximum tolerated dose of 460 mg/m^2 when the drug was given as a 1-h infusion and 800 mg/m^2, as a 24-h infusion. Although no life-threatening toxicity prevented continued dose escalation, we felt it unlikely that patients would tolerate higher doses and that it would be unethical to pursue this.

Since the completion of this study, MZPES has been given at 400 mg/m^2 over 1-h in an EORTC phase II study of soft tissue sarcoma. Of 12 patients treated, 4 developed marked neurological toxicity (3 grand mal convulsions, 1 case of hallucination) immediately following the infusion (G. R. P. Blackledge, personal communication). In view of this and of the lack of any anti-folate or anti-tumour activity in the present study, further clinical trials have been stopped. Additional toxicological studies are prosposed together with in vitro studies in methotrexate-resistant cell lines.

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Received April 11, 1988/Accepted September 23, 1988