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A Phase II Study of High-dose Hydroxyurea and Dacarbazine (DTIC) in the Treatment of Metastatic Malignant Melanoma

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Dacarbazine (DTIC) exerts its major biochemical effect through the formation of methylated DNA adducts. Hydroxyurea (HU) is a ribonucleotide reductase inhibitor which blocks DNA excision-repair by the depletion of intracellular ribonucleotides. Combination of HU and DTIC was used to enhance the activity of DTIC by inhibiting DNA repair. 16 patients with metastatic malignant melanoma were treated with the combination. All patients had measurable disease and none had received prior systemic therapy. Hydroxyurea was given as a continuous intravenous (i.v.) infusion of 1 g/h (total 36 g) and DTIC 1 g/m^2 i.v. over 1 h, 23 h from the start of hydroxyurea infusion. 4 patients achieved partial remission with an objective remission rate of 25% [95% confidence interval (CI) 7–52%]. Median duration of response was 3.5 months. 3 of the responding patients had predominant visceral metastases. Disease was stabilised in 5 patients with a median time to progression of 16 months. The predominant toxicity to this treatment was gastrointestinal, with 3 patients developing grade 3 nausea/vomiting. Only 1 patient developed grade 3 leucopenia complicated by septicaemia. It is concluded that the combination of hydroxyurea and DTIC is a well-tolerated regimen with activity against visceral metastases from malignant melanoma but the duration of response to this treatment is short.

Key words: hydroxyurea, dacarbazine, melanoma, DNA repair
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INTRODUCTION

THE PROGNOSIS of metastatic malignant melanoma remains very poor despite various systemic treatments [1]. Dacarbazine (DTIC) is generally considered the most active agent in treating malignant melanoma with response rates ranging from 16 to 31% [2].

The major anti-tumour activity of DTIC results from the methylation of DNA which is dependent on its metabolic activation by *N*-demethylation to MTIC [5-(3-methyl-1-triazeno) imidazole-4-carboxamide] [3, 4]. MTIC methylates DNA at a number of sites and the formation of O^6-methylguanine–DNA adduct is probably very important in the cytotoxicity of DTIC. Repair of the O^6-methylguanine proceeds via the enzymatic reaction mediated by O^6-alkylguanine transferase and there is a suggestion that the activity of this enzyme may be responsible for some of the resistance exhibited against DTIC [5]. Another mechanism of DNA repair after damage with DTIC involves the removal of the modified base by a glycosylase to leave an apurinic site: the DNA strand is then cut by an endonuclease close to this site to excise a portion of the DNA and the remaining intact strand acts as a template for synthesis of new DNA which is subsequently ligated to complete the repair process [6]. N^3-Methylguanine and adenine and N^7-methylguanine DNA adducts are removed by this process. Single-strand DNA breaks resulting from the process of excision repair have been demonstrated in peripheral lymphocytes after treatment with DTIC [7].

Hydroxyurea (HU) is a ribonucleotide reductase inhibitor which inhibits DNA repair by the critical reduction of intracellular deoxyribonucleotide levels [8]. Previous studies have shown that in cultured cell lines, inhibition of ADP-ribosylation, an important DNA repair mechanism, resulted in enhanced cytotoxicity to MTIC. The effect was more marked when cells expressed O^6-alkylguanine-DNA transferase compared to cells which were deficient in that enzyme [9, 10]. The combination of HU and DTIC may thus provide a rational approach in the treatment of malignant melanoma. We have previously shown that it would be feasible to inhibit DNA synthesis in tumours from patients with solid tumours by employing a continuous infusion of HU [11].

The aims of this study were to determine the anti-tumour efficacy and toxicity of a combination of high-dose HU and DTIC in patients with metastatic malignant melanoma. HU was given as an intravenous (i.v.) infusion and continued for 12 h after the administration of DTIC in order to maintain the depletion of deoxyribonucleotides and maximise DNA repair inhibition. The timing of DTIC administration in relation to HU was based on our previous experience with high-dose HU [11]. Mean steady state plasma HU levels of 1.67 mM were achieved after 23 h of a continuous infusion of HU, at which time there was no uptake of 5-iodo-2-deoxyuridine by tumour cells, indicating inhibition of DNA synthesis by HU.

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MATERIALS AND METHODS

Patients

16 consecutive patients with measurable metastatic malignant melanoma took part in this phase II study. Table 1 gives patients' characteristics. The mean age was 54 years (range 29–73) with a median pretreatment ECOG performance status score of 1. None of the patients had previously received systemic therapy for metastatic disease. All patients gave informed consent prior to treatment and the study was approved by the Central Oxford Research Ethical Committee. Patients had adequate haematological and renal function prior to commencing treatment.

Treatment

Hydroxyurea (Hydrea, Bristol-Myers Squibb, U.K.) was administered as a controlled i.v. infusion of 36 g (in 2 l of isotonic saline 0.9%) over 36 h. Dacarbazine (DTIC-dome, Bayer, U.K) was given in a dose of 1 g/m^2 dissolved in 0.5 l isotonic saline and infused i.v. over 1 h starting 23 h from the commencement of hydroxyurea infusion. Anti-emetics comprised dexamethasone 8 mg twice daily orally/i.v. and metoclopramide 10 mg/kg i.v. Cycles were repeated every 3 weeks.

Assessment of response and toxicity

Patients were assessed for objective tumour response after the completion of two to three cycles of therapy. Patients with brain or bone metastases were not eligible for response assessment unless accompanied by other evaluable or measurable metastases. A decrease by at least 50% in the product of the greatest perpendicular parameters of all measurable tumour lesions indicated partial remission. Stable disease was defined as less than a 25% increase or less than a 50% decrease in the parameters for at least 12 weeks. Progressive disease was defined as greater than a 25% increase in disease parameters. Duration of response (or stable disease) and survival were measured from the first day of treatment to the date of disease progression and death, respectively. Grading the severity of toxicity was according to the World Health Organization's criteria for toxicity grading.

RESULTS

Table 2 summarises the outcome of this study. The median number of courses received by the patients was three. 2 patients received concomitant radiotherapy to single disease sites. Partial remission was achieved in 4 patients [25%; 95% confidence interval (CI) 7–52%] lasting 1.5, 3, 4 and 8 months, respectively. 3 of those patients had visceral metastases, 1 with a brain metastasis. Objective remission was accompanied by a significant improvement in the performance status in 2 patients. 5 patients had stable disease lasting 5–22+ months. One patient who had stable disease after three courses of treatment died suddenly of probable pulmonary embolus, 4 weeks from stopping therapy. The median duration of survival of all patients was 9.5 months.

Table 1. Patients' characteristics

Characteristics	No. of patients
Total number	16
Median age, years (range)	54(29–73)
Male:female	8:8
ECOG performance status	
0	2
1	12
2	2
Prior chemotherapy	0
Evaluable for toxicity	16
Evaluable for response	16
Visceral dominant metastases	8
Central nervous system	1
Liver	4
Lungs	8
Bone	2
Gastrointestinal tract	1
Soft tissue metastases only	8

Table 2. Response data

Response	No. of patients	Disease sites	Time to progression (months)
PR	4	ST (1), VD (3)	1.5, 3, 4, 8
SD	5	ST (4)*, VD (1)	5, 12, 16, 22+
PD	7	ST (3), VD (4)	1,1,1,1,1, 2, 2

PR, partial remission; SD, stable disease; PD, progressive disease; ST, soft tissue; VD, visceral disease. * One patient with stable disease died suddenly of probable pulmonary embolisation.

All patients were evaluable for toxicity. No treatment-related deaths were observed. Grade 3 nausea/vomiting was seen in 3 patients. One patient developed septicaemia due to grade 3 leucopenia which was not life-threatening. Alopecia was not significant and none experienced greater than grade 1 toxicity. Grade 1 diarrhoea and mucositis were observed in only 1 patient, respectively.

DISCUSSION

Enhanced DNA repair is a recognised phenomenon of drug resistance and failure of chemotherapy [12]. A number of anti-metabolites inhibit DNA repair and the degree of such inhibition is dependent upon the concentration of the anti-metabolite and the proliferative rate of the malignant cells [8]. Biochemical modulation of the activity of cytotoxic drugs that have some activity in malignant melanoma is one way of enhancing tumour cell kill in a population of patients whose prognosis is very poor.

Results of this study show that it is feasible to administer the combination of high-dose HU and DTIC with acceptable toxicity. Patients included in this study had advanced disease and nearly half of those had visceral metastases which are associated with poor response to chemotherapy. The objective remission rate to HU plus DTIC was 25%, which is within the confidence intervals of previous studies using single-agent DTIC, and three out of the four responses were in patients with predominant visceral metastases.

Lack of significant potentiation of DTIC's antitumour effect may be explained on the basis that repair of DNA apurinic sites generated after glycosylase activity is only one component of resistance to DTIC in patients with malignant melanoma. Repair of the O^6-methylguanine adducts by O^6-alkylguanine-DNA alkyltransferase is a major mechanism in the resistance to DTIC in such patients [5] and the activity of this enzyme would not be expected to be influenced by HU. Alternatively, the degree of DNA repair inhibition of DNA adducts achieved with the current regimen of HU may not be sufficent to produce significant tumour cell kill.

Based on information gained from *in vitro* work and animal experiments, a number of factors govern the outcome of treatments designed to biochemically modulate cytotoxic therapy.

Amongst those, the choice of anti-metabolite(s) and schedule of administration are probably the most important variables. For example, rapidly growing tumours are less sensitive to the inhibitory effects of HU because of the higher levels of intracellular nucleotide pools [8]. The use of anti-metabolites with different biochemical effects (e.g. cytosine arabinoside, gemcitabine), singly or in combinations with HU, should be considered in order to inhibit different key DNA synthetic pathways inside the cell. Solveing *et al.* [7] have shown that the highest level of DTIC-induced DNA damage (due to adduct formation) in peripheral lymphocytes from treated patients was achieved within 5 h with daily treatment with DTIC 250 mg/m^2 and most of the DNA damage was repaired within 20 h. This may indicate that longer infusions of HU may be required to effectively block DNA repair synthesis after DTIC, because the duration of depletion of intracellular deoxyribonucleotides by antimetabolites may be critical in preventing cells from repairing damaged DNA. Longer infusions of HU will, however, increase the risk of toxicity to HU [13]. The use of other agents that are more active after depletion of O^6-alkylguanine-DNA alkyltransferase, such as fotemustine [14], should be assessed in combination with standbreak repair inhibition and DTIC, to maximise the inhibition of repair pathways.

Biochemical modulation of the activity of cytotoxic drugs remains an intriguing aspect of cancer therapy and requires further exploration. Information obtained from experimental systems may not be predictive of outcome in humans because of the difference in cell kinetics and biochemical characteristics of animal and human tissues.

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Feature Article

Cancer Vaccines

A.G. Dalgleish

FASHION TENDS to come full circle every decade or so. Consider tumour immunology and the attempted development of cancer 'vaccines' as treatment. Those few individuals attempting to treat human cancer with 'immunotherapy' a decade or more ago were very much marginalised by the mainstream therapists. Today they are undergoing a rehabilitation, in part made possible by the biotechnology revolution and the ability to dissect and alter the immune responses to tumours. A century ago, dogs and donkeys were used to raise sera against human tumours to treat cancer patients. Similar clinical 'trials' were to be practised for the next few decades even though regressions and clinical improvements were rarely seen (for a review, see Oettgen and Old [1]). Similar studies were revived in the 1950s and 1960s without much success. The advent of monoclonal antibodies and the ability to epitope map immune responses has not had the clinical impact that had been expected except for a few anecdotal remissions. Early studies in mice, where methylcholanthrene-induced sarcoma cells could immunise syngeneic mice so they