injections of 6 mg/kg DOX-SL or 1 mg/kg VIN-SL are moderate protocols with only mild systemic toxic side-effects (temporary weight loss during weekly injections), the significant prophylactic and therapeutic benefits observed are encouraging.

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Opposite Effect of Miltefosine on the Antineoplastic Activity and Haematological Toxicity of Cyclophosphamide

J. Stekar, P. Hilgard and T. Klenner

The effect of pretreatment with miltefosine (MIL) on the antineoplastic activity of cyclophosphamide (CPA) was evaluated in subcutaneous benzo(a)pyrene-induced sarcomas (BPS) of the rat. MIL alone had no antineoplastic effect on this autochthonous tumour, but enhanced the chemotherapeutic effect of CPA. Conversely, MIL counteracted the myelotoxicity of CPA in normal adult rats. Although the nadir of the leucocyte count remained unchanged, the recovery phase was considerably shortened, an effect which resembled the pharmacological action of GM-CSF.

Key words: miltefosine, cyclophosphamide, interactions

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INTRODUCTION

MILTEFOSINE (MIL) IS a cell membrane active alkylphosphocholine with considerable activity against autochthonous, chemically induced mammary carcinomas; however, some autochthonous and transplantable tumours are resistant to this new antineoplastic agent [1]. In this study, we assumed that even in tumours such as benzo(a)pyrene-induced sarcomas (BPS), which appear unaffected by MIL [2], some latent damage to cancer cell membranes might be induced by the administration of this alkylphosphocholine and could be detected by subsequent treatment with some other antineoplastic agent. To test this supposition, BPS-bearing rats were treated sequentially with MIL and cyclophosphamide (CPA) and the result of the experiment compared with that of a single-agent treatment.

Unlike classical DNA-interactive cytotoxic agents, MIL induces leucocytosis and thrombocytosis in rats [2]. This was confirmed in clinical trials where an increase in white blood cells (WBC) and the platelet count occurred in a large proportion of patients treated with MIL [3]. Preliminary evidence suggested that this peculiar effect was caused by a stimulation of stem cell activity in the presence of lymphokines [4]. In view of these haematological effects, it seemed reasonable to expect that MIL should protect tumour bearing rats from myelosuppression brought about by CPA. Alternatively, considering the synergism between MIL and CPA with respect to antineoplastic action, the possibility that MIL could potentiate rather than mitigate the haematological toxicity of CPA could not be ruled out. Since repeated assessment of WBC in the group undergoing a two-drug treatment would impose an additional stress on the animals, the haematological toxicity of the combination MIL-CPA was first studied in normal, rather than tumour-bearing rats.

MATERIALS AND METHODS

Animals and tumour induction

Female Sprague-Dawley rats (Moellegaards Breeding Center, Ejby, Denmark) were used throughout this study. The animals were kept under specific pathogen-free (SPF) conditions, fed with standard pellet diet (Altromin® 1324) and had unrestricted water (acidified to pH 3) supply.

For the induction of subcutaneous sarcomas, 5 mg of benzo(a)pyrene (Serva, Heidelberg, Germany) dissolved in 1 ml of olive oil were injected s.c. into the neck of 90 day old animals. Approximately 3 months later single solid tumours appeared at the site of carcinogen application. Tumour measurements were performed as described previously [2].

Treatments

MIL, with a chemical purity of greater than 98% was manufactured by ASTA Medica AG (Frankfurt, Germany). The compound was dissolved in normal saline and administered through a stomach tube in a 1 ml volume. Commercial grade CPA (ASTA Medica AG) was dissolved in normal saline and injected i.v. at 0.1 ml/100 g body weight; when only saline was given, the volumes injected corresponded to those of MIL- and CPA-solutions, respectively.

BPS. When the weight of the BPS reached approximately 1 g, the rats were randomised to experimental groups consisting of 10 animals each. Two groups were treated orally with 10 doses of MIL (46.4 mg/kg per day, 5 times weekly), followed by a single i.v. administration of either 121 mg/kg CPA or 0.9% NaCl. The remaining two groups first received a 2-week oral pretreatment with 0.9% NaCl; thereupon the rats were given one i.v. injection of CPA (121 mg/kg) or normal saline (controls).

Haematological experiments. Two groups of 10 normal female rats were treated either with 1 × 46.4 mg/kg CPA i.v. (day 0) or with 10 daily doses of 46.4 mg/kg MIL, given 5 times weekly (days 0–4 and 7–11); the third group was given a combination of both treatments. WBC was determined from sublingual venous blood at regular intervals using standard haematological techniques.

Statistical analysis

The significance of differences between the groups injected with single-agent CPA and those given combination treatment was determined by two-tailed Student's *t*-test.

RESULTS

The slight differences between MIL-treated BPS and the controls (Figure 1) did not reach statistical significance. Single-agent CPA retarded tumour growth, but no regressions were observed. However, with both treatments combined, the BPS regressed to approximately 50% of the weight observed on the day of CPA-injection (Figure 1); even at day 35, the tumours weighed only 50% of those in the CPA alone group. From day

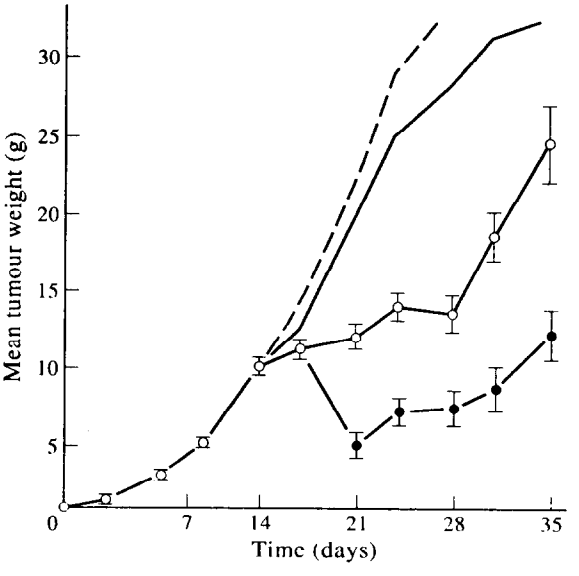

Figure 1. Antineoplastic activity of the combination therapy MIL plus CPA in rats bearing BP-induced sarcomas. Animals were pretreated orally either with MIL or with 0.9% NaCl (controls) according to the same schedule. All i.v. treatments were administered on day 14. Group size: 10 animals. Data are expressed as means ± SEM (bars). ---- controls; —— pretreatment with MIL only; ○—○ No MIL pretreatment, CPA only; ●—● pretreatment with MIL plus CPA.

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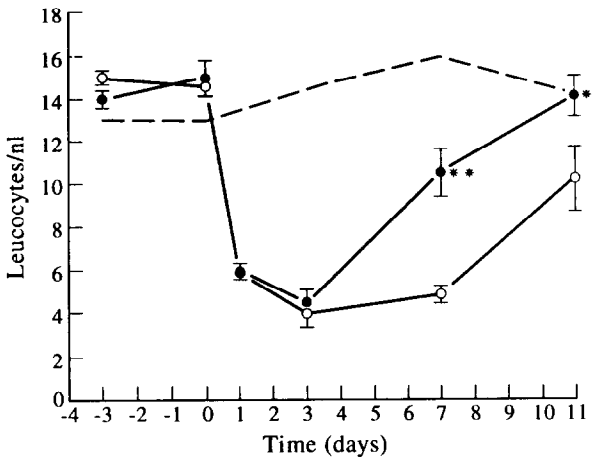

Figure 2. Influence of oral MIL treatment on the recovery of the leucocyte counts following a single i.v. injection of CPA given on day 0 to adult healthy rats (46.4 mg/kg). Group size: 10 animals. Data are expressed as means ± S.E.M. (bars). ---- MIL alone; ○—○ CPA alone; ●—● CPA plus MIL; * $P<0.05$ versus CPA alone; ** $P<0.001$ versus CPA alone.

21 onwards, the combination treatment was significantly better than the treatment with CPA alone ($P<0.05$). Similar results were recently obtained using the combined treatment in the dimethylbenz(a)anthracene-induced mammary carcinoma of the female Sprague-Dawley rat. Treatment with a single CPA dose (100 mg/kg i.v.) or with low dose MIL (14-day oral administration of 4.6 mg/kg/day) barely influenced the tumour growth. In contrast, the combination of the two treatments (CPA injection preceded by a 2-week oral dosing of MIL) resulted in a complete arrest of tumour growth for 10 days. At this time, the mean tumour weight amounted to only 40% of that registered in the groups receiving single-agent treatment (data not shown).

Figure 2 shows WBC of normal rats treated with MIL, CPA and the combination of both. MIL treatment was not able to counteract the kinetics of the CPA-induced decrease in WBC and to influence its nadir. Yet the recovery of WBC was more rapid in the CPA-group which also received MIL treatment. At day 7 the WBC was higher in the CPA-MIL group than in rats injected with CPA alone ($P<0.001$), and 4 days later the WBC count returned to normal (Figure 2). In contrast, complete recovery following single-agent CPA required more than two weeks.

DISCUSSION

The present investigation suggests a potential benefit of the combination treatment with oral miltefosine and cyclophosphamide; the efficacy of the combination was clearly supra-additive.

It has been shown that a variety of chemically unrelated protein kinase C inhibitors can enhance the antiproliferative activity of cisplatin and nitrogen mustard [5]. MIL also inhibits this enzyme, therefore the synergistic effects observed in the present study may conceivably be an expression of the same phenomenon. Although the main target for the cytotoxic activity of cyclophosphamide is still considered to be the DNA, it has been demonstrated that alkylating agents affect several transport systems of the plasma membrane [6]. Considering the important role of the plasma membrane in the regulation of cell division, it cannot be ruled out that, in our experiment, both MIL and CPA exerted their effect at the same cellular level.

In view of the therapeutic synergy between MIL and CPA, it is surprising that MIL counteracted the CPA-induced myelosuppression. Interestingly, this effect was observed at a miltefosine dose level which on its own did not significantly elevate the WBC in normal animals. The myeloprotective effect of MIL was qualitatively similar to the action of GM-CSF [7]. Recent studies indicated that MIL increased the CSF-dependent haemopoietic progenitor cell colony growth [8].

In summary, it was shown experimentally that MIL modulated the antineoplastic activity and the haematological toxicity of CPA in opposite directions. If the findings reported here can be substantiated in the clinic, concomitant MIL therapy might improve the therapeutic index of certain cytostatic agents.

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