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A Phase I Evaluation of N^{10}-Propargyl-5,8-dideazafolic Acid

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Abstract—*The quianazoline antifolate* N^{10}*-propargyl-5,8-dideazafolic acid (ICI* 155,387), *an inhibitor of thymidylate synthetase (TS), was evaluated for clinical toxicity in a phase I trial. The compound was given once every week as a bolus injection. Fourteen patients with advanced cancer were treated at doses of* 10–30 mg/m^3. *Four patients from the lowest to the highest dose developed severe renal toxicity, detected by a reversible decrease in the* Cr-*EDTA clearance.*

Hepatotoxicity was observed with transient elevations of alanine aminotransferase (ALT) in 10 *patients and alkaline phosphatase in nine patients. Neither the incidence nor the severity of these toxicities was dose related. Two patients developed feelings of fatigue, which in one patient coincided with a decrease in* Cr-*EDTA clearance. No myelotoxicity, dermatological, gastrointestinal toxicity or mucositis was seen. No tumour responses due to ICI* 155,387 *occurred.*

The severity and the erratic nature of the renal side-effects suggest that this schedule cannot be recommended for further development of this compound in Phase II trials.

INTRODUCTION

Two commonly used cytostatic agents, methotrexate (MTX) and 5-fluorouracil (5-FU), are known to inhibit thymidylate synthesis. MTX inhibits dihydrofolate reductase, which results in a depletion of intracellular reduced folates necessary both for purine and thymidylate synthesis. 5-FU is metabolized in both ribo- and deoxyribonucleotide derivatives, which may bind tightly to thymidylate synthetase (TS). The action of 5-FU can also be explained by incorporation of 5-FU into RNA/DNA. It is therefore conceivable that a drug only inhibiting TS might have increased antitumour activity, lesser side-effects and not be affected with MTX resistance [1, 2].

N^{10}-Propargyl-5,8-dideazafolic acid (CB 3717, NSC 327182, NSC 373233 and ICI 155,387) was found to possess such a selective inhibitory activity against TS purified from L1210 leukaemic cells with an inhibitor constant (K_i) of 1.14 nM [1]. ICI 155,387 has been shown also to inhibit the growth of human hepatocellular carcinoma *in vitro* [ID_{50} of drug (dose at which cell number is 50% of control) for PLC/PRF/5 is 1.48 ± 0.55 μM and for Hep 3B is 1.95 ± 1.57 μM] [3] with an ID_{50} which falls within the range of plasma levels achieved when ICI 155,387 infusions have been administered to patients in early clinical trials [4]. In a number of methotrexate resistant human and rodent cell lines ICI 155,387 has been demonstrated to be highly active [2, 5], regardless of whether the resistance was due to an elevated level of dihydrofolate reductase or reduced membrane transport of methotrexate [2]. ICI 155,387 has been shown to have high antitumour activity at doses of 125–200 mg/kg i.p. in the L1210 i.p. implanted tumour in mice [1] with nine out of 10 long-term survivors and ICI 155,387 has also demonstrated inhibition of growth of human hepatocellular carcinoma in athymic mice (200 mg ICI 155,387 kg^{-1} body wt day^{-1} i.p. for 5 days) [3].

Animal toxicology studies of ICI 155,387 indicated that the main target organs for toxicity were the liver and the gastrointestinal tract in dogs, liver and kidney in rats and kidney and testes in mice [6, 7]. Because of the specific mechanism of action and its preclinical antitumour activity ICI 155,387 was selected for clinical development. Various dose schedules were explored initially in the U.K. [8] and later within the Early Clinical Trials Group of the EORTC [9]. This article contains the data from such a phase I trial performed in Copenhagen using a weekly schedule of ICI 155,387.

MATERIALS AND METHODS

All patients entering the study had a microscopi-

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cally confirmed malignant solid tumour and they had either been unsuccessfully treated with standard therapy or were untreated because no recognized effective therapy was available. All patients had normal renal function evaluated by Cr-EDTA clearance and normal liver function confirmed by normal *s*-bilirubin, *s*-alanine aminotransferase (ALT) and *s*-alkaline phosphatase. In addition haematologic status consisting of haemoglobin, platelets and white blood cells had to be within normal limits. Verbal informed consent from the patients was obtained following the guidelines of the Copenhagen Ethical Committee. No patients had received systemic antineoplastic therapy within the previous 4 weeks (6 weeks for nitrosoureas and mitomycin C) or had unresolved toxicity from prior anticancer therapy before entering the study with the exception of alopecia in some patients. Clinical assessment of haematologic status, measurement of serum values of sodium, potassium, calcium, creatinine, ALT, alkaline phosphatase, total bilirubin, total protein, albumin, LDH, prothrombin time and urea were performed before and weekly after the start of treatment. Cr-EDTA clearance together with urinanalysis for albumin, haemoglobin and glucose were performed every 3 weeks. Chest X-ray was performed in all patients before the first treatment, and if abnormal every 3 weeks during treatment. In patients with measurable disease the various parameters used for evaluation were examined every 3 weeks applying WHO criteria (1979) [10]. ICI 155,387 was supplied by Imperial Chemical Industries Limited in ampoules containing 5 ml of the disodium salt in bicarbonate buffer, at a concentration equivalent to 10 mg/ml. The drug was administered by intravenous bolus injection once a week.

At least three patients were entered at each dose level with starting dose being at 10 mg/m^2. This dose was chosen as a safe starting dose because an earlier phase I trial with ICI 155,387 administered every 3 weeks i.v. only discovered minimal toxicity at 140 mg/m^2. A Fibonacci dose scheme was followed for dose escalation. Dose escalation did not take place within individual patients. All patients were treated until progression of disease or signs of severe toxicity occurred.

RESULTS

Fourteen patients with a mean age of 60 years (range 40–72 years) were admitted to the study. One patient did not fulfil the inclusion criteria (performance 4) and was subsequently excluded from further analysis. Among the 13 patients, six patients had lung cancer, two ovarian cancer and the remaining five patients miscellaneous types of malignant tumours. One patient was previously untreated while 12 patients had received either

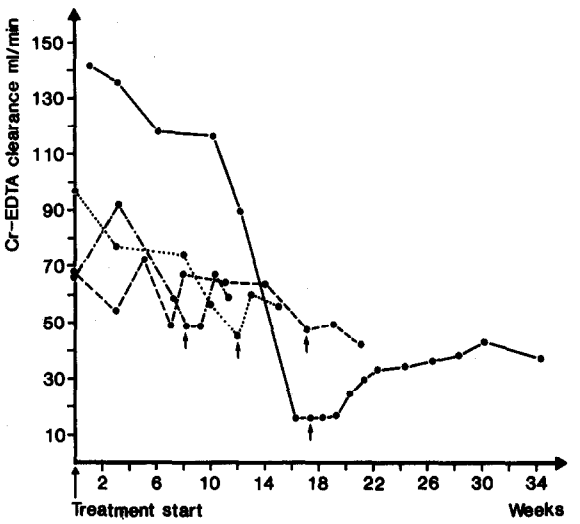

Fig. 1. Renal toxicity. Cr-EDTA clearance (CrEC) vs. time in patients with renal toxicity. One patient at dose 10 mg/m^2 ●—●; *one patient at dose* 20 mg/m^2 ●····●; *one patient at dose* 20 mg/m^2 ●-·-·-●; *one patient at dose* 30 mg/m^2 ●---●; *discontinuation of treatment* ↑.

prior chemotherapy, radiotherapy or both. With respect to WHO performance status 10 patients had performance status 0–1 while three patients were classified as performance status 2 and 3. Three patients received 10 mg/m^2 of ICI 155,387 once weekly for a duration of 5–17 weeks, six patients received 20 mg/m^2 once weekly for 8–15 weeks and four patients received 30 mg/m^2 once weekly for 5–16 weeks.

The main toxicity observed in this study was linked to renal and hepatic function while no myelotoxicity was observed.

The dose-limiting toxicity was renal, which was observed in four patients. As depicted in Fig. 1 one patient treated at a dose level of 10 mg/m^2 developed serious reduction in Cr-EDTA clearance after 17 treatments (total dose 170 mg/m^2). After cessation of treatment Cr-EDTA clearance increased slowly but it did not return to normal levels. Among the other three patients, two patients at dose 20 mg/m^2 with a total dose of 220 and 160 mg/m^2 and one patient at dose 30 mg/m^2 and a total of 480 mg/m^2 developed moderate reduction in Cr-EDTA clearance, which after discontinuation of treatment returned to normal. Patients, who developed reduction in Cr-EDTA clearance during treatment with ICI 155,387 were examined with ultrasound of abdomen, urine and blood analysis in order to exclude other causes of renal insufficience. No deaths due to renal toxicity occurred and the four patients with renal toxicity were not examined *post mortem* because of death at home or at local hospitals.

The other patients received up to a total dose of 510 mg/m^2 without reduction in Cr-EDTA clearance. No proteinurea was observed.

Table 1. *Hepatoxicity*

Dose (mg/m²)	Number of patients with *s*-alanine aminotransferase toxicity grade					Number of patients with *s*-alkaline phosphatase toxicity grade				
	0	1	2	3	4	0	1	2	3	4
10		1	1					2		
20		3		2	1		1	3	1	
30		1	1				1	1		

As to hepatotoxicity, it was observed at all dose levels in 10 patients with transient elevations of ALT levels in all patients (Table 1). The first sign of elevation of ALT occurred at a total dose of ICI 155,387 varying from 10 to 510 mg/m².

With successive courses ALT was increased in some patients while ALT in other patients returned to normal by continued treatment. Increases in alkaline phosphatase levels were also induced in nine of the 10 patients with ALT elevation, but the severity was less pronounced (Table 1). The first sign of a rise in alkaline phosphatase occurred at a total dose of ICI 155,387 of 20 mg/m². No patient developed elevated alkaline phosphatase without an increase in ALT, and in eight of nine patients the increase in ALT was discovered before the increase in alkaline phosphatase. Only one patient at a dose of 20 mg/m² developed slight hyperbilirubinaemia (toxicity grade 1) 2 weeks after increasing ALT (toxicity grade 3)—bilirubin and ALT returned to normal despite continued treatment. All patients with hepatic toxicity were examined for the presence of other causes—none such were observed. Prolongation of prothrombin time was not observed.

The development of abnormal renal function coincided with feelings of fatigue during 3 weeks in one patient (10 mg/m²). One patient at a total dose of 300 mg/m² had a feeling of fatigue, which disappeared after discontinuation of treatment. The patient's feeling of fatigue was not related to hepatotoxicity.

No myelotoxicity, dermatological, gastro-intestinal toxicity or mucositis has been noted.

No tumour responses were observed during treatment with ICI 155,387.

DISCUSSION

In this schedule with ICI 155,387 given once a week, the dose limiting factor was renal toxicity evidenced by decreasing Cr-EDTA clearance. Another clinical study of ICI 155,387, given once every 3 weeks, has recently [8] been reported also to reveal dose-related renal toxicity, but not dose-limiting. Pharmacokinetic studies [4] have indicated that the urinary excretion of ICI 155,387 is approx. 30% of the administered dose within 24 h of treatment and no ICI 155,882 metabolites have yet been found in urine. ICI 155,387 is a weak acid and insoluble at acid pH. This could possibly explain both the results of the preclinical toxicology studies in mice in which the renal failure was due to precipitation of ICI 155,387 in the renal tubules [6] and the toxicity observed in human studies. Noteworthy in that respect is the observation that residual drug has been detected in *post mortem* kidney tissue in one patient 8 days after treatment [4]. The renal toxicity which has become more pronounced with ICI 155,387 administered once every week instead of once every 3 weeks can possibly be explained by accumulation of the compound in the renal tubules. The renal toxicity can probably be overcome by development of water-soluble analogue of ICI 155,387 or by maintaining alkaline diuresis during treatment [8]. The liver toxicity was self-limiting, and appeared in most patients. Nine of 10 patients, who had received prior treatment with cytostatic agents, developed hepatic toxicity and two of two patients not previously treated with chemotherapy also developed hepatic toxicity. Clinical pharmacokinetic studies have revealed a tentative correlation between the incidence of hepatic toxicity and peak ICI 155,387 plasma levels [4].

In this study malaise could not be related to the hepatic toxicity, as was reported earlier with administration of ICI 155,387 given once every 3 weeks [8] and neither was the malaise dose-limiting. In conclusion ICI 155,387 results in reversible renal and hepatic toxicity when given weekly at a low dosage but the severity and the erratic nature of the renal toxicity is such that this schedule cannot be recommended for further development of this compound in phase II trials.

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