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Cisplatin with high-dose infusions of hydroxyurea to inhibit DNA repair

A phase II study in non-small-cell lung cancer

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Summary. A total of 45 patients with locally advanced and/or metastatic non-small-cell lung cancer (NSCLC) were treated in a phase II trial with high-dose i.v. infusions of 24 g hydroxyurea over 24 h, with 50 mg/m^2 i.v. cisplatin 8 h after the start of hydroxyurea infusion. Hydroxyurea, a cell-cycle-specific inhibitor of ribonucleotide reductase, inhibits DNA repair by depleting nucleotide pools. We gave hydroxyurea to achieve steady-state levels of ≥ 1 m*M* and to potentiate therapy by inhibiting repair of DNA damage produced by cisplatin. Among 21 patients with squamous cell lung cancer, there were 1 complete response (CR), 2 partial responses (PR) and 3 minor responses (MR). Of 13 patients with adenocarcinoma of the lung, 2 had MRs; of 11 patients with large-cell anaplastic lung cancer, none responded. The dominant toxicity was nausea and vomiting, which was manageable and mainly related to cisplatin. The response rate in squamous cell lung cancer was similar to responses obtained with cisplatin alone. The relative ineffectiveness of high-dose 24-h infusions of hydroxyurea in inhibiting repair of DNA damage produced by cisplatin may be due to the low growth fraction of human NSCLC. The high-dose hydroxyurea approach may be more applicable in tumours with a high growth fraction.

Introduction

Hydroxyurea is an S-phase cell-cycle-specific agent that inhibits DNA synthesis by inhibiting ribonucleotide reductase and depletes cells of nucleotide triphosphates [19]. At higher doses, it inhibits DNA repair [7, 12, 17], probably by preventing the filling of gaps in DNA with nucleotides. Cisplatin has moderate single-agent anti-tumour activity against non-small-cell lung cancer (NSCLC), [1] and in human cell lines it induces DNA inter-strand cross-links reaching a maximal level 6–12 h after exposure to the drug [5]. Hydroxyurea rapidly affects the nucleotide pools within 1 h after its addition to cultured cells [6]. We therefore designed a clinical study combining cisplatin with high-dose intermittent infusions of hydroxyurea to prevent repair of DNA damage induced by the former. Since hydroxyurea is a cell-cycle-specific agent, it would therefore be most effective when given such as to ensure continuous exposure of tumour cells to the drug [10]. We [15] and others [2] have shown that high-dose i.v. infusions of hydroxyurea, achieving levels that inhibit repair in vitro, are possible with mild toxicity in humans.

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Patients and methods

A total of 45 patients with histologically diagnosed, locally advanced and/or metastatic NSCLC were treated with hydroxyurea and cisplatin. All patients had assessable disease, and their performance status was graded by WHO criteria [18]. Patient characteristics are given in Table 1, and Table 2 shows details of the courses of treatment. Hydroxyurea powder was aseptically prepared for i.v. use by the Pharmacy, Newcastle General Hospital, and was given at a dose of 24 g dissolved in 3 l 4% glucose and 0.18% sodium chloride by i.v. infusion over a 24-h period. In addition, 8 h after the start of the hydroxyurea infusion 50 mg/m^2 cisplatin in 100 ml 0.9% sodium chloride solution was infused i.v. over 15 min. Since steady-state levels of hydroxyurea are achieved within 8 h of a constant i.v. infusion [15], cisplatin was given at this time and hydroxyurea was continued over the time course predicted for subsequent repair of DNA adducts, i.e. 16 h. Courses were repeated every 3 weeks, to a maximum of six courses in any one patient.

Before each treatment course, haemoglobin, WBC count, serum urea, creatinine, electrolyte and liver function tests were carried out. Response was assessed clinically and by serial chest X-rays (CXR), and some patients also underwent computerized axial tomography. Routine anti-emetics were given with each chemotherapy course, usually i.v. metoclopramide at an initial dose of 2 mg/kg followed by 100 mg by 8-h infusion. Alternatively, patients were given 4 mg qd oral dexamethasone for 1 day and 30 mg qd oral domperidone for 2 days or longer.

Results

One patient had a complete response (CR, complete disappearance of all pre-treatment evidence of tumour), and two others had $\geq 50\%$ decreases in tumour volume designated as partial responses (PRs). These three objective responses occurred in patients with squamous cell lung cancer. In addition, three patients with squamous cell lung cancer had $< 50\%$ regression in tumour volume designated as minor responses (MRs). Thus, the percentage of response rate for conventionally defined objective responses, i.e. CRs + PRs, was 14.2% (95% confidence limits, 3.0%–36.3%). If CRs, PRs and MRs are combined, the

Table 1. Patient characteristics in 45 patients with NSCLC

Median age (range) in years	62 (29–73)
Median performance status (range)	1 (0–3)
	Number of patients
Male	35
Female	10
Locally advanced inoperable tumour	24
Metastases identified	21
Histological cell type	
Squamous cell	21
Adenocarcinoma	13
Large-cell anaplastic	11
Previous treatments[a]	
Surgery	7
Chemotherapy	2
Radiotherapy	0

[a] Despite these therapies, patients had assessable tumours prior to treatment with hydroxyurea plus cisplatin

Table 2. Courses and doses of hydroxyurea and cisplatin

Median number of courses (range)	2 (1–6)
Number of patients receiving six courses	9
Number of patients refusing because of GI toxicity or hospitalisation	4
Dose reduction	5
Number of patients with progressive disease	32

response rate in squamous cell lung cancer was 29% (95% confidence limits, 11.2%–52.1%). Of 13 patients with adenocarcinoma of the lung, 2 had MRs, and of 11 patients with large-cell anaplastic lung cancer, none responded.

Survival

In all, 37 patients who did not respond to therapy had a median survival of 10 weeks, and one was alive 200 weeks after the initiation of therapy. After progression of the tumour despite hydroxyurea and cisplatin, this patient had an objective PR to subsequent radiotherapy. The median survival for eight patients with evidence of response (CR, PR and MR) was 115 weeks (range, 50–220 weeks); the patient attaining CR continued to survive relapse-free at 220 weeks after the initiation of hydroxyurea and cisplatin. The latter patient received no other anti-tumour therapy after hydroxyurea and cisplatin.

Toxicity

After the first course, one patient developed left ventricular failure considered to be due to infusion fluids. Another patient unresponsive to hydroxyurea and cisplatin developed a peripheral neuropathy, but this could have been related to his underlying cancer. Although full blood counts and serum biochemistry were only estimated immediately prior to treatment cycles and not between courses, myelosuppression and hepatic and renal toxicities were mild if at all present. WHO grade 1 leucopenia was detected in one patient; WHO grade 1 elevation of serum urea occurred on two occasions, and grade 1 elevation of serum creatinine was detected on one occasion. The dominant toxicities were gastrointestinal. Two patients had diarrhoea after therapy, most likely due to cisplatin. Despite routine pretreatment with anti-emetics, 13 patients had grade 1 WHO gastrointestinal toxicity (nausea). A further 16 patients had WHO grade 2 emesis, and one patient, grade 3 emesis. A substantial number of dose reductions were necessary because of toxicity (Table 2).

Blood levels of hydroxyurea. We measured serum hydroxyurea levels as previously described [15]. During the last 8 h of hydroxyurea infusion in six patients the mean serum level was 1.97 m*M* (range, 0.8–3.09 m*M*) and levels of ≥ 1 m*M* are sufficient to cause 99% inhibition of A549 lung cancer cell growth in culture [15].

Discussion

High-dose i.v. infusions of hydroxyurea can be safely given to humans with cancer [15], and the present study confirms the feasibility of combined high-dose i.v. hydroxyurea and i.v. cisplatin. Significant toxicities were nausea and vomiting, which were manageable and presumably due to cisplatin, as these toxicities are much milder with hydroxyurea alone [15].

Because hydroxyurea is an S-phase cell-cycle-specific agent, it is likely to be most effective when given such as to ensure continuous exposure of tumour cells to the drug; Belt et al. [2] designed clinical studies to examine this issue. In addition to inducing cell kill in the S phase, hydroxyurea blocks cells at the G^1-S border and sensitises these cells to radiation or cytotoxic drugs [11]; therefore it may be a useful synchronisation agent, particularly when given by continuous infusion [2]. The clinical value of hydroxyurea as a synchronising agent given with radiotherapy is unproven, although some encouraging results have been reported in head and neck and cervical cancer. These studies have been criticised because of their lack of information about the circulating and tissue pharmacokinetics of hydroxyurea as well as the cell kinetics of the tumours studies [11].

We gave high-dose infusions of hydroxyurea to inhibit repair of DNA damage caused by cisplatin rather than to attempt cell synchrony. Serum hydroxyurea levels achieved in the present study as well as a previous phase I study [15] were highly tumouricidal as assessed in an appropriate in vitro model [15]. Our response rates suggest that the high-dose hydroxyurea approach may be of clinical value only in squamous cell lung cancer. However, in that tumour cell type the anti-tumour activity of the combination is not any greater than that of cisplatin alone. Single-agent cisplatin gave a mean response rate of 20% (range, 6%–32%) in a combined series of 5 trials involving 140 evaluable patients [1]. Doses in these trials ranged from 75 to 120 mg/m^2 every 3–6 weeks [1] and were higher than the dose used in the present study. In our study, survival in patients with evidence of response to therapy was associated with considerable survival advantage in comparison with those not achieving objective response. Inferences about this observation cannot be made, as there are a variety of reasons for bias in comparing survival of responders with that of non-responders [9]. Furthermore, some of the responding patients underwent radiotherapy for relapse after the completion of cisplatin and hydroxyurea therapy.

Since hydroxyurea affects S-phase cells, cells that are in cycle may be most susceptible to the modification of repair of cisplatin cross-links. Even with a low growth fraction, those cells that are dividing should be more susceptible to this drug interaction. NSCLC does not possess a high growth fraction [14], and this could account for our low response rate. Early studies of hydroxyurea given at a continous, minimal oral dose of 40 mg/kg gd showed very poor anti-tumour activity in lung cancer patients [3]. The use of a cell-cycle-specific agent in combination with cisplatin to inhibit DNA repair might more appropriately be applied to tumours possessing high growth fractions. However, there are several mechanisms of drug resistance to cisplatin, including increased repair, reduced uptake, protection by glutathione and other uncharacterised mechanisms [8]. One or several of these may be present in any particular tumour. Furthermore, nucleoside salvage pathways may bypass the effects of hydroxyurea on de novo biosynthesis. The detectability of the marked effect of a particular biochemical modulation will depend on the relative importance of these pathways in the patient population.

Hydroxyurea is the only specific inhibitor of ribonucleotide reductase currently in clinical use, but its effectiveness is limited because it is a weak inhibitor of ribonucleotide reductase in vivo [4]. Ribonucleotide reductase remains an important target for chemotherapy because it catalyses the reductive conversion of ribonucleotides to deoxyribonucleotides, which is the first reaction in the biosynthetic pathway specifically committed to DNA synthesis [13]. The development of more potent inhibitors of ribonucleotide reductase [16] will allow further exploration of target inhibition as well as future combination therapies. This study shows the safety and feasibility of such an approach, but future studies will require pre-treatment evaluations of possible resistance mechanisms in biopsies to select patients for randomised studies of the appropriate biochemical modulation.

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