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Phase II Study of Cystemustine in Metastatic Colorectal Carcinoma

A Trial of the EORTC Clinical Screening Group

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27 patients with metastatic colorectal carcinoma were treated, every 2 weeks, with 60 mg/m² cystemustine, a new chloro-2-ethyl nitrosourea derivative. Haematological toxicity was the major side-effect including neutropenia and thrombocytopenia. We did not observe any complete or partial response. Cystemustine, with this dose and this schedule, has no activity in colorectal cancer.
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INTRODUCTION

COLORECTAL CARCINOMA causes 10–15% of morbidity encountered in western countries. This particular type of tumour is resistant to most of the chemotherapeutic drugs, except for fluoro-pyrimidine derivatives, especially 5-fluorouracil [1]. These drugs have a consistent antitumour activity in colorectal tumours. Interestingly, some studies report activity of another class of drugs, the nitrosoureas, CCNU, BCNU and methyl CCNU [2].

In order to investigate the therapeutic value of these results, the EORTC Clinical Screening Group performed a phase II trial using a new nitrosourea compound cystemustine.

PATIENTS AND METHODS

Patient eligibility was defined by a measurable metastatic colorectal tumour within a 3-months period prior to the beginning of treatment, a WHO performance status of 0–1, a normal white blood cell and platelet count and normal renal and

Table 1. Patients' characteristics

Patients eligible	26
Fully evaluable patients	24
Sex (male/female)	17/9
Age (years)	
Median	59
Range	17–69
WHO performance status	
Grade 0	11
Grade 1	13
Grade 2	2
Previous radiotherapy	7
Previous chemotherapy	20
Adjuvant therapy with nitrosourea	0
Site of disease	
Local relapse	1
Liver	18
Lung	5
Other	6

liver functions. In addition, no previous chemotherapy with nitrosourea was a criterion for inclusion of patients. All patients provided written consent.

Treatment

Cystemustine was given intravenously at a dose of 60 mg/m^2, infused in 100 ml 5% dextrose. The treatment plan consisted of one administration every 2 weeks for four cycles. Disease response was assessed 2 weeks later. In patients with non-progressive disease, this schedule was continued until progression or excessive toxicity.

Dose modification

The injection was postponed for 1 week if, at day 15, the granulocyte count fell below 1500/mm^3, or the platelet count below 100 000/mm^3. WHO grade III or IV thrombocytopenia necessitated a dose reduction at 45 mg/m^2. If treatment had to be delayed for more than 3 weeks, the patient was taken out of the study.

Response evaluation

The response was evaluated according to the WHO criteria [3] after a minimum treatment period of four cycles. Patients progressing before the end of the 8 weeks of treatment were included in the category of progressive disease.

RESULTS

27 patients were included into this phase II study. One was considered ineligible because of age; 26 were evaluable for the toxicity of the treatment and 24 for response. Patients' characteristics are shown in Table 1. In the patients evaluable for response, 7 patients had stable disease and 17 had progressive disease. No complete or partial responses were observed.

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Table 2. Haematological toxicity (evaluation for 26 patients after induction chemotherapy)

	Median	Range	Grade 3–4 (no. of patients)
White blood cells (10^9/l)	4.6	1.2–14.2	1
Neutrophils (10^9/l)	2.5	0.4–10.6	2
Platelets (10^9/l)	179	24–471	2
Haemoglobin (mmol/l)	7.9	5.9–12.7	0

The haematological toxicity is given in Table 2: 7.7% of patients experienced grade 3 or 4 toxicity for neutrophils or platelets nadir. The non-haematological toxicity was mild (Table 3). Nausea (grades 1–2: 12 patients; grade 3: 1 patient), diarrhoea (grade 1: 2 patients), infection (grade 2: 2 patients) and asthenia (grades 1–2: 6 patients) were the main toxicities encountered.

CONCLUSION

Cystemustine is a biochemical compound derived from chloro-2-ethyl nitrosourea, after reduction of disulphur bonds, S-methylation and oxidation at the sulfur position. This molecule presents different and interesting properties, for example, solubility and stability in aqueous solution and a good diffusion coefficient through the blood–brain barrier. In animal models this compound is active on murine tumours, with mainly moderate haematological toxicity, compared to other nitrosourea molecules, this one slightly inhibits glutathione reductase [4]; the drug was therefore selected for the phase II trials undertaken by the EORTC Clinical Screening Group.

Our results confirm that this molecule induces essentially a haematological toxicity in induction phase, with very minor other side-effects: unfortunately, the compound has no beneficial effect in colorectal cancer, although non-hepatic metastases have been included in the protocol.

In recent years, nitrosoureas have been used in phase II trials with low response rates of 9–13% [5,6]. Although nitrosoureas have often been used in association with 5-fluorouracil, their exact contribution has not been clearly defined: Moertel *et al.* have reported an increase in response rate with 5-fluorouracil + methyl CCNU + vincristine compared with 5-fluorouracil alone [7]. However, Richards did not observe the same positive results [8].

As adjuvant therapy in stage II and III rectal cancers, similar results were achieved. A combination of radiotherapy and 5-fluorouracil with and without methyl CCNU has been tested by the NCCTG. More relapses occurred in the nitrosourea arm of

Table 3. Non-haematological toxicity (evaluation for 26 patients after induction chemotherapy)

	Grade 0	Grade 1	Grade 2	Grade 3
Nausea	13	8	4	1
Diarrhoea	24	2	—	—
Liver toxicity	25	—	1	—
Infection	24	—	2	—
Mucositis	24	2	—	—
Neurotoxicity	25	1	—	—
Asthenia	20	4	2	—

the protocols; therefore, the analysis of the results did not show any beneficial role of nitrosourea [9].

In conclusion, the present results and those in the literature confirm that cystemustine should not be used in the treatment of colorectal carcinomas.

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The Immunohistochemical Expression of Proliferating Cell Nuclear Antigen (PCNA/Cyclin) in Malignant and Benign Epithelial Ovarian Neoplasms and Correlation with Prognosis

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Proliferating cell nuclear antigen (PCNA)/cyclin is considered to be a marker of cell proliferation. The aim of this study was to evaluate the expression of PCNA/cyclin in epithelial ovarian neoplasms (EON) as well as the possible correlation with degree of differentiation, tumour stage and overall survival. The material consisted of 34 benign and 40 malignant EON. Positive nuclear staining was detected in 2/34 (6%) of benign and 23/39 (59%) malignant EON ($P < 0.001$). Most cases in the high proliferation group were diagnosed in advanced clinical stages. There was no difference in overall survival between nuclear PCNA positive and negative patients, as well as the high and the low proliferation group. In conclusion, the role of PCNA as a marker of malignant potential and prognosis in EON merits further investigation.
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INTRODUCTION

CURRENTLY THERE is growing information regarding monoclonal antibodies that recognise a 36 kD, S-phase associated, nuclear protein called proliferating cell nuclear antigen (PCNA)/cyclin. PCNA/cyclin plays a critical role in DNA synthesis and the initiation of cell proliferation [1]. PCNA/cyclin has been detected immunohistochemically on paraffin sections of normal and malignant tissues such as gastrointestinal lymphomas, breast, gastric, pancreatic, prostatic and renal cell carcinomas and haemangiopericytomas [2–7].

The association of PCNA/cyclin with the rate of proliferation using monoclonal anti-PCNA/cyclin antibodies has been investigated with the aid of immunofluorescence microscopy and flow cytometry [8, 9]. Monoclonal antibodies PC-10 and 19A2 that recognise the acidic protein of PCNA have shown a linear correlation with other proliferation indices such as Ki-67 and flow cytometry in lymphoid malignancies, whereas in other tumours such as breast and gastric carcinomas no such correlation has been found [2].

The aim of this study was to investigate PCNA expression in benign and malignant epithelial ovarian neoplasms (EON). In addition, an attempt was made to study the possible correlation of PCNA with other parameters such as histological type and grade, clinical stage and overall survival.

MATERIALS AND METHODS

A retrospective analysis of tumour tissue obtained from the Department of Pathology, University of Athens Medical School was performed. The tissue specimens were obtained from a total of 73 women with EON of which 34 were benign and 39

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