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SHORT COMMUNICATION

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Initial clinical trial and pharmacokinetics of Thymitaq™ (AG337) by 10-day continuous infusion in patients with advanced solid tumors

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Abstract *Purpose*: To establish the maximum tolerated dose (MTD), dose-limiting and other major toxicities and the major pharmacokinetic parameters of a 10-day infusion of the nonclassical antifolate Thymitaq™. *Methods*: The drug was given by 10-day infusion via a portable pump. The starting dose was 286 mg/m^2 per day with escalation to 572 and 716 mg/m^2 per day. Thymitaq in plasma was assayed by a validated isocratic reverse-phase HPLC assay with detection at 273 nm. *Results*: The dose of 716 mg/m^2 per day × 10 was considered too high as none of three patients completed a 10-day infusion and two of three developed grade IV myelotoxicity. At 572 mg/m^2 per day three of four patients completed a 10-day infusion. Dose-limiting myelosuppression was seen in one of four but owing to a high incidence of thrombotic phenomena, no further patients were added. *Conclusion*: Continuous 10-day infusions of Thymitaq should be limited to low doses until further studies can be done.

Key words Thymitaq™ · Phase I · 10-Day infusion · Pharmacokinetics

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Introduction

Thymitaq™ (AG337) is an inhibitor of thymidylate synthase (TS) recently introduced into clinical trial. It is a nonclassical antifolate with the structure 2-amino-3,4-dihydro-6-methyl-4-oxo-5(4-pyridylthio)-quinazoline dihydrochloride. Synthesis of the compound was based on the three-dimensional structure of the active site of TS determined by X-ray crystallography. The compound was designed to undergo tight binding to the cofactor binding site of TS. It has a K_i of 11 n*M* for human recombinant TS, an IC_{50} of 0.39 μ*M* for L1210 murine leukemia in vitro and is curative in vivo against the intraperitoneally and intramuscularly implanted L5178Y/TK$^-$lymphoma [11]. It has negligible intracellular retention and thus continuous exposure is required to maintain inhibition of TS [11].

In a phase I study of a 24-h continuous infusion (CI), doses up to 1073 mg/m^2 (as free base) were explored [7]. The maximum tolerated dose (MTD) was not reached. Primary toxicity was local tenderness and swelling at the site of injection. No venous thrombosis was seen. In studies of a 5-day CI [1, 5, 6], the MTD was 898 mg/m^2 per day. Dose-limiting toxicity (DLT) was myelosuppression and mucositis. Phlebitis was noted when the drug was given into a peripheral vein but not when it was given through a central line. Pharmacokinetics were nonlinear with a reduction in plasma clearance at higher doses.

In phase II studies, at a starting dose of 795 mg/m^2 per day × 5, over 150 patients have been entered. Myelosuppression, mucositis and skin reactions have been major toxicities [2, 4]. No thrombotic phenomena have been reported. Activity has been seen in squamous cell carcinoma of the head and neck, carcinoma of the colon, non-small-cell carcinoma of the lung, and in hepatocellular carcinoma [2, 4, 10].

Because of the demonstrated increase in antiproliferative activity of Thymitaq on prolonged exposure and the good tolerability of the drug when given by central

Table 1 Toxicity of Thymitaq 572 and 716 mg/m^2 per day

Patient no.	Dose (mg/m^2/day)	Duration (days)	No. of courses	Leucopenia		Thrombocytopenia		Thrombotic phenomena	C_{ss} (μg/ml)
				Nadir of WBC	Grade	Nadir of platelet count	Grade		
6[a]	572	10	4	2800	II	41,000	III	–	–
7	572	10	2	3800	I	432,000		–	2.2
8[b]	572	10	2	3100	I	52,000	II	Bilateral subclavian and innominate vien thrombosis	5.3
13	572	5	1	960	IV	48,000	III	Thrombosis of mediport. Deep vein thrombosis right leg	–
10	716	8	1	1700	III	15,000	IV	–	7.0
11	716	5	1	3200	I	140,000	I	Mediport occlusion	4.1
12	716	7	1	890	IV	37,000	III	Thrombosis left innominate vein	10.5

[a] Patient also had grade III esophagitis
[b] Patient also had grade III vomiting

line for 5 days, a phase I study of a 10-day CI through a central line was explored. The objectives of the study were to define the tolerability, MTD, DLT and other major toxicities, pharmacokinetics and recommended phase II dose of a 10-day CI of the drug.

Materials and methods

Patients

Patients ≥18 years of age with histologically confirmed solid tumors not amenable to treatment of known effectiveness, with normal bone marrow, hepatic and renal function and a performance status (ECOG) of 0 or 1, who gave written informed consent were entered. Other entry and exclusion requirements were as previously reported [3].

Drug administration

All patients had central venous access. Thymitaq was dissolved in 5% dextrose in water and the resulting solution (maximum concentration 99 mg/ml) infused at 18–35 ml/day by a CI ambulatory pump (CADD Pharmacia, Deltec St. Paul Minn.) with cassette changes every 3–5 days.

Monitoring of toxicity

Vital signs were measured every 15 min for 2 h after the start of the initial dose. Blood was drawn for complete blood count twice weekly (daily in the presence of myelosuppression) and for blood chemistry profile weekly until values returned to normal.

Pharmacokinetic monitoring

Blood was collected in heparinized tubes at the following timepoints: pretreatment, at 1, 2 and 4 h on day 1; at 72, 144 and 168 h during the 7-day infusion (see below) and at 72, 144, 216 and 240 h during the 10 day infusion. Following the end of the infusion, samples were obtained at 5, 15, 30 and 45 min and at 1, 2, 4, 6, 8, 12 and 24 h. All samples were brought to the laboratory on ice and centrifuged immediately. Plasma was stored at −20 °C until analysis.

Analysis of Thymitaq in plasma

An isocratic reverse-phase HPLC procedure validated as an external standard method was used. Plasma deproteinized with acetonitrile was evaporated to dryness under nitrogen, the dry residue reconstituted with water and an aliquot injected into the HPLC apparatus. The HPLC conditions were as follows: column, Altech C6 (15 cm × 4.6 mm; 5 μm); mobile phase, sodium phosphate buffer (10 m*M*, pH 7.0), acetonitrile (70:30); flow rate, 0.8 ml/min; detection at 273 nm. A six-point standard curve in the range of 0.2 to 20 μg/ml in plasma was used for quantitation of the unknowns. Quality control (QC) samples (2 μg/ml and 15 μg/ml in plasma) were prepared in bulk and aliquots frozen at −20 °C. Two sets of QC samples were analyzed with each set of patient samples.

Data analysis

Pharmacokinetic parameters were derived using the noncompartmental data analysis program LAGRAN [8]. Steady-state concentrations (C_{SS}) reported are mean observed values.

Results

A total of 13 patients were entered (6 with colorectal carcinoma, 2 each with non-small-cell lung carcinoma and squamous cell carcinoma of the head and neck and 3 with miscellaneous tumors). The median age was 59 years (range 41–67 years), performance status ECOG was 0 (four patients) or 1 (nine patients). All had had prior chemotherapy (one regimen – five patients, two regimens – seven patients, three regimens – one patient). Two patients had incomplete courses unrelated to drug toxicity and were not evaluable for toxicity.

Three dose levels were explored: 286, 572 and 716 mg/m^2 per day[1]. Because the effect of prolonging the infusion was not known, the first two patients at 286 mg/m^2 per day were treated for 7 days only.

[1] Doses are given as free base. The corresponding doses of the dihydrochloride salt are 360, 720 and 900 mg/m^2.

At 286 mg/m^2 per day, four evaluable patients received eight courses. The only grade II toxicity seen was fatigue (two) and anorexia (one). All other toxicities were grade I. One patient noted neck fullness, facial swelling and jugulovenous distension towards the end of the 10-day infusion which resolved rapidly on completion of the infusion. These events recurred during the second course but again resolved.

The toxicities seen at 572 and 716 mg/m^2 per day are shown in Table 1. At 716 mg/m^2 per day, two of three patients developed grade IV myelotoxicity after 8 and 7 days, respectively, and this dose level was considered too high. This was considered to be at or above the MTD; a 10-day infusion could not be completed in any of the three patients. A fourth patient was added at 572 mg/m^2 per day. The purpose of adding more patients to the 572 mg/m^2 per day dose level was to determine whether this would be a suitable phase II dose. However, the study was stopped when this patient developed deep vein thrombosis and this, together with thrombotic phenomena in other patients (see Table 1), led to discontinuation of the study.

Pharmacokinetic measurements were compatible with the nonlinear pharmacokinetics previously described [7]. However, the small number studied at each dose level and the substantial interpatient variability make conclusions from the pharmacokinetic data in this study necessarily tentative. The mean AUC 0–144 h values ($\mu g \cdot h \cdot ml^{-1}$) for the three dose levels were 308 ($n = 4$), 393 ($n = 2$) and 1206 ($n = 2$), respectively (Table 1).

Discussion

This phase I study was undertaken to increase intracellular exposure to Thymitaq. Since the molecule lacks a glutamate moiety its intracellular retention is poor compared with that of the classic antifolates and thus prolonged exposure is required for optimal antitumor effect. As noted above, in phase I and phase II studies, 795 mg/m^2 per day $\times$ 5 is well tolerated and has been used in more than 160 patients. No excess thrombotic episodes have been reported when the drug is given by a central venous access. In the present study, of the seven evaluable patients who received 572 or 716 mg/m^2 per day, two developed thromboses in the veins into which the drug was infused, one patient developed a thrombosis in the mediport and deep vein thrombosis of the lower extremity in spite of coumadin prophylaxis and one developed an occlusive fibrin sheath at the tip of the catheter. In addition, two patients at the lowest dose had thrombotic episodes which, in retrospect, might be considered to have been possibly drug related. One patient, several weeks after completing four cycles at 286 mg/m^2 per day $\times$ 7, was found to have a thrombosis of the axillary and subclavian veins on the side the drug was infused and one patient, as noted above, had facial swelling with both courses of 286 mg/m^2 per day $\times$ 10 (this patient was on coumadin for a previous deep vein thrombosis).

Unlike in previous and other ongoing studies of Thymitaq, in the present study a number of episodes of thrombosis were seen. The reason for this disparity is unknown. The pH of the drug infusate was low, but was the same as that used in other studies. The placement of the central venous access was carried out by the same team of surgeons employed for our study of etoposide phosphate which was given by CI for 42 days to 22 patients with no problems with thrombosis [9]. Prothrombin time and partial thromboplastin time were measured in the later patients in the present study but showed no abnormality except in patients receiving coumadin.

From the data presented, it would seem advisable to limit 10-day infusions of Thymitaq to low doses. However, the safety of such low-dose 10-day infusions was not established by the present study because at the lowest dose evaluated there was suggestive evidence of thrombotic phenomena. Lower doses would need to be studied to establish the safety of this dosage schedule

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