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Initial Clinical Experience with a Simultaneous Combination of 2,4-Diamino-5(3′,4′-Dichlorophenyl)-6-Methylpyrimidine (DDMP) with Folinic Acid

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Summary. *DDMP, a diaminopyrimidine folate antagonist, was given to 26 tumor patients in a dosage of 50 mg/m² per week orally, simultaneously with 3 mg CF i.m. or i.v. The CF dose was increased to 30 mg in patients showing evidence of toxicity, and withdrawn in the absence of toxicity. The dose-limiting toxicity was seen in myelosuppression, particularly thrombopenia and skin rashes. At the 3 mg CF level, 18 out of 26 patients developed toxicity. No toxicity was seen at the 30 mg CF level in 11 patients. After cessation of CF, toxicity occurred in five out of seven patients. After the onset of toxicity, CF was added as a delayed rescue, in a dosage of 15 mg every 8 h or 30–60 mg daily. One patient died of sepsis with agranulocytosis. All other patients recovered from myelosuppression within 1 or 2 weeks. Objective responses were observed in seven patients, four of the ten with epidermoid cancer of the head and neck, two out of eight with epidermoid cancer of the lung, and one out of three with melanoma.*

Introduction

2,4-diamino-5(3′,4′-dichlorophenyl)-6-methylpyrimidine (BW 197U, DDMP, Metoprine) is one of the antimalarial diaminopyrimidines (Falco et al., 1951). DDMP is water-insoluble and highly lipid-soluble. It reaches a high concentration in the brain and other lipid-rich tissues. Its half-life in plasma is uncommonly long and averages 180 h (Nichol et al., 1977). Diaminopyrimidines are inhibitors of dihydrofolate reductase (Hitchings et al., 1952; McCormack et al., 1969; Nichol et al., 1977). The inhibition is reversed by folinic acid (citrovorum factor, CF) (Philips et al., 1952; Hamilton et al., 1954). The antitumor activity of DDMP has been observed in a number of animal tumors and cell cultures (Clarke et al., 1952; Burchenal et al., 1952; Nadel et al., 1953; Sugiura, 1953; Goldie et al., 1973; Denlinger et al., 1976). Hill et al. (1977) have demonstrated in cultures of L5178Y cells that simultaneous addition of CF to DDMP reduced the influx of DDMP into the cells. This effect of CF was obtained with a methotrexate (MTX)-sensitive but not with a MTX-resistant cell strain. The difference in CF protection between MTX-sensitive and MTX-resistant cells was markedly reduced when CF was added as a delayed rescue.

Clinical experience with DDMP is still very limited. Murphy et al. (1954) observed three remissions in 12 children with acute leukemia. No responses were observed in six adult patients with leukemias or in various types of solid tumor. The drug was administered in daily doses of 2.5–20 mg until the appearance of myelosuppression. The very long half-life of the compound was not suspected and this schedule led to sustained marrow toxicity, resulting in the withdrawal of DDMP from clinical use. 20 years later a new interest in DDMP and other diaminopyrimidines was generated by the observation by Geils et al. (1971) that oral pyrimethamine was active in the treatment of meningeal leukemia and by the demonstration by Nichol (1968) and by Hill et al. (1973, 1975) of an activity of DDMP in MTX-resistant tumors. The treatment schedule proposed by Price and Hill (1976) and Price et al. (1975) was characterized by long intervals between DDMP doses and by simultaneous CF protection.

DDMP was given orally, in single weekly doses of 1.5–4 mg/kg. CF was given in single weekly doses of 45–90 mg orally, immediately before DDMP. The tolerance to the treatment was reportedly good, characterized by reversible myelosuppression and occasional skin rashes. A few patients complained of haziness or headache. Responses were observed in patients with cancer of the lung (squamous cell and anaplastic small cell types), kidney, and rectum. No explanation was given as to how a single short-lived dose of CF could afford pro-

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tection from the toxic effects of DDMP, and this point remains unclear. The authors considered, however, that this protection was highly effective and that the DDMP dose used would not have been tolerated without simultaneous CF. In another Phase I/II study (Miller et al., 1976) an attempt was made to maintain a constant DDMP blood level by adding to a single initial dose of 120 mg/m^2 a supplementary daily dose of 5–15 mg. CF was given orally in a dose of 5–20 mg daily. Responses were seen in cancer of lung and bladder, mycosis fungoides and leukemia. Besides myelosuppression and skin rash, mental disturbances were considered a major limitation to the clinical use of DDMP.

The pilot study reported here was undertaken in order to investigate the tolerance and antitumor effects of DDMP when given at a lower dose than previously used. The protection afforded by CF when given simultaneously in a low or high dose was also investigated, as was the effectiveness of a delayed CF rescue in case of toxicity.

Patients, Materials and Methods

All patients with advanced or recurrent histologically proven solid tumors were eligible, provided they were not amenable to conventional therapy, did not present side effects from a previous antitumor treatment, and had a performance status superior to 40 (Karnofsky). One or more measurable tumor lesions, a sufficient renal function as shown by a serum creatinine level of less than 1.4 mg/100 ml, and digestive functions compatible with oral chemotherapy were also required. Pregnant patients and children under 16 years were excluded. The following parameters were recorded before initiation of the treatment and followed up weekly: tumor measurements, performance status, weight, temperature, pain, need for analgesics, blood transfusions, other treatments, drug side effects, complete blood count. Serum creatinine, alkaline phosphatase, and transaminase were checked every 2 weeks.

DDMP was kindly provided by the Wellcome Research Laboratories, Beckenham, Kent, England, as 25 mg tablets and was stored at room temperature. CF was purchased from commercial sources as calcium leucovorin in 3-mg ampules for intramuscular or intravenous injection. DDMP was administered in 1 weekly dose of 50 mg up to 1.5 m^2, 75 mg between 1.5 and 2.0 m^2, and 100 mg over 2.0 m^2. The dosage was never increased or reduced, but medication was temporarily omitted in case of toxicity. A weekly dose of CF was injected by the i.m. or i.v. route simultaneously with DDMP. The initial CF dose was 3 mg. In case of toxicity, as expressed by a fall in the blood count, including reticulocytes, or by itching or an incipient skin rash, a CF rescue treatment was immediately started. After recovery the DDMP–CF combination was resumed but the dosage of CF was increased to 30 mg weekly. If no toxicity was observed after the first 3 weeks, CF was omitted in future courses. The treatment schedule is illustrated in Table 1. The CF rescue program initially scheduled was 15 mg every 6 h for 3 days or more. During the last months of the study however, some of the patients were treated with one daily dose of 30–60 mg for 1 or more days. This unusual schedule was adopted as a consequence of the very rapid reversibility of toxicity with the 'classic' rescue program and of the absence of toxicity when DDMP was combined with a single CF dose of 30 mg.

Table 1. Treatment schedule for the study of a combination DDMP + CF

DDMP 50 mg/m^2 p.o. weekly with CF 3 mg i.m. or i.v. weekly	
If no toxicity occurs during the first 3 weeks	If toxicity occurs during the first 3 weeks
DDMP 50 mg/m^2 p.o. weekly No CF	CF rescue 15 mg q 6 h i.m. or i.v. × 12 or more or 30–60 mg daily × 1 or more
	DDMP 50 mg/m^2 p.o. weekly with CF 30 mg i.m. or i.v.

The tolerance was evaluated separately for each level of CF protection and also expressed by the relative number of patients for whom the dose of CF had to be increased or could be omitted. An objective response was defined as a reduction of 50% in the product of two measurable perpendicular diameters in one or more tumor lesions without concurrent progression at other sites. Bone and brain lesions and pleural and peritoneal effusions were not considered measurable.

Results

26 patients were treated between May 1976 and May 1977, 11 with epidermoid cancer of the lung, 10 with epidermoid cancer of the head and neck, one with epidermoid cancer of the penis, three with melanoma and one with hypernephroma. 21 patients were men and five women. Their ages ranged from 42–78 years, with a median of 63 years. The total duration of DDMP treatment ranged from 1–49 weeks, with a median of 6 weeks. The total amount of DDMP per patient ranged from 50–1876 mg, with a median of 400 mg. Six patients did not receive more than 3 weeks of treatment. Four of them had a CF rescue. Three died within 2 weeks after a single dose, one of brain metastases, one of pneumonia, possibly resulting from a rapidly reversible toxic granulopenia, and one of agranulocytosis with concomitant sepsis. In two patients the treatment was interrupted after 3 weeks because of tumor progression. The last patient presented an almost complete regression of a locally advanced head and neck tumor without prior treatment. The residual tumor was irradiated and the chemotherapy stopped.

20 patients were treated for more than 3 weeks. 14 developed drug-related toxicity during the first 3 weeks. All received rescue treatment. In all cases the toxicity was reversible. In 11 patients the treatment was resumed with the 30 mg CF dose. In seven it was eventually possible to reduce the dose of CF to 3 or 9 mg weekly, and in three cases CF was completely withdrawn. No toxicity was observed with 30 mg CF. When CF was

Table 2. Toxicity and treatment response for 26 patients treated with a combination DDMP + CF

No. of patients	Tumor	DDMP + 3 mg CF				DDMP + 30 mg CF				DDMP alone/reduced CF dose				Treatment response[b]
		No. of doses	Toxicity			No. of doses	Toxicity			No. of doses	Toxicity			
			Leuco[a]	Thrombo[a]	Skin		Leuco[a]	Thrombo[a]	Skin		Leuco[a]	Thrombo[a]	Skin	
1.	Lung	1	2.8	105	no									X
2.	Lung	1	0.4	75	no									TD
3.	H + N	1	2.5	1	yes									PD
4.	H + N	1	2.0	19	no									(TD?)
5.	Lung	3	10.1	120	no									PD
6.	Lung	3	0.9	17	no									OR
7.	H + N	4	5.4	120	no									OR
8.	H + N	4	4.8	50	yes									OR
9.	Melanoma	4	2.6	120	no									OR
10.	Kidney	6	3.8	94	no									PD
11.	H + N	6	3.5	23	yes									PD
12.	Lung	2	3.4	10	no	2	10.2	246	no					PD
13.	Lung	2	2.9	68	no	2	8.0	179	no					PD
14.	Lung	1	11.0	380	yes	3	6.6	420	no					X
15.	Melanoma	2	10.7	106	no	5	6.5	100	no					NC
16.	Melanoma	1	3.9	204	no	1	7.9	217	no	2	7.7	250	no	PD
17.	H + N	1	3.0	390	no	3	8.3	593	no	5	11.5	637	no	PD
18.	H + N	2	12.7	100	no	2	7.7	340	no	6	8.2	289	no	OR
19.	Lung	2	1.9	16	yes	5	6.9	131	no	2	4.5	23	no	OR
20.	Penis	2	6.0	26	yes	3	5.4	160	no	14	4.8	92	yes	NC
21.	Lung	1	2.3	270	no	3	13.0	493	no	17	4.5	88	no	NC
22.	H + N	1	0.6	1	yes	18	2.5	130	no	6	4.4	200	no	OR
23.	H + N	3	9.3	200	no					2	6.5	45	no	PD
24.	H + N	3	6.4	156	no					3	9.2	140	no	NC
25.	Lung	3	5.9	183	no					3	2.7	19	no	PD
26.	Lung	4	4.6	236	no					8	3.4	6	yes	PD

[a] Nadir counts $\times 10^3/mm^3$

[b] X = inevaluable; TD = toxic death; PD = progressive disease; OR = objective response; NC = no change

reduced or withdrawn three patients developed a second episode of toxicity, which was reversible by a second CF rescue. In three patients with mild toxicity the treatment was resumed with the initial low CF dose. No further toxicity was observed. Of the six patients who showed no toxicity during the first 3 weeks, two were treated further with the low CF dose. In the others the weekly CF dose was omitted. In three of them a second episode of toxicity was reversed by a second CF rescue. In one case no further toxicity appeared.

During the initial phase at the low CF level, the median nadir leukocyte count was 3,700/mm³. Three patients reached a nadir of less than 1,000/mm³. The median nadir for thrombocytes was 102,000/mm³. A nadir of less than 30,000/mm³ was observed in eight cases. Seven patients presented an erythematous and morbilliform skin rash, often preceded by pruritus, appearing on localized areas of the trunk or limbs and extending progressively to become generalized in severe cases. The rashes appeared, in all cases but one, shortly before or immediately after the first evidence of leuko- or thrombopenia, and some of them developed secondary purpura. In the last case the rash was the only sign of toxicity. All rashes cleared rapidly under CF rescue. During second episodes of toxicity nadir leukocyte counts ranged from 4,800 to 2,700/mm³ and thrombocyte counts from 92,000 to 6,000/mm³. Two skin rashes were observed. In the whole study neurological toxicity was observed in only two patients, who complained of haziness and asthenia, in one case followed by headache. Another patient presented a transitory coma, which was reversible by discontinuation of DDMP. This patient, however, was known to have brain metastases, and died as a result of these shortly thereafter in recurrent coma.

24 rescue treatments were performed in 18 patients. All were initiated at the first signs of marrow or skin toxicity. In 14 cases the schedule consisted in six to 16 i.m. or i.v. injections of 15 mg at 6-h intervals. In eight cases the 'rescue' was limited to a single injection of 30 mg. In two cases 30 or 60 mg was injected daily for 2 or 4 days. 23 rescue treatments were rapidly followed by recovery. Normal blood counts were registered 2 weeks after the nadir in five cases and 1 week after the nadir in all other cases. Skin rashes cleared within 2 weeks. One

Table 3. Characteristics of patients with positive response to DDMP + CF

Age	Sex	Tumor	Weeks of treatment	Weeks of response	Site of responding lesions
57	m	Head and neck	49	31	Primary, skin
78	m	Lung	18	29	Primary, lymph nodes
64	m	Lung	4	6	Lymph nodes
59	m	Melanoma	6	4	Liver, skin
59	f	Head and neck	11	3	Primary
68	m	Head and neck	4	—[a]	Primary
69	f	Head and neck	1	—[a]	Primary, lymph nodes

[a] Residual tumor irradiated during remission

rescue treatment did not reverse the marrow toxicity and the patient died of sepsis with 400 WBC/mm^3 and 75,000 thrombocytes/mm^3 11 days after a single dose of DDMP, on the second day of rescue treatment consisting in 15 mg every 8 h.

23 patients were evaluable for tumor response. Two were considered unevaluable: one because of early death from brain metastases 6 days after the onset of treatment and one because of concurrent palliative radiation therapy on the measurable lesions. The outcome of the treatment was left unclassified for the last patient with lung cancer, who died of myelosuppression with an histological picture of extensive tumor destruction. There were four objective responses in ten patients with epidermoid cancer of the head and neck, two in eight patients with epidermoid cancer of the lung, and one in three patients with melanoma. The duration of responses ranged from 3–31 weeks. In two patients the duration of response could not be measured because the residual tumor was irradiated during remission. The main characteristics of patients with objective response are summarized in Table 3.

Discussion

The main reasons for the choice of a low dose of DDMP in this study were to avoid, if possible, the neurological complications encountered at higher doses, and also to reduce the dosage and hence the cost of CF. In mice and rats the acute neurological toxicity becomes manifest in convulsions and is not prevented by CF (Hamilton et al., 1954). In previous clinical experience, even if used in a relatively high dose, CF did not prevent the appearance of neurological complications. The principle of a simultaneous low dose of CF was employed for this study. This was based on the experimental work of Hill et al. (1973, 1975, 1977), and also on personal exchanges with the principal investigators concerned in previous clinical studies with DDMP, which suggested that this agent would be too toxic if used alone. The present results demonstrate that a simultaneous low-dose DDMP-CF combination is tolerable, that its toxicity is rapidly reversible, and that objective tumor regressions can be obtained at this dose level.

Tolerance to DDMP was definitively better when the agent was simultaneously combined with 30 mg CF rather than with 3 mg CF. With 30 mg CF no drug toxicity was observed, whereas it occurred in 14 of 20 patients at the 3-mg level. Of seven patients who received DDMP without simultaneous CF, five showed toxic symptoms. These findings show that a single weekly dose of CF can modify tolerance to the long-lived DDMP. This observation remains unexplained and suggests that the interrelationship at the cellular level of CF and DDMP is not identical with that of CF and MTX. The fact that DDMP is a weaker inhibitor of dihidrofolate reductase than MTX (Nichol et al., 1977) by itself can hardly explain the prolonged protection afforded by a single CF dose. Though the present study clearly demonstrated the 'protective' effect of a weekly CF dose of 30 mg, the role of the 3-mg dose could not be ascertained. It might be that DDMP alone would have given the same effects with the same tolerance. The results of other, still unpublished studies with DDMP alone (Hansen et al., 1978; de Jager et al., 1978) may go some way toward answering this question.

The toxic effects of DDMP encountered in the present series are similar to the ones previously reported, with the important exception that neurological complications, if any, were rare and mild and did not necessitate modification of the treatment. Only two patients complained of haziness and asthenia, in one case accompanied by headache. Myelosuppression was the main toxic effect of DDMP. In the present series thrombopenia was more pronounced than leukopenia. Anemia was frequent and preceded by a fall in the blood reticulocyte count. These alterations appeared during the first 3 weeks of treatment, frequently after the first dose. The nadir count was reached rapidly and recovery was achieved within 1–2 weeks. Skin rashes, often preceded by pruritus, cleared rapidly under CF rescue. Pruritus

and rashes were not accompanied by epigastric pain or other histaminic effects, and were not reversed by antihistaminics. The similarity of skin reactions observed after DDMP, MTX, and triazinate strongly suggests a common effect of different folate antagonists. Alterations of the digestive mucosae were not observed.

Besides its simultaneous combination with DDMP, CF was also used as a delayed rescue. In contrast to the preventive rescue technique, where CF is added at a predetermined time after a high dose of MTX, a CF rescue was only administered after the first signs of marrow or skin toxicity.

The effectiveness of delayed CF rescue is suggested by the remarkably rapid recovery even in the case of severe thrombopenia or generalized skin rash. A single CF dose or a few daily doses seemed to be as efficient for the recovery as the maintenance of a constant CF serum level by means of fractionated doses over many days. However, it must be stated that, for obvious ethical reasons, there was no attempt to compare the reversibility of DDMP toxicity with and without CF rescue.

Objective tumor regressions were observed in patients with epidermoid tumors of the head and neck and the lung, and with melanoma. Two responses were obtained in patients previously treated with MTX. The present data confirm the effectiveness of DDMP in various human tumors, and suggest that it can be active at a well-tolerated dose in tumor types that respond poorly to other antitumor agents.

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