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Short Communication

EO9 Phase II Study in Advanced Breast, Gastric, Pancreatic and Colorectal Carcinoma by the EORTC Early Clinical Studies Group

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In a phase II trial, the activity of EO9, a new bioreductive alkylating agent, was assessed. EO9 was used as second-line chemotherapy in breast cancer patients and as first-line chemotherapy for patients with gastric, pancreatic and colorectal cancer. EO9 was given as a 5 min i.v. infusion at a weekly dose of 12 mg/m². 92 patients were entered; 22 with breast cancer, 26 with colon cancer, 24 with pancreatic cancer and 20 with gastric cancer. In general, the drug was well tolerated with nausea and vomiting occurring in 26.42 and 13.3% of courses, respectively. Reversible proteinuria was the main toxicity occurring in 45% of courses. Antitumour activity was not observed. At this dose and schedule, EO9 is not an active drug in the type of tumour studied. Copyright © 1996 Elsevier Science Ltd

Key words: EO9, breast cancer, pancreatic cancer, gastric cancer, colorectal cancer, proteinuria, renal toxicity, phase II study

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INTRODUCTION

EO9 (3-hydroxy-5-aziridinyl-1-methyl-2-(1H-indole-4,7-dione)-prop-β-en-α-ol) was selected for clinical evaluation as the leading compound of a series of bioreductive cytotoxic agents. EO9, an indoloquinone synthesised by Oostveen and Speckamp [1] is structurally related to mitomycin C [1, 2]. Its mechanism of action is considered to be a bioreductive alkylation, with the activation being catalysed by the two-electron donating flavoenzyme, DT-diaphorase (EC 1.6.99.2) [2]. This reduction generates DNA damaging species *in vitro*, with the development of DNA single strand breaks and cross-links [3].

Different *in vitro* studies have suggested a close relationship between cellular DT-diaphorase content and sensitivity of a particular cell line to EO9 [4–6].

The preclinical evaluation in the disease-oriented cell line panel of the National Cancer Institute showed a preferential activity against solid tumour-derived cell lines, with lack of activity against lympho-leukaemic cell lines [7]. The *in vivo* activity was quite similar. Toxicity data showed a LD₁₀ in mice after a single administration of 9 mg/kg (27 mg/m²).

The increased efficacy under hypoxic conditions and the lack of haematological toxicity in animals were reasons to select this agent for phase I studies within the framework of the Early Clinical Studies Group of the European Organization for Research and Treatment of Cancer

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(EORTC). In the first phase I study, using a 3-weekly schedule, starting at 2.7 mg/m² and escalating up to 27 mg/m², dose-limiting toxicity (DLT) proved to be proteinuria, salt and water retention and elevation of serum creatinine [8]. The recommended dose for phase II studies was 22 mg/m². Pharmacological studies showed a rapid elimination with a *t*_{1/2} of 0.8 to 19 min. The AUC was linearly related to the administered dose. Pharmacodynamic analysis indicated a sigmoidal relationship between the AUC and the proteinuria. In a second phase I study, the drug was given weekly and the DLT also proved to be proteinuria, reached with a dose of 15 mg/m² once weekly. A dose of 12 mg/m² proved to be safe. On the basis of these two studies, the dose of 12 mg/m² weekly was selected since the total dose of EO9 given in 3 weeks exceeded the 3 weekly dose (36 mg/m² over 22 mg/m²).

The Early Clinical Studies Group (ECSG) of the EORTC initiated phase II studies with EO9 as a weekly bolus injection of 12 mg/m² in patients with breast, pancreatic, gastric and colon cancer.

PATIENTS AND METHODS

Eligibility

All patients entered into these four trials had to have histologically or cytologically verified advanced measurable malignant disease beyond resectability. In patients with pancreatic cancer with only local disease present, the indicator lesion needed to be at least 3 cm in diameter. Patients had to be at least 18 years of age, have a WHO performance status < 2, a WBC > 2000/μl and a platelet count > 75 000/μl. Normal liver (exception in case of liver metastases) and kidney function were required and proteinuria was not allowed. Retrospectively, patients with minimal initial proteinuria which was within the normal limits of their institute were accepted since analysis of toxicity data did not show any difference between this group and the group without pretreatment proteinuria. Patients with pancreatic and stomach cancer could not have received any prior chemotherapy. Prior treatment with adjuvant chemotherapy was allowed for patients with colorectal carcinoma, provided the treatment-free interval was more than a year. For patients with breast cancer, chemotherapy both in the adjuvant set-

ting and/or first line for metastatic disease was allowed. Pretreatment with mitomycin C was not permitted.

Formulation, dosage and treatment procedures

EO9 was provided by Kyowa-Hakko Kogio (Tokyo, Japan) as a freeze-dried powder. It was dissolved in sterile saline to a concentration of 0.5 mg/ml and administered as an intravenous bolus injection over 5 min. The drug was given at a dose of 12 mg/m² every week for an initial period of 6 consecutive weeks. After this period, patients with tumour response or disease stabilisation were allowed to continue treatment.

Treatment was interrupted for one week in case of any proteinuria or other renal toxicity, grade 3 haematological toxicity or any other grade 2 non-haematological toxicity. If the toxicity persisted for more than one week without treatment, patients went off protocol.

No preventive anti-emetic treatment was given during the first administration, thereafter it was left to the investigator to select a particular regimen if needed.

Response and toxicity evaluation

Follow-up studies included weekly complete blood counts, serum creatinine and urine analysis. Every 3 weeks, a complete clinical evaluation and full biochemistry analysis was also carried out. Tumour evaluation occurred every 6 weeks.

The objectives of the studies were response and toxicity evaluation in patients with advanced breast, colorectal, pancreatic and gastric cancer. Standard WHO response criteria were used. Toxicities were graded according to the NCI Common Toxicity Criteria for Cancer clinical trials.

RESULTS

Patients' characteristics are shown in Table 1.

Gastric cancer

20 eligible patients with advanced untreated gastric cancer were entered in the study. All patients were eligible. A total of 132 courses was administered. 3 patients were not evaluable for response. 2 due to persistent proteinuria and 1 patient received only 2 courses because of rapid progression

Table 1. Patient characteristics

	Gastric cancer	Colorectal cancer	Pancreas cancer	Breast cancer
Number of patients	20	26	24	22
Number eligible	20	25	23	22
Characteristics				
Median age	61	61	54	57
Age range	36-83	33-74	44-83	38-75
Women/men	6/14	14/12	11/13	22/0
Performance status				
0	9	14	4	8
1	9	10	16	10
2	2	2	4	4
Previous therapy				
Surgery	13	25	12	19
Radiotherapy	0	5	0	17
Immunotherapy	0	0	0	1
Hormone therapy	0	0	1	16
Chemotherapy	0	6	0	21

Table 2. Toxicity

	Gastric cancer			Colorectal cancer			Pancreatic cancer			Breast cancer			Total (%)
Number of patients	20			25			23			22			90
Number of courses	132			175			129			120			556
Toxicity grade	I	II	III	I	II	III	I	II	III	I	II	III	All grades
Nausea	27	6	0	34	5	1	24	6	0	27	16	1	147 (26.4)
Vomiting	8	4	0	7	4	1	18	2	1	6	22	1	74 (13.3)
Proteinuria	58	7	0	56	14	0	45	19	0	42	9	1	251 (45.1)
Creatinine elevation	2	2	0	8	0	0	0	0	0	2	0	0	14 (2.5)
Oedema	0	2	0	2	5	0	2	0	0	2	3	1	17 (3.0)

of disease. No tumour regressions were observed. Stable disease was observed in 6 patients.

Colorectal cancer

26 patients with advanced colorectal cancer were entered in the study. 1 patient did not start therapy. 6 patients had been treated with adjuvant chemotherapy, completed more than 1 year prior to disease progression. The remaining 25 patients received in total 175 drug administrations without dose modifications, but with delay in 17 instances. These delays were drug-related in 13 instances and all due to proteinuria. 3 patients were not evaluable for response—1 stopped because of proteinuria, and 2 others only received 2 courses because of progressive disease. In the 22 patients evaluable, no responses were observed. 6 patients had stable disease.

Pancreatic cancer

24 patients with untreated advanced pancreatic cancer were entered. 1 patient was not eligible because of the lack of a measurable lesion and never started treatment. The 23 remaining patients received 129 drug administrations. No dose reductions were needed but a delay in dosing occurred in 23 instances, mainly due to proteinuria. Of these 23 patients, 4 were not evaluable for response. 1 patient died prior to evaluation and 3 received only one cycle. No anti-tumour activity was observed in any of the remaining 19 patients. Stable disease was observed in 4 patients.

Breast cancer

22 patients with breast cancer resistant to one line of chemotherapy were entered in the study. All patients were eligible. A total of 120 courses was administered. 3 patients were not evaluable for response—1 patient received only one course, a second one only two courses, and a third patient had persistent proteinuria and went off study. No antitumour effect was observed. 3 patients had stable disease after six courses, while the other patients all progressed.

Toxicity

In this phase II study, a total of 556 courses were administered to 90 patients. A summary of toxicity details is given in Table 2. All courses were evaluable for toxicity. Toxicity was generally mild. Nausea grade I and II were observed in 145/556 (26%) courses. Only two grade III toxicities were recorded. Vomiting was equally mild and mainly limited to grade I and II. In total, 13.3% of courses was accompanied with some degree of vomiting. Asthenia,

mentioned during 149 courses (26.8%), was also limited to grade I and II. The most important toxicity was renal with proteinuria in 251 instances (45.1%). This was grade I for 201 (36.2%) and grade II for 49 (11.3%) courses. Only one grade III proteinuria was recorded. 4 patients had interrupted treatment because of persistent proteinuria. 12 patients had grade I renal toxicity with creatinine elevations. No haematological toxicity was observed in this group of patients. Oedema was seen in 17 courses. There was no apparent relationship between the occurrence of proteinuria and oedema, or the presence of some degree of creatinine elevation.

In some institutes, patients with minimal proteinuria, detected with sensitive biochemical urine analysis, were included into the study. This minimal initial proteinuria did not affect the incidence or severity of treatment-related proteinuria, nor did it worsen the risk for renal function disturbances or for the development of oedema.

DISCUSSION

EO9 is one of a series of new bioreductive alkylating agents selected for clinical testing. In preclinical models, the role of DT-diaphorase in activation of EO9 is considered to be important for its cytotoxic activity under aerobic conditions [9]. Under hypoxic conditions, the importance of DT-diaphorase activation is less clear. Even against a cell line that lacks DT-diaphorase activity, hypoxic sensitisation both for mitomycin C and EO9 is evident [10, 11]. These different modes of activation and the selective efficacy against solid tumour cell lines were reasons to select EO9 for clinical testing.

A weekly dose of 12 mg/m² was selected for our phase II trial, but this schedule showed no sign of antitumour activity. The patterns of toxicity previously described in the phase I study were confirmed. In total, 90 patients received 556 drug administrations. Main toxicities were mild nausea and vomiting rarely in excess of grade II and easily controllable with standard anti-emetic agents, such as metoclopramide or alizapride. No haematological toxicity was observed. The clinically most important toxicities were renal. In 14/556 courses, a moderate creatinine elevation, grade I or II, was observed, but this was always reversible. Moderate grade I or II oedema was observed on 17 occasions (3.0%). However, proteinuria was much more frequent. Grade I or II urinary protein loss was noticed in 251/556 (45%) of courses. One case of grade III proteinuria was recorded. These toxicities are not dissimilar from those observed in the phase I study and confirm that the selected dose can be considered as the highest feasible dose. We

could not demonstrate a relationship between the proteinuria and the appearance of oedema.

The lack of activity is disappointing and needs some comment. As stated, the toxicity analysis in this trial, confirmed that EO9 was administered at an adequate dose. Further dose escalations with this weekly schedule are not possible. Therefore, one possible explanation for the lack of activity despite the promising *in vitro* data might be given by the recent data from Collard and associates [12]. These investigators examined the relationship between DT-diaphorase activity and sensitivity to EO9 in a panel of human and rodent tumours. In cell lines, a possible relationship between enzyme activity and the chemosensitivity of cell lines was confirmed. Once grown in nude mice DT-diaphorase activity in most tumours fell significantly. It may well be that the tumours from the patients studied did not contain adequate DT-diaphorase levels. These data suggest a cautious approach when developing strategies of rational drug design based on enzyme activity of cell lines, even if those are of human origin. We conclude that EO9 at this dose and schedule is not an active drug in the types of tumour studied.

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