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Sequential Administration of Dacarbazine and Fotemustine in Patients with Disseminated Malignant Melanoma—an Effective Combination With Unexpected Toxicity

Steinar Aamdal, Béatrice Gerard, Tone Bohman and Maurizio D'Incalci

Resistance to alkylating agents is partly due to the presence of the DNA repair enzyme, termed O^6 alkyltransferase (O^6AT). Preclinical evidence of the transient restoration of sensitivity of cells resistant to nitrosoureas by pretreatment with a methylating agent, whose role is to deplete cells of O^6AT activity and clinical evidence of such a depletion in patients lymphocytes, led us to test the sequential administration of dacarbazine 3 h prior to fotemustine, a chloroethylnitrosourea derivative. 24 patients with measurable advanced melanoma entered the trial and are evaluable. Toxicity was mainly haematological with early neutropenia and/or thrombocytopenia. Clinical activity (33%) was impressive especially on lung metastases with high complete response rate for that site (7/14). Unfortunately, the occurrence of a rapidly fatal pulmonary toxicity precludes further use of the regimen before a plausible explanation for this unexpected toxicity is obtained. Indeed, similar cases have been reported in other trials using the sequential schedule while no lung toxicity was reported in single agent or alternated administrations. Preclinical studies are ongoing to test the hypothesis of a glutathione depletion and the possibility of a rescue treatment.
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INTRODUCTION

CHEMOTHERAPY IN the treatment of disseminated malignant melanoma has been disappointing for many years. Very few agents have demonstrated significant activity and dacarbazine, with a 20% response rate (RR) when used as single agent, remains the most widely used chemotherapeutic agent. Randomised studies with a combination of two or more cytotoxic drugs, with or without dacarbazine failed to demonstrate a clear advantage when compared with dacarbazine alone [1]. Dacarbazine is presently under investigation in combination with hormonal agents and biological response modifiers.

Recently, a nitrosourea derivative, fotemustine, has been tested both as a single agent and in combination with dacarbazine. Both regimes proved to be active with 24% [confidence interval (CI) 17–31%] and 32% (CI 21–45%) response rate, respectively. The limiting toxicity was thrombopenia. The only other relevant toxicity was transient and reversible hepatotoxicity [2, 3].

During the last few years, more data concerning the resistance mechanisms to nitrosoureas has become available and it seems that the presence of a DNA repair system termed O^6 alkylguanine alkyl transferase (O^6AT) plays a major role in the resistance to some alkylating agents [4].

This repair system is made of a protein complex that removes methyl or ethyl radicals from the O^6 position of the guanine to a cysteine thiol residue. The enzyme O^6AT is a 'suicide' enzyme that needs to be regenerated [5]. It is found at various concentrations in approximately 75% of tumour cells and normal cells but may vary considerably from one individual to the other [6]. It is genetically determined and its level is probably modulated by previous exposure to mutagenic agents [4]. In cell lines and in animal models, the amount of O^6AT has been clearly correlated to the therapeutic response to nitrosoureas [7–9].

In vitro and *in vivo* it has been shown that pretreatment with methylating agents can reverse the resistance to nitrosourea by depleting cells of O^6AT for a few hours [10–14].

Among the clinically useful agents, dacarbazine is probably the most potent agent to saturate the O^6AT system and restore the sensitivity of a resistant cell to nitrosourea [15]. Moreover, in 13 patients receiving dacarbazine at a dose of 500 mg/m^2 or 800 mg/m^2, O^6AT was found to be decreased by 23% to 100% (median 70%) in blood lymphocytes with a maximum depletion after 3–4 h [16]. This interval can be extrapolated for tumoural tissues. Preclinical confirmation of the synergy has been obtained in a L_{1210}/carmustine resistant cell line which had been pretreated at a non-cytotoxic dose with temozolomide—the prodrug of MTIC, the active metabolite of dacarbazine—and further treated with the chloroethyl nitrosourea, fotemustine [17].

In the present study, the clinical application of such a synergism, using sequential administration of dacarbazine and fotemustine in patients with disseminated malignant melanoma, was tested.

PATIENTS AND METHODS

Patients with progressive, disseminated and histologically proven malignant melanoma, including patients with brain metastases, were eligible, providing they had received no prior

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Table 1. Patients' characteristics

Entered	24
Evaluable	24
Male/female	14/10
Median Karnofsky performance status (range)	90% (60–100)
Median age (range)	47 years (34–73)
Prior chemotherapy	0
Prior radiotherapy	3
Prior immunotherapy	0
No. of sites of metastases	No. of patients
1	11
2	4
3	5
≥4	4

Table 2. Activity

	No. of patients	CR	PR	SD	PD	RR
Overall	24	4	4	4	12	33%
Target site						
Brain	4	0	0	3*	1	0/4*
Lung	14	7	0	4	3	7/14
Liver	5	2	1	0	2	3/5
Nodes	6	2	3	1	0	5/6
Skin	4	1	1	0	2	1/4

*2 patients had a minor response > 25% < 50%.
CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, RR = relative response.

treatment with dacarbazine or a nitrosourea. Other inclusion criteria were: Karnofsky performance status (KPS) ≥ 60%, measurable disease and standard biological parameters.

Therapeutic regime

Dacarbazine 500 mg/m^2 on day 1, intravenously over 10 min followed 3 h later by fotemustine 100 mg/m^2 intravenously over 15 min. The treatment was repeated every 4 weeks if complete haematological recovery was obtained. In this trial dacarbazine is used not only for its cytotoxic effect, which would have required a much higher dose (800–1000 mg/m^2), but mainly for its ability to deplete cells of the O^6AT system.

The fotemustine dose used is the same dose recommended in the phase I trial for weekly administrations [18]. The O^6AT depletion by dacarbazine was expected to increase the bone marrow toxicity and the treatment was repeated only on every day 29. A minimum of two cycles were planned unless there was a clear progression of the disease already after the first cycle. Evaluation of response was made following the WHO criteria and documented by extramural review.

RESULTS

From April 1989 to January 1990, 24 patients entered the study. Patients characteristics are listed in Table 1. Most of the patients (22/24) had visceral involvement. 4 patients had brain metastases. Nuclear magnetic resonance (NMR) was used in all patients to detect brain metastases. The median Karnofsky performance status (KPS) was 90%. 3 patients had previously received radiotherapy (2 brain irradiation; 1 limb irradiation). None of the patients had received previous chemotherapy. The patients received from one (early progressive disease) to eight cycles, with a median number of three cycles. 4 complete responses (CR) and 4 partial responses (PR) were observed. The overall response rate is 8/24 (33%).

4 patients had stabilised disease (SD) and 12 had progressive disease (PD). Among the patients with overall progression or stable disease, dissociated response was observed in 2 patients: in abdominal masses in 1 patient and in lymph nodes in the other. The median duration of response was 28 weeks (12–40 weeks). 2 patients responding to therapy refused continuing the treatment for personal convenience.

The four complete responses were observed in patients with visceral metastases. In 1 patient CR was obtained in lung, liver and lymphnode metastases. In another patient CR was obtained in lung and lymphnode metastases. The 2 other patients with CR had lung metastases only. The four partial responses were observed in 3 patients with visceral metastases and in 1 patient with nonvisceral metastases. In 1 of these patients PR was documented in spleen, adrenal and lymphnodes metastases, while CR was documented in liver metastases. In 1 patient, PR was the result of a CR in lung and minor response (44% decrease) in cerebral metastases for 7 months. In another patient PR was the result of CR in lung, PR in liver and skin metastases and, clinically and radiologically, improved brain metastases. The fourth PR was obtained in lymphnodes. Table 2 presents the antitumour activity of the treatment by site. Importantly CR was obtained in 7/14 patients with lung metastases.

Toxicity

Haematological toxicity was the main toxicity with grade 0–1 in 13 patients, in 7 patients a grade II–III and in 1 patient a grade IV toxicity for both platelets and leucocytes. The median day of nadir was day 21 both for leucopaenia and thrombocytopaenia, i.e. 2–3 weeks earlier than commonly seen during nitrosourea treatment. A cumulative haematological toxicity was observed in 4 patients after 2, 3, 4 and 6 cycles, respectively.

Treatment was postponed until day 40 in 1 patient because of delayed haematological recovery or at the patient's request in case of prolonged treatment. In 2 patients, the drug dose was reduced (75 and 60%) from the second and fifth course, respectively due to thrombocytopenia WHO grade 2 and 3 during the previous cycle. 2 patients had a transient increase in transaminase values (grade 3) and in alkaline phosphatase (grade 2).

Nausea and vomiting were prevented with standard antiemetic treatment or with 5 HT_3 antagonists. 4 patients had grade III vomiting episodes.

No other relevant toxicities were reported except in 1 patient who presented pulmonary toxicity. The patient was 61-years-old and had no previous lung disease. He had clinical signs of a rapidly progressing pneumonia and died 1 week later. There was no documented infection and broadspectrum antibiotics had no effect. The patient was not leucopenic. An X-ray of the lungs showed bilateral rapidly progressing alveolar shadowing. The patient had received three treatment cycles. The cumulated dose of dacarbazine and fotemustine was 1.500 mg/m^2 and 300 mg/m^2, respectively and the lung metastases had completely disappeared during the treatment. Autopsy was not permitted.

DISCUSSION

The present study indicates an interaction between dacarbazine and fotemustine when the drugs are given sequentially in

patients with metastatic melanoma. This is suggested by several observations: (i) high response rate, especially in visceral metastases; (ii) onset of unexpected pulmonary toxicity; (iii) different haematological toxicity when dacarbazine is added to the treatment.

The overall response rate of the dacarbazine + fotemustine sequential combination is 33% (4 PR and 4 CR). The complete responses were all seen in patients with visceral metastases. If we consider the activity by site, 12 CR + 5 PR out of 33 measurable sites were observed. Firm conclusions cannot be drawn as the number of patients in this trial is small and the 95% confidence limits are 16–62%. Nevertheless, in two other studies, using the same sequence of drug administration and a dose of dacarbazine of 800 mg/m^2 and 1000 mg/m^2 per cycle, respectively 50% and 33% response rates for lung metastases are reported [19]. Studies *in vitro* and *in vivo* have shown that nitrosourea resistant cells, after pretreatment with a methylating agent, regain their sensitivity to nitrosoureas [10–14]. The most likely explanation for the activity of our sequence is that the depletion of the DNA repair system, O^6 alkyltransferase, by the pretreatment with dacarbazine as observed in the blood lymphocytes [16], permits a longer lasting effect of fotemustine and consequently more severe DNA damage. Indeed, if we compare with the schedule using the alternative administration of dacarbazine and fotemustine [3], we obtain a similar overall response rate with half of the two drug doses and better response rate in visceral metastases.

Unfortunately, this efficacy is accompanied by an unexpected acute pulmonary toxicity. The responsibility of the sequential combination dacarbazine + fotemustine for developing lung toxicity is supported by the report of a clinically and radiologically similar pattern of toxicity in patients receiving a cumulated dose of dacarbazine $\geq$ 1500 mg/m^2 in other trials using the same drug schedule [19, 20], while patients receiving single agents or alternated administration never present such toxicity. All patients with pulmonary toxicity were responders to therapy and had no previous or concomitant chemotherapy or radiotherapy. The possible pathological mechanisms of this lung injury are competition for glutathione or alterations of the glutathione transferase activity [21, 22], direct effect of the increased alkylating power at the DNA level due to O^{6}AT inhibition [4] and the possible role of cytokines [23]. These mechanisms are most probably coresponsible for the onset of this adverse effect and will be discussed in detail in another paper. The particular sensitivity of lung tissue—if glutathione metabolism is implicated—could be due to differential metabolic rates between lung and main sites like liver or kidney tissue [24] despite an equal alkylating activity of dacarbazine in all organs [25].

A further indication of synergistic interaction between dacarbazine and fotemustine is suggested by the data on haematological toxicity. We observed a much earlier onset of the haematological toxicity compared to the previous studies with fotemustine or any other nitrosourea given at standard dose. In this case, the bone marrow, which has low basal O^{6}AT levels, showed a sensitivity comparable to the one observed when a high dose nitrosourea treatment is administered [26]. The study also illustrates the limitations of chemotherapy when efficacy and toxicity are closely related. To improve the therapeutic ratio, i.e. reduce lung toxicity and retain the antitumour activity, studies are now being undertaken with locoregional administration of temozolomide—which spontaneously decomposes to MTIC without requiring metabolic biotransformation—in combination with fotemustine. Preclinical studies are ongoing to document the toxicity and test a rescue treatment, if glutathione pool depletion is implicated.

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Course, Patterns, and Risk-factors for Chemotherapy-induced Emesis in Cisplatin-pretreated Patients: a Study with Ondansetron*

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Vomiting and nausea are the most distressing side-effects of cancer chemotherapy. With standard antiemetic regimens (e.g. metoclopramide based combinations) sufficient antiemetic control is achieved in 50–70% of cisplatin treated patients. Ondansetron, a selective 5-HT$_3$-receptor antagonist has shown efficacy in cisplatin-induced emesis. In the present study, we evaluated the safety and efficacy of ondansetron in cisplatin pretreated patients who had suffered from severe emesis in spite of antiemetic prophylaxis. Complete antiemetic control was reached in 43.5% on the day of treatment and in 27.2% of the patients regarding a worst day analysis. 25% of the patients suffered from severe cisplatin-induced emesis (> 5 emetic episodes per 24 h). We try to characterise risk-factors for cisplatin-induced emesis by performing a multivariate analysis. Sex, cisplatin dose, and combination therapy with cisplatin plus anthracyclines seem to be independent risk-factors for vomiting on day 1 and on worst day. Delayed emesis occurred less often when sufficient antiemetic protection from acute vomiting had been obtained. Female sex, cisplatin dose and recurrent disease seem to influence the probability for occurrence of delayed vomiting.
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INTRODUCTION

NAUSEA AND vomiting are distressing side-effects of tumour drug therapy [1] and are held responsible for discontinuation of therapy in 3–19% of cancer patients [2, 3]. Cisplatin leads to nausea and vomiting in almost all patients [4, 5]. Without any antiemetic medication, cisplatin-induced vomiting will commence after 1–4 h [6–8]. If antiemetic drugs are given, vomiting will on average start after 4, 5h (metoclopramide), and only after 6–24 h using 5-HT$_3$-antagonists [9–12]. Besides acute vomiting, cisplatin will also cause delayed emesis, which has been observed even if acute vomiting was completely suppressed [13]. However, sufficient therapy of acute vomiting seems to affect delayed emesis in a positive way [14]. Assumed risk factors for increased chemotherapy-induced vomiting under cisplatin therapy are female sex [10, 15, 16], patient age [17], a history of prior chemotherapy [16, 18], and a combination of cisplatin with anthracyclines [14].

Serotonin-receptors, and among them especially 5-HT$_3$-receptors seem to play a central role in chemotherapy-induced emesis [19]. The latter have been shown to be present in the chemotherapy trigger zone (CTZ), and on peripheral afferent vagal neurons. Blocking of 5-HT$_3$-receptors or of serotonin synthesis will prevent cisplatin induced emesis in animal models

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