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Phase I Clinical Trial and Pharmacology of 2,4-Diamino-5-(3′,4′-Dichlorophenyl)-6-Methylpyrimidine (Metoprine) (DDMP) and Folinic Acid (CF)

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Summary. *Escalating doses of DDMP (metoprine) (15–280 mg/m²) were administered as single oral doses 24 h before a fixed leucovorin (CF) rescue (15 mg IM every 6 h for 72 h). CNS toxicity was dose-limiting and cumulative when the drug was given more frequently than at 3-week intervals. DDMP has a very long half-life (150 h) and is extensively bound to serum proteins (88%). It diffuses into the CSF and concentrates in brain tumours and normal brain tissue (brainserum ratio 3.8–5.3). DDMP is a potentially useful drug against brain tumors. Tumor regressions were seen in two patients with epidermoid carcinomas.*

Introduction

2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methyl pyrimidine (metoprine) (DDMP) is a folate antagonist with several unique properties making it a potentially attractive chemotherapeutic agent. Although its affinity for dihydrofolate reductase (DHFR) is 1/100 that of methotrexate (MTX) [3], it is still an effective inhibitor of this enzyme. The absence of inhibition by DDMP of other folate-metabolizing enzymes suggests that DHFR inhibition may be an important mechanism of action of the drug [3, 4, 12, 19, 20].

DDMP is highly lipophilic and penetrates into the intracellular space by passive diffusion without competing for the membrane transport mechanism shared by reduced folates and MTX [16, 25, 26]. The concomitant administration of DDMP and folinic acid (CF) may result in the selective protection of folate-dependent normal tissues (skin, gut, bone marrow) and in selective toxicity of MTX-resistant tumors lacking the folate transport mechanism or of tumors of the central nervous system (CNS) which are poorly permeable to CF [7, 10, 13, 14, 21].

Experimental data have shown the lack of cross resistance between DDMP and MTX in selected tumour systems where MTX resistance is due to a cellular membrane transport defect (L5178Y/MTX and Walker carcinoma) [13, 21]. The therapeutic index of DDMP against intracranial sarcoma 180 was improved on specific multiple-dose schedules by CF rescue [24].

DDMP crosses the blood-brain barrier well and concentrates in the brain. Constant brain-to-plasma concentration ratios were measured during peak drug levels and plasma decay in rats [27]. Furthermore, the therapeutic activity of pyrimethamine, also a 2,4-diaminopyrimidine, against meningeal leukaemia after oral administration was reported by Geils et al. [9].

Several clinical trials of DDMP alone or in combination with concomitant or delayed CF have shown therapeutic activity against leukemias, lymphomas, and solid tumors [2, 5, 6, 17, 18, 22, 23].

Price et al. favored the use of DDMP plus concomitant CF in their clinical studies [22, 23], on the basis of experimental in vitro studies [13, 14]. The optimal dose and schedule of administration of DDMP with or without CF remains to be established, however.

The purpose of this study was to determine the maximum tolerated dose and the dose-limiting tox-

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icity of DDMP administered 24 h before a given dose and schedule of CF, in a manner known to prevent toxic reactions, to high-dose MTX. CF administration was started 24 h later, rather than concomitantly, to avoid a potential negative effect of CF in MTX-sensitive tumors.

In addition, pharmacokinetic data were obtained to determine whether significant DDMP levels existed in the CSF and whether the drug concentrates in the brain and brain tumors.

Materials and Methods

Clinical Study. DDMP was given orally 24 h before CF, 15 mg IM every 6 h for 3 days, was started. Whereas CF administration was kept constant, the DDMP was escalated from 15–280 mg/m^2. At least three patients per dose level received injections of 15, 30, 45, 60, 90, 120, 180, and 280 mg/m^2. Initially some patients received a second dose at the next higher level 14 days after the first one in the absence of toxicity. At the highest dose level, 280 mg/m^2, no patient had received prior DDMP and no second dose was given. In cases of severe toxicity: skin rash, leukocyte count < 1,500/mm^3 and platelet count < 50,000/mm^3, CF rescue was resumed with 15 mg IM or PO every 6 h for 3 days. Timed sera, CSF, and 24-h urine specimens for pharmacokinetic studies were collected in some patients receiving DDMP at doses between 90 and 280 mg/m^2.

In addition, two patients received DDMP 24 h before craniotomy for brain tumors. Biopsies were obtained at the time of surgery after informed consent given according to hospital regulations.

All patients had advanced, biopsy-proven, malignant solid tumors and were not candidates for conventional chemotherapy. Clearly evaluable lesions were not a prerequisite for the phase I study. Performance status of 40 or above, serum creatinine < 1.5 mg/100 ml and bilirubin < 2 mg/100 ml were required. All patients were hospitalized; blood counts were obtained daily, serum creatinine and liver function tests, twice a week.

Response to treatment was defined as at least a partial remission (PR): a decrease by 50% or more of the product of the largest perpendicular diameters of measurable lesions, and a decrease by 50% of the measurable diameters in evaluable lesions, in the absence of progression or appearance of other tumor manifestations.

Pharmacology. DDMP was measured by gas-liquid chromatography, 2,4-diamino-5-(3′,4′-dibromophenyl)-6-methyl pyrimidine (DDMP-Br) being used as internal standard.

Gas Chromatography. A Hewlett-Packard gas chromatograph 5840A with a detector sensitive to nitrogen was used. The helium carrier gas was 99.999% pure (Gardner Cryogenics Europe, Belgium) and the stationary phase was 2% OV 17 onto chromosorb 100–120 mesh W-HP (Filter Service, Belgium). The working temperatures were 250° C for the column, 280° C for the injection port, and 300° C for the detector.

Reagents. DDMP and DDMP-Br were gifts of Wellcome Laboratories Beckenham, UK. Other reagents were Merck's P.A. grade: ethyl and amyl acetate, Na_3PO_4, Na_2HPO_4, and KH_2PO_4.

Extraction and Analysis. Of sample (serum, urine, CSF), 2 ml was mixed with 0.2 ml internal standard (DDMP-Br at 8 μg/ml) and 0.4 ml saturated Na_3PO_4 (pH 11) and then extracted for 20 min with 7 ml ethyl acetate. After centrifugation (10 min at 2,000 rpm), the ethyl acetate fraction was evaporated to dryness and the residue redissolved in 0.5 ml amyl acetate. One to five microliters of this final solution was injected into the column and eluated with an helium flow of 30 ml/min. Retention times were 3.10 min for DDMP and 6.05 min for DDMP-Br.

Protein Binding. The determination of the protein binding was made with a magnetically stirred ultrafiltration cell Amicon model 12 and Amicon Diaflo ultrafilters type YM10 (10,000 mol. wt cut-off). Filtration pressure was 5 kg/cm^2. The study was carried out at 25° C and pH 7.4 (Na_2HPO_4 and KH_2PO_4 0.067 *M*).

Results

Clinical Study

Twenty-one patients with epidermoid carcinomas of the head and neck (nine patients), lung (four patients), cervix (three patients), anal margin (one patient), adenocarcinomas of lung (two patients) or breast (one patient), or unknown origin (one patient) received 27 treatments, 23 of which were evaluable for toxicity and/or response. Three patients dying from progressive disease within 2 weeks of DDMP administration without evidence of drug-induced toxicity and one patient receiving intrathecal MTX 4 days after DDMP because of meningeal carcinomatosis were considered unevaluable.

The maximum tolerated dose of DDMP is 280 mg/m^2 for this particular schedule of administration with CF. The dose-limiting toxicity is neurological. Two types of CNS toxicity were observed: (a) severe headaches occurring within 72 h of DDMP administration, while patients were still receiving CF, and lasting up to 2 weeks; and (b) intermittent agitation, confusion, and disorientation appearing later, around day 15, and lasting up to 4 weeks. Since neurologic toxicity was not concomitant with hematologic depression in the patients studied, no CF was given and therefore it is unknown whether it could be rapidly reversed by CF, as suggested by Currie and Young [5]. Electroencephalograms showed no focal abnormalities, but did reveal generalized slow dysrythmia characterized by delta waves (1-2 cycles/s).

Hematologic toxicity was not seen with DDMP doses below 180 mg/m^2 but was severe in three of four patients who received 280 mg/m^2, possibly contributing to death by bleeding in one case. At DDMP doses of 280 mg/m^2, WBC nadirs of 700, 1,600, and 1,900/mm^3 were observed on days 19, 26, and 38, platelet nadirs of 20,000, 46,000, and 47,000/mm^3 on

days 21, 24, and 38. Toxic effects lasted 8–21 days. It is not possible to know whether the CF rescue given to all patients with hematologic toxicity contributed or not to blood count recovery (Table 1).

Pruritis and a toxic morbilliform rash preceded hematologic toxicity in two cases. Mild nausea and/or vomiting were seen in five patients. There were no cases of renal or hepatic toxicity.

One patient with epidermoid carcinoma of the cervix showing tumor regression developed vaginal and vesical bleeding and died 45 days after receiving DDMP 280 mg/m^2. Autopsy showed toxic cystitis and near-complete regression of disease.

Another patient receiving 90 mg/m^2 developed without toxicity a complete remission of a recurrent epidermoid carcinoma of the anal margin invading the pelvis. She has continued to receive DDMP and remains in complete remission more than 7 months later.

Pharmacology

All results were the mean of three experiments. A standard concentration curve was obtained by adding pure DDMP to serum. The method of extraction was reproductible and 95%–99% of DDMP was recovered. The limit of detection was 0.01 μg/ml (0.037 μ*M*) and the standard serum concentration curve was linear up to 10 μg/ml. Binding of DDMP to plasma proteins was 88% at the concentration of 1 μg/ml, studied by ultrafiltration.

After oral administration of DDMP, sera were collected for 7 days from patients receiving 90–280 mg/m^2. Peak serum concentrations varied with the administered dose but were not strictly dose-related. This may reflect variable absorption, excretion, and/or metabolism [15] of DDMP. Maximal concentrations occurred between 12 and 18 h after a single dose of 90 mg/m^2 and at 48 h for doses of

Table 1. DDMP phase I trial with CF-toxicity

DDMP dose (mg/m^2)	Number of evaluable treatments	Number of patients treated	Number of patients with toxicity			
			CNS	Hematologic	Skin	Nausea, vomiting
15	1	3	–	–	–	–
30	3	3	–	–	–	–
45	3	3	1[a]	–	–	–
60	3	3	2[a]	–	–	–
90	3	3	1[b]	–	–	2
120	2	3	1[b]	–	–	–
180	4	5	3[a]	2	1	1
280	4	4	4	3	1	2

[a] Major CNS toxicity following a second dose of DDMP given 14 days after a lower dose without toxicity

[b] Headaches only

Table 2. Pharmacokinetics of oral DDMP in nine patients

No. of patient	Dose (mg/m^2)	Peak serum concentration (μg/ml)	Time after DDMP administration (h)	Half-life (h)
1	280	4.70	48	150
2	280	8.90	48	106
3	280	3.10	48	122
4	180	3.50	48	166
5	120	4.10	48	150
6	120	3.40	48	165
7	90	1.07	18	140
8	90	0.94	18	183
9	90	0.68	12	175

Table 3. Five-day cumulative urinary excretion of DDMP in six patients

No. of patient	Dose (mg/m^2)	Cumulative 5-day urinary excretion (mg)	Cumulative 5-day urinary excretion (% dose)
2	280	14.8	3
3	280	45.8	10
4	180	61.4	2
5	120	3.4	1.7
7	90	14.0	9
8	90	5.0	3.3

Table 4. DDMP concentration in CSF in five patients

No. of patient	Dose	Time after DDMP administration (h)	CSF concentration (μg/ml)	Serum concentration (μg/ml)	CSF concentration (% serum concentration)
1	280	24	0.16	4.63	3.5
3	280	24	0.23	2.90	8.0
9	90	50	0.05	0.66	7.6
10	90	24	0.12	1.85	6.5
11	90	24	0.09	0.97	9.3

120–180 mg/m^2. These serum concentrations represent total DDMP, protein-bound and unbound. The median serum half-life was 150 h (range 106–183 h) (Table 2).

The cumulative 5-day urinary excretion of DDMP was 1.7%–10% of the dose in six patients (Table 2). However, small amounts of the drug were still being eliminated on the tenth day (0.7% and 1% of the total dose) in patients 3 and 8.

DDMP concentrations were measured simultaneously in the CSF and serum of five patients (Table 4). The CSF concentration was 3.5%–9.3% of the serum concentration. These results are consistent with the hypothesis that only free DDMP (~ 10% of the total) not bound to proteins enters the CSF. DDMP was also measured in normal brain tissue and brain tumor of two patients who received 90 mg/m^2 24 h before craniotomy. The first patient had a DDMP concentration of 5.58 μg/g (wet weight) in the brain tumor versus 7.95 μg/g in normal brain and 0.4 μg/ml in the CSF. The second patient had similar results: 5.14 μg/g in the brain tumor versus 7.03 μg/g in normal brain. No CSF was available for the second patient. Although serum samples were not received from these two patients, one can extrapolate from Table 4 a 3.8–5.3 : 1 ratio of brain drug concentration to serum concentration. This is similar to the findings of Stickney et al. in dogs and rats [27].

Discussion

The maximum tolerated dose of DDMP combined with a 72-h CF rescue is about five times that of DDMP alone [5]. Whereas thrombocytopenia is the dose-limiting toxicity for DDMP alone [5], CNS toxicity is dose-limiting for the combined treatment.

Our data do not permit us to know whether the acute neurologic toxicity could be reversed by IV leucovorin, as suggested by Currie et al. [5]. The mechanism of the CNS toxicity is unknown and it is possible that DDMP or a metabolite interferes with cellular metabolism upon a different target enzyme. The lack of reversal by CF of the acute CNS toxicity induced in mice receiving an LD_{50} dose of DDMP supports this assumption [11]. Furthermore, severe headaches occurring independently of the more acute syndrome of hyperactivity and disorientation starts within 72 h of DDMP administration while patients are still receiving CF. CNS toxicity appears to be dose-dependent and cumulative, since it occurs at low DDMP levels when the drug is repeated at intervals of less than 3 weeks (Table 1).

The pharamacokinetic data confirm the long half-life of DDMP in serum (median 150 h) (Table 2), the persistent low daily urinary excretion (Table 3) and the high extent of protein binding. This probably

results in extensive binding to tissue lipoproteins as well as to plasma proteins explaining the long persistence of the drug in the blood.

The low CSF : serum ratio could be explained by the fact that only free DDMP, which represents less than 10% of the total DDMP, may diffuse into the CSF.

The measurements of DDMP in brain tumors and brain tissue in two patients demonstrate that the drug penetrates and concentrates in both. Similar data were obtained in rats carrying brain tumors induced by ethylnitrosoureas [7] and DDMP did increase survival in this animal model. These results and the reports that DHFR, the drug receptor for 2,4-diaminopyrimidines, is present in human primary brain tumors [1, 8] make DDMP an attractive drug to test against human CNS neoplasms.

This phase I clinical trial combining intermittent DDMP administration with CF may serve as the basis for the design of a phase II protocol. Better understanding of the mechanism of the acute CNS toxicity and its control by pharmacological means may yet improve the therapeutic index of this combined treatment.

From other studies, DDMP is known to have a broad spectrum of therapeutic activity [2, 5, 6, 17, 22]. Our data do not permit an analysis about cross resistance between MTX and DDMP [12, 23]. The two responders had not received prior treatment with MTX; of the remaining 21 patients, nine had received a prior chemotherapy combination containing MTX.

The clinical evaluation of a drug given along with its antagonist raises complicated methodological problems to determine an optimal dose and schedule of administration for each. However, the accumulated experimental and clinical data are such that further development of DDMP is warranted.

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References

1. Abelson HT, Gorka C, Fosburg M, Kornblith P (1978) Identification of dihydrofolate reductase in human central-nervous-system tumors. Lancet 1: 184
2. Alberto O, Peytremann R, Medenica R, Beretta-Piccoli M (1978) Initial clinical experience with a simultaneous combination of 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methylpyrimidine (DDMP) with folinic acid. Cancer Chemother Pharmacol 1: 101
3. Browman GP, Calvert AH, Taylor GA, Hart LI, Harrap KR (1980a) The intracellular mechanism of action of metoprine (DDMP). Eur J Cancer 16: 1547
4. Browman GP, Gorka C, Mehta B, Lazarus H, Abelson H (1980b) Studies with a 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methyl-pyrimidine (DDMP)-resistant L1210 leukemia cell line without cross-resistance to methotrexate. Biochem Pharmacol 29: 2241
5. Currie VE, Young CW (1980) Phase I study of 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methylpyrimidine (metoprine) with and without leucovorin. Proc Am Soc Clin Oncol 21: 359
6. de Jager R, Rodzynek JJ, Klastersky J, Kenis Y (1978) Phase I study of DDMP (2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methylpyrimidine) and folinic acid (CF) administration. Proc Am Assoc Cancer Res 19: 403
7. Denlinger RH, Nichol CA, Cavallito JC, Sigel CW (1976) Chemotherapy of primary brain tumors in rats with two lipid soluble diaminopyrimidine folate antagonists. Proc Am Assoc Cancer Res 17: 95
8. Duch DS, Bigner DD, Bowers SW, Nichol CA (1979) Dihydrofolate reductase in primary brain tumors, cell cultures of central nervous system origin and normal brain during fetal and neonatal growth. Cancer Res 39: 487
9. Geils GF, Scott CW, Bauch CM, Butterworth CE (1971) Treatment of meningeal leukemia with pyrimethamine. Blood 38: 131
10. Goldie JH, Harrison SI, Price LA, Hill BT (1971) Impaired responsiveness to folinic acid protection in methotrexate-resistant L5178Y cells. Eur J Cancer 11: 627
11. Hamilton L, Philips F, Sternberg S (1954) Hematological effects of certain 2,4-diaminopyrimidines, antagonists of folic acid metabolism. Blood 9: 1062
12. Hill BT, Price LA (1980) DDMP (2,4-diamino-5-(3′,4′-dichlorophenyl)-methylpyrimidine). Cancer Treat Rev 7: 95
13. Hill BT, Price LA, Goldie JH (1975) Methotrexate resistance and uptake of DDMP by L5178Y cells. Selective protection with folinic acid. Eur J Cancer 11: 545
14. Hill BT, Price LA, Harrison SI, Goldie JH (1977) The difference between "selective folinic acid protection" and "folinic acid rescue" in L5178Y cells in culture. Eur J Cancer 13: 861
15. Hubbel JP, Henning ML, Grace ME, Nichol CA, Sigel CW (to be published) *N*-Oxide metabolites of the 2,4-diaminopyrimidine inhibitors of dihydrofolate reductase, trimethoprim, pyrimethamine and metoprine. In: Gorrod JW (ed) Biological oxydation of nitrogen. Elsevier North-Holland Biomedical Press Amsterdam, pp 77–182
16. Levitt M, Nixon PF, Pineus JH (1971) Transport characteristics of folates in cerebrospinal fluid: a study utilising doubly labeled 5-methyl tetrahydrofolate and 5-formyl tetrahydrofolate. J Clin Invest 50: 1301
17. Miller DS, Rundles RW, Nichol CA, Nichol JL, Wooley JL, Sigel CW (1976) Phase I/II experience with a lipid soluble folate antagonist: 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-metyhlpyrimidine. Proc Am Soc Clin Oncol 17: 263
18. Murphy M, Ellison RR, Karnofsky DA, Burchenal JH (1954) Clinical effects of the dichloro and monochlorophenyl analogues of diaminopyrimidine: antagonists of folic acid. J Clin Invest 33: 1388
19. Nichol CA (1967) Studies on dihydrofolate reductase related to the drug sensitivity of microbiologic and neoplastic cells. Adv Enzyme Regul 6: 305
20. Nichol CA (1977) Pharmacokinetics. Selectivity of action related to physicochemical properties and kinetic patterns of anticancer drugs. Cancer 46: 519
21. Nichol CA, Cavallito JC, Wooley JL, Sigel CW (1977) Lipid-soluble diaminopyrimidine inhibitors of dihydrofolate reductase. Cancer Treat Rep 61: 559

22. Price LA, Goldie JH, Hill BT (1975) Methodichlorophen as an antitumor drug: preliminary report. Br Med J 2:20
23. Price LA, Hill BT, Goldie JH (1977) DDMP and selective folinic acid protection in the treatment of malignant disease: a further report. Clin Oncol 3:281
24. Sirotnak FM, Moccio DM, Dorick DM (1977) Experimental chemotherapy with 5-aryl pyrimidine antifolates: further studies on toxicity and the responsiveness of ascitic, solid and intracranial Sarcoma 180 to DDMP and DDMP with Citrovorum factor. Cancer Treat Rep 61:1325
25. Spector R, Lorenzo AV (1975a) Folate transport by the choroid plexus in vitro. Science 187:540
26. Spector R, Lorenzo AV (1975b) Folate transport in central nervous system. Am J Physiol 229:777
27. Stickney DR, Simmons WS, DeAngelis RL, Rundles RW, Nichol CA (1973) Pharmacokinetics of pyrimethamine (PRM) and 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methylpyrimidine (DDMP) relevant to meningeal leukemia. Proc Am Assoc Cancer Res 14:52

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