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Phase II Study of Diamino-Dichlorophenyl-Methylpyrimidine (DDMP) with Folinic Acid (CF) Protection and Rescue*

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Abstract—*DDMP, a folate antagonist with a plasma half-time of* 180 hr *in man, was given to* 128 *patients with squamous cell carcinoma (lung, head and neck or other primary), melanoma, renal cell carcinoma or soft tissue sarcoma. The dosage was* 50–100 mg *weekly (according to body surface) orally simultaneously with leucovorin (CF),* 3 *or* 9 mg *weekly i.m. or i.v. Supplementary CF rescue was given in case of toxicity,* 15 mg *every* 6 hr *or* 30 mg *daily. Among* 115 *evaluable patients* 11 *partial responses were observed,* 7 *in squamous cell tumors and* 4 *in other types, with durations from* 29 *to* 170 days. *Ten of eleven responses were obtained when DDMP was combined with the lower CF dose. Also, intensity and duration of drug toxicity were related to the dosage of CF. The main toxicity consisted in myelosuppression and skin rashes. Nausea, vomiting, anorexia, diarrhea, headache and haziness were observed but remained mild and easily reversible. CF given as a true "rescue" reversed the toxic effects within* 4–12 days.

DDMP is active against squamous cell carcinoma of various sites when combined with simultaneous CF at dose levels producing a tolerable and reversible amount of toxicity.

INTRODUCTION

2,4-DIAMINOPYRIMIDINES are antagonists of pteroylglutamic acid [1]. Many of them have antimalarial activity [2]. These compounds are known to inhibit the reduction of dihydrofolate by binding to dihydrofolate reductase [3]. The antitimor activity of diaminopyrimidines was first described in 1952 in sarcoma 180 [4], subsequently in methotrexate-sensitive and methotrexate resistant strains of Ak4 leukemia [5] and in L1210 leukemia [6] in mice.

DDMP, or metoptine (2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methyl-pyrimidine), is one of the diaminopyrimidines with anti-tumor activity, particularly in methotrexate resistant cells in animals (Walker 256) [7] and *in vitro* (L5178Y) [8], and in ethylnitrosourea induced brain tumors in rats [9]. Compared to methotrexate, DDMP is a weaker inhibitor of dihydrofolate reductase activity [8, 10]. DDMP is a highly lipid-soluble and water-insoluble compound. Studies in rats have indicated that the concentration in brain and brain tumor tissue is several-fold higher than the concentration in plasma, and that the curves of elimination of DDMP from the CSF and from the plasma are parallel [10]. The plasma half-time of DDMP is unexpectedly long and averages 180 hr in man [10].

In 1954, Murphy *et al.* [11] obtained three objective improvements in 12 children with acute leukemia treated with DDMP on a

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daily schedule. However, delayed toxicity, particularly myelosuppression, was severe and prevented further use of the compound. Geils *et al.* [12] induced remissions of CNS leukemia with pyrimethamine, another lipid-soluble diaminopyrimidine. More recently, DDMP was reintroduced as an antineoplastic compound by Price *et al.* [13, 14], using large single weekly doses concurrently with folinic acid (CF). This simultaneous "protective" use of CF was based on observations by Hill *et al.* in L5178Y cells that the cell uptake and lethal effect of DDMP could be prevented by simultaneous application of CF in methotrexate-sensitive cells but not in methotrexate-resistant cells [15, 16]. Hansen *et al.* [17] concluded from observation in a phase I study including 15 patients that the toxicity of DDMP given weekly without CF was unpredictable. Alberto *et al.* [18] compared the tolerance of DDMP 50–100 mg weekly given simultaneously with CF 3 mg or 30 mg, then concluded that the tolerance was related to the CF dose. No toxicity was observed with 30 mg CF weekly, whereas definite but acceptable toxicity occurred with 3 mg CF weekly. Toxic symptoms consisted in myelosuppression and skin rashes and they were rapidly reversed by supplementary "rescue" with CF. Objective tumor responses were seen in patients with squamous cell carcinoma of the head and neck, lung and melanoma.

Based on these latter results a co-operative multicentric Phase II trial was initiated by the Early Clinical Trials Group of the EORTC using a weekly DDMP schedule and a low simultaneous CF "protection". The present paper is the final report on this study.

MATERIALS AND METHODS

Patients

The tumor screening panel selected for this study included the following types: squamous cell carcinoma of the head and neck, squamous cell carcinoma of the lung, squamous cell carcinoma of other origin, melanoma, renal cell carcinoma and soft tissue sarcomas.

All patients admitted to one of the participating institutions with a histological diagnosis of one of the tumor types selected were eligible, provided they fulfilled the following requirements: age of more than 15 yr, Karnofsky performance score of 40 or more, no concomittant second tumor, no psychosis or major neurological disease, no specific antitumor treatment for the last 3 weeks with exception for a limited radiation therapy outside the measurable tumor lesions, normal renal, liver and bone marrow functions as objectivated by serum creatinine less than 1.5 mg/100 ml (130 μmole/l), serum bilirubin less than 1 mg/100 ml (17 μmole/l) and leucocyte and thrombocyte counts superior to 3500 and 120,000/mm^3, respectively. In addition, the following conditions were required: one or more tumor lesions measurable in two perpendicular diameters or evaluable in one diameter, tumor progression before treatment and histological, cytological or clinical evidence that the lesions taken as objective criteria were part of the tumor.

Treatment

All patients were treated with weekly DDMP and CF taken at the same time. DDMP was given orally, 50 mg up to 1.5 m^2 body surface, 75 mg between 1.5 and 2.0 m^2 body surface and 100 mg beyond 2.0 m^2 body surface. CF was given i.m. or i.v. The initial weekly dose was 3 mg. However, the last 42 evaluable patients were treated with 9 mg CF weekly in order to reduce the level of toxicity as observed in the 3 mg level of CF. A CF rescue treatment was initiated immediately at the first appearance of a skin rash or whenever the leucocyte or thrombocyte counts reached values lower than 2500 and/or 80,000/mm^3 or lower than 40% of the pretreatment counts. Initially, the rescue consisted in 15 mg CF every 6 hr i.m. i.v. for 3 days or more. Thirty-eight patients received this program. Forty-one patients were rescued with a simplified program consisting in one daily 30 mg CF dose to be repeated according to toxicity. After rescue and recovery the DDMP–CF combination was resumed with an unchanged dose for DDMP but with the CF dose increased to 9 mg for those patients who had received 3 mg weekly previously and 15 mg for those patients who had received 9 mg.

DDMP was kindly supplied by the Wellcome Research Laboratories, Beckenham, Kent, U.K. as 25 mg tablets.

Evaluation of results

Tumor responses were defined as follows: complete response was disappearance of all measurable or evaluable lesions with disappearance of all symptoms and signs of the disease. Partial response was a decrease of at least 50% of the sum of all measurable lesions (as measured by the product of the largest

perpendicular diameters), or for evaluable lesions, a decrease corresponding at least to 75% of the estimated volume. Stabilisation or "no change" was less than 50% variation during at least 6 weeks. Tumor progression was a 50% increase of measurable or evaluable lesions as objectivated by one diameter or the product of two diameters. Early death was death within the first 3 weeks after initiation of treatment. Every attempt was made to distinguish between toxic death and tumor related death.

All patients were seen at weekly intervals during the whole period of treatment. Clinical and laboratory data were regularly reported on standardized study forms and sent to the EORTC Data Center, where intermediate and final evaluations were prepared. All positive tumor responses were individually reviewed with all available documents during the semi-annual groups meetings.

Hematologic toxicity was expressed by median values for nadir counts, time from day one to day nadir and time from day one to recovery (as defined by leucocyte and thrombocyte counts of more than 3500 and 120,000 per mm^3). The statistical analysis of the difference observed for the hematological toxicity was made according to a Chi square test for trends as described by Sylvester [19]. For this purpose, blood nadir counts (in counts/mm^3) were classified in 5 categories: less than 1000, 1000–1999, 2000–2999, 3000–3999, 4000 or more for leucocytes; less than 25,000, 25,000–49,999, 50,000–99,999, 100,000–149,999, 150,000 or more for thrombocytes.

RESULTS

One hundred and twenty-eight patients were entered in the study between June 1977 and March 1978. Thirteen patient observations were discarded as inadequate, either for major protocol violation, loss to follow-up or patient refusal. Among the 115 evaluable patients, 73 received the starting CF dose of 3 mg weekly and 42 patients started with a CF dose of 9 mg weekly.

Tumor response

Table 1 shows the distribution of patients by response categories and by tumor sites. Seven responses were observed in squamous cell tumors consisting of 5 head and neck, 1 lung and 1 uterine cervix. Isolated responses were observed in soft tissue sarcoma, melanoma and renal-cell carcinoma. All responses but the one in cervix cancer were observed with the lower (3 mg) weekly CF dose. From 11 responders 4 were previously treated with other chemotherapeutic agents. No objective responses were obtained. Only one of them was pretreated with methotrexate. For squamous cell carcinoma in general, the overall response rate was 15% (7/46), and 21% (6/29) for the 3 mg CF subgroup. For head and neck carcinoma, the corresponding rates were 28% for the whole group and 33% for the 3 mg CF subgroup. The duration of responses ranged from 2 to 16 weeks with a median value of 7 weeks (Table 2).

Toxicity

The dose limiting toxicity was myelosuppression and skin rash. Table 3 summarizes the degree and duration of toxicity according to the CF dose for hemoglobin concentration, leucocyte and thrombocyte counts and skin lesions. These data indicate that the level of toxicity correlates with the dose of CF protection. The differences in leucocyte and thrombocyte counts in Table 2 are statistically significant. In the 3 mg CF group 54 of 69 patients (78%), fully evaluable for toxicity, received a CF rescue treatment. In the 9 mgCF group, the number of rescued patients

Table 1. *Distribution of patients by tumor sites and by response categories*

Tumors	Total	Evaluable	Complete response	Partial response	No change	Progression	Early death
Squamous cell, lung	24	22	0	1	7	11	3
Squamous cell, head and neck	21	18	0	5	5	7	1
Squamous cell, other	7	6	0	1	3	2	0
Renal cell carcinoma	32	30	0	2	13	15	0
Melanoma	31	27	0	1	4	18	4
Soft tissue sarcoma	13	11	0	1	2	8	0
Total	128	115	0	11	34	62	8

is 21 of 44 evaluable (48%). In both groups the median first day of rescue was day 15 of treatment. As indicated in the tables the time from rescue to recovery was short, generally one week or less. Table 4 also shows that the fraction of patients with high grade hematological toxicity was higher in the 3 mg CF group as compared to the 9 mg CF group. Two drug related deaths were observed, in both cases due to hemorrhage secondary to thrombopenia, one in the 3 mg and one in the 9 mg CF group.

Table 5 indicated that the time to recovery after CF is related to the dose of rescue CF. For those patients having received 15 mg CF every 6 hours for 3 days or more, the duration of leucopenia and thrombocytopenia is clearly shorter than for the patients having received one or more single daily CF doses of 30 mg. However, both rescue types were effective.

The other observed side effects were mainly digestive, including nausea, vomiting, anorexia and diarrhea. Neurological symptoms were recorded in 8 out of 72 evaluable patients. Seven were quoted as mild and one as moderate. They consisted of headache and reversible changes in the level of consciousness.

DISCUSSION

The present results confirm other observations [11, 13, 14, 17, 18, 20–22] of therapeutic activity of DDPM in human tumors. However, they differ in many aspects from previous reports. The most obvious concerns the dosage of DDMP and CF. Price and co-workers used a weekly DDMP dose of 1.5–4 mg/kg simultaneously with 45–90 mg CF while Miller and co-workers [20] used an initial loading dose of 120 mg whereas CF was given daily, 5–20 mg orally. Hansen and Hansen [17] applying a low weekly dose of DDMP without supplementary CF had to discontinue their phase I trial because of unpredictable toxicity, in particular thrombocytopenia observed at a dose level of 50 mg/m^2 weekly. In the present study, it was accepted that DDMP should be given simultaneously with "protective" CF. However, based on an earlier phase I study [18], the CF dose was kept as low as possible in order to avoid unnecessary drug expenses and to explore the clinical effectiveness of

Table 2. Duration of response

Tumor type	CF dose (mg)	Day of response	Day of relapse
Head and neck	3	29	64
Head and neck	3	23	64
Head and neck	3	13	102
Head and neck	3	14	27
Head and neck	3	7	25
Melanoma	3	16	128
Soft tissue	3	7	71
Lung	3	33	88
Renal	3	9	29 (toxic death)
Renal	3	63	170
Cervix	9	28	99

Table 3. Median values for level and duration of toxicity related to CF dose

	CF 3 mg	CF 9 mg
Leucocytes		
Nadir count/mm^3*	3200	4300
Day nadir	15	15
Day recovery	21	17
Thrombocytes		
Nadir count/mm^3†	65,000	98,000
Day nadir	18	22
Day recovery	27	28
Hemoglobin		
Nadir g/100 mls‡	9.6	10.9
Day nadir	29	28
Day recovery	36	37
Skin rash		
Day appearance	15	15
Day recovery	22	21

Statistical significance (see text): *$P=0.0006$; †$P=0.0099$; ‡not significant.

Table 4. Hematologic toxicity related to CF dose

Nadir counts	CF 3 mg* (%)	CF 9 mg* (%)
Leucocytes < 1000/mm^3†	6	0
Thrombocytes < 25,000/mm^3‡	25	9

*Percentage of evaluable patients.
Statistical significance (see text): †not significant; ‡$P=0.049$.

Table 5. Median time in days from nadir to recovery related to CF rescue program

Treatment group	CF 15 mg q6H × 12 or more	C F 30 mg daily
DDMP + 3 mg CF		
Leucocytes	5	12
Thrombocytes	5	11
DDMP + 9 mg CF		
Leucocytes	3.5	6
Thrombocytes	5	5

DDMP when given at a lower dose than used before. Contrarily to what could have been (and actually was) speculated from results obtained with higher dosages of DDMP and CF our results indicate that DDMP may be active at a relatively low dose without large amounts of CF.

Another difference concerns the activity of DDMP in renal cell carcinoma where Price observed objective responses in 2/4 patients treated. In the present study only one response was observed in the 30 patients with renal cell tumor treated. This result corresponds statistically to a less than 5% chance of an actual response rate equal or superior to 20% [23]. In another recent report [24], no significant tumor responses were observed in 10 patients with renal cell carcinoma treated with DDMP and CF weekly doses corresponding to that used by Price.

DDMP is active in squamous cell carcinoma of head and neck, and probably also of other primary sites. No sufficient activity was observed in soft-tissue sarcoma, melanoma and renal-cell carcinoma. In a previous phase I study with a similar schedule [18], 6 responses were observed in squamous cell carcinomas, 4 out of 10 head and neck tumors and 2 out of 8 lung tumors. Two responses were obtained in patients previously treated with methotrexate. Although MTX tumor resistance could not be documented in these patients this suggests that the lack of cross resistance against DDMP and MTX observed experimentally is confirmed in clinical use. Our results also suggest that there is a relation of tumor response to the dose of protective CF. Of 11 objective tumor responses observed, 10 were obtained with 3 mg simultaneous CF and only 1 with 9 mg simultaneous CF.

Neurological toxicity was minor in the present study and never lead *per se* to dose modifications. This is in contrast to what was observed with higher doses. In a phase I/II study, Miller and co-workers [21] observed severe neurotoxicity consisting of mental confusion. Price mentions the occurrence of haziness and frontal headache [13], while Hindmarsh and co-workers [24] observed severe, neurotoxicity described as frontal headache, lethargy and muzziness. In all these reports DDMP was used at a higher dosage than in the present series and the large dose of CF given concomitantly does not seem to have prevented neurotoxicity. In animals, the acute neurotoxicity of DDMP could not be prevented by amounts of CF as large as 30 mg/kg, even given before DDMP [22]. These findings suggest either that the neurological toxicity of DDMP is not related to folic acid antagonism or that the non-liposoluble CF cannot interfere with DDMP in lipid-rich tissues.

The effectiveness of CF "rescue" in case of hematological or cutaneous toxicity was remarkable. This is in striking contrast to what is observed with methotrexate, where CF has to be given before the onset of toxicity in order to cause a reversing effect. With DDMP, this advantage was particularly appreciated because of the often sudden and severe blood and skin toxicity encountered. The actual role of CF rescue in the reversibility of DDMP toxicity is not yet completely defined, the duration and intensity of DDMP toxicity with or without CF rescue having never been compared adequately. However, our results indicate that the duration of toxicity was longer with 30 mg CF daily than with 15 mg CF every 6 hr, thus suggesting a relationship between toxicity and the amount of CF used as a rescue.

In conclusion, further investigation of the antitumor effect of DDMP in man is warranted. Isolated responses have been observed in leukemia, lymphoma, bladder cancer, mycosis fungoides, small cell carcinoma of the lung and rectum carcinoma [11, 13, 18, 25]. Phase II trials of DDMP should be particularly necessary in brain tumors, meningeal leukemias and lymphomas. Other treatment schedules should also be investigated. Due to the very long plasma T/2 of DDMP in man, a weekly schedule, as used here and in other series, may result in cumulative plasma concentrations and longer intervals between single doses with or without "protective" CF might be more advantageous to use. Finally, the mechanism of action of CF on the toxicity and effectiveness of DDMP should be explored further in order to define the role of "protective" and "rescue" CF.

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