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Phase I study of the anthrapyrazole biantrazole: clinical results and pharmacology

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Summary. In a phase I study the anthrapyrazole biantrazole (Warner-Lambert Company) was given to 41 patients with tumour refractory to existing therapy. The drug was given i.v. weekly for 3 weeks, with a 3-week interval between courses. At the 1st week a full pharmacokinetic study was performed, and at weeks 2 and 3, blood samples were taken at 1 and 6 h following treatment to check for drug accumulation. Biantrazole pharmacokinetics were linear with respect to the AUC ($r = 0.924$) over the full range of doses studied (4–36 mg/m^2) but exhibited large inter-patient variations at each dose level. Elimination was triphasic, comprising two rapid early phases and a long terminal half-life (mean, 14.1 ± 7.8 h). There was no evidence of drug accumulation over the 3-week treatment period. Approximately 12% of the parent drug was excreted unchanged in the urine together with two non-circulating, more water-soluble metabolites. Biantrazole was well tolerated but did cause moderate emesis at doses of >18 mg/m^2 and mild alopecia. The dose-limiting side effect was leucopenia, with no other major toxicity being observed. One patient developed biventricular failure that was not clearly related to biantrazole administration. On the present schedule, the recommended dose of biantrazole is 24 mg/m^2. No response were seen in this patient population.

Introduction

Doxorubicin has established itself as a highly active cytotoxic agent, particularly against lymphoma and breast and lung cancer, but also against a wide range of malignancies [13]. Unfortunately, this drug is associated with long-term cardiotoxicity in addition to myelosuppression, alopecia and emesis. The cardiotoxicity is related to the total delivered dose [11], probably to the schedule employed [12], and to peak blood levels [1]. Many attempts have been made to develop a drug that retains the spectrum of activity of doxorubicin without expressing its cardiotoxic properties. The anthracenedione mitozantrone is capable of intercalating DNA and although it has distinctly less cardiotoxic potential than doxorubicin, it appears to show generally reduced clinical efficacy [8]. The paraquinone group in the doxorubicin molecule is capable of generating the reactive free-radical species that are strongly implicated in the drug's cardiotoxicity [7].

In an attempt to synthesise an active anti-neoplastic drug that would display activity comparable with that of doxorubicin but without paraquinone groups, the Warner-Lambert Company synthesised biantrazole, one of three leading compounds in a series of anthrapyrazoles [2, 9, 10]. In several model systems, biantrazole showed activity similar to that of doxorubicin and better than that of mitozantrone [6]. Toxicity revealed the LD_{10} (lethal dose for 10% of the animals tested) in mice to be 20 mg/kg on an i.v. bolus schedule, with myelosuppression being the major toxicity and with convulsions being observed in animals treated at superlethal doses [3], related to the peak level of the drug. Studies have demonstrated that biantrazole causes only minimal free-radical generation as compared with doxorubicin and that the former drug can inhibit free-radical generation induced by the latter [4]. The purpose of the present study was to determine the limiting dose of biantrazole given to patients on a weekly schedule, to document any resulting toxicity, to define the human pharmacokinetics of the drug and to monitor patients for signs of therapeutic response.

Patients and methods

Patients. Subjects between the ages of 15 and 70 years who were suffering from malignant disease for which no satisfactory treatment was available were eligible for study, providing that the following criteria were met. All patients were required to give informed consent to partici-

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Table 1. Patients' characteristics

Patients (*n*)	41
Sex:	
Men	19
Women	22
Age	23–69 (median, 59) years
ECOG performance status:	
0	10
1	24
2	6
Tumour type:	
Bowel	9
Ovary	7
Breast	7
Sarcoma	5
Renal	4
Non-small-cell lung cancer	4
Melanoma	3
Non-Hodgkin's lymphoma	13
Previous treatment:	
None	9
Radiotherapy only	5
Chemotherapy[a]	14
Chemo/radiotherapy	13

[a] 16 patients received ≥2 regimens

pate, must not have received chemotherapy during the previous 3 weeks (6 weeks for nitrosoureas and mitomycin C), had to have a life expectancy of at least 3 months and an ECOG (Eastern Cooperative Oncology Group) performance status of ≤2, and must not have had a history of pre-existing heart disease or moderate/severe hypertension or a history of seizures (unless they remained stable on anti-convulsant therapy for ≥6 months; Table 1). Patients had to have normal urea, electrolyte and creatinine levels, a haemoglobin value of >10 g/dl, a WBC of $>3 \times 10^9$/l and a platelet count of $>100 \times 10^{12}$/l. Initially, only patients with normal liver-function tests (with the exception of a raised alkaline phosphatase value) were considered to be eligible for study, but subjects with abnormal values were subsequently accepted unless they exhibited obvious biliary obstruction or deranged liver function.

Pre-treatment investigations included those necessary to establish eligibility, determination of plasma lactate dehydrogenase (LDH) activity, an ECG and a chest X-ray and appropriate scans or X-rays to establish disease measurability. Between courses of treatment, patients underwent weekly complete blood counts (CBC) and determinations of urea, creatinine, electrolyte and LDH levels as well as liver-function tests; these investigations were repeated together with a chest X-ray and an ECG before each treatment course and at the end of treatment.

Drug administration and dose escalation. Biantrazole was formulated as a lyophylised powder in glass vials. Doses were given once weekly for 3 consecutive weeks, followed by a 3-week interval during which patients were observed for toxicity, the next course being started at the beginning of week 7. The appropriate dose was reconstituted with water for injection and then given as an i.v. bolus over 5 min into the side of a freely running i.v. infusion of sodium chloride. The starting dose was 2 mg/m^2, with initial escalations being planned per three patients treated and guided pharmacokinetically by comparison of the AUC at each dose level against that at the LD_{10} in mice. In the presence of either marked inter-patient variations in pharmacokinetics or severe toxicity, a conventional dose escalation was to be performed in increments of 40% as long as no serious side effects were seen (WHO grade 1).

Pharmacokinetics sample collection. In consenting patients an i.v. cannula was inserted from which blood samples were obtained at the following time points around the time of the biantrazole bolus, commencing from time zero: 5, 10, 15, 30 and 60 min and 2, 4, 8, 12 and 24 h. In addition, at weeks 2 and 3 of biantrazole bolus injection, further samples were drawn at time zero and at 1 and 6 h to investigate possible drug accumulation from variable pharmacokinetics after repeated dosing. Serum was collected and frozen at –20° C, being subsequently thawed immediately prior to analysis. Urine was collected in plastic containers over a 6-h period for 24 h, the total volume of each collection was measured and a 25-ml sample was separated and stored at –20° C prior to analysis.

Drug analysis. Biantrazole concentrations in patients' plasma and urine were determined by high-performance liquid chromatography (HPLC) using solid-phase sample preparation according to the method of Graham et al. [5]. Briefly, the stationary phase consisted of a 15-cm-long stainless steel column with an inside diameter of 4.6 mm that was packed with Spherisorb C6 silica gel particles and a pre-column packed with the same material (supplied by Crawford Scientific, Strathaven, Scotland). The mobile phase consisted of a 1:1:8 (by vol.) mixture of acetonitrile: methanol: 0.25 M ammonium formate (adjusted to pH 3 with formic acid) eluting isocratically at a flow rate of 1 ml/min. Detection by visible absorption was carried out at a wavelength of 492 nm using the orange chromophore of biantrazole for high selectivity. Solid-phase sample preparation used 500 mg of Bound Elut C_2 silica gel packed into 2.4-ml reservoirs (supplied by Crawford Scientific, Strathaven, Scotland) and a ten-place manifold operating under negative pressure. The mini-columns were activated with 1 ml methanol, then washed with 1 ml water. Next, a 1–2 ml sample of plasma or urine was loaded onto the column and, after a wash with 2 column volumes of water, biantrazole was eluted in 2 ml methanol: concentrated hydrochloric acid (19:1, v:v), which was dried and then reconstituted in a small volume of mobile phase prior to HPLC. The efficiency of the extraction technique was 90% ± 7% over a wide range of drug concentrations encompassing those normally found in plasma samples. The limit of detection of the assay was 1 ng/ml in plasma or urine.

Pharmacokinetic analysis. Plasma drug concentration-time profiles were best fitted to a triexponential decline describing a three-compartment open pharmacokinetic model using the "ELSFIT" computer programme (L. Sheiner, University of California, San Francisco). By this method, plasma concentrations = $Ae^{-ta} + Be^{-tb} + Ce^{-tc}$, where A, B and C are constants and a, b and c are the elimination rate constants (K_{el}). AUC extrapolated from time zero to infinity ($AUC_{0-\infty}$), clearance (C) and elimination half-life ($t_{1/2}$) were calculated using the following formulae:

$$AUC_{0-\infty} = \frac{A}{a} + \frac{B}{b} + \frac{C}{c}$$

$$C = \frac{\text{Dose}}{\text{AUC}}$$

$$t_{1/2} = \frac{0.693}{K_{el}}$$

To identify metabolites of biantrazole in urine and plasma, a diode-array high-speed scanning HPLC spectrophometric detector was used.

Results

Clinical results

The reconstitution and administration of biantrazole was easy and no local inflammatory problems were encountered. Dose escalation based on pharmacokinetics was not possible due to the large inter-patient variability (see below); thus step-by-step escalation was dose based on traditional criteria (Table 2). The dose-limiting side effect was leucopenia, with toxicity of WHO grade 2 or greater being consistently seen at doses of ≥18 mg/m^2. Profound leucopenia was observed at doses of 32–36 mg/m^2, with

Table 2. Dose escalation and evaluable courses of biantrazole

Biantrazole dose (mg/m^2)	Number of evaluable courses (6-week cycles)	Patients (*n*)
2	3	3
4	4	3
6	3	2
8	12	7
10	4	2
12	7	3
15	6	5
18	4	4
24	5	5
32	11	6
36	3	3

Table 3. Haematological toxicity of biantrazole

Dose (mg/m^2)	Number of patients with leucopenia of WHO grade				
	0	1	2	3	4
2	3	0	0	0	0
4	3	0	0	0	0
6	1	1	0	0	0
8	4	2	1	0	0
10	1	0	1	0	0
12	1	2	0	0	0
15	1	2	2	0	0
18	0	0	4	0	0
24	0	1	2	3	0
32	0	0	0	1	5
36	0	0	0	1	2

Table 4. Patients with nausea and vomiting

Biantrazole dose	Episodes experienced
18 mg/m^2	2 × WHO grade 1
24 mg/m^2	1 × WHO grade 1, 2 × WHO grade 2
32 mg/m^2	3 × WHO grade 1, 4 × WHO grade 2, 1 × WHO grade 3
36 mg/m^2	1 × WHO grade 1, 1 × WHO grade 3

one infective episode occurring in a patient who received 36 mg/m^2 (Table 3). Two patients who had undergone previous chemotherapy developed thrombocytopenia: one had received a dose of 32 mg/m^2 (nadir, 52×10^9 platelets/l) and the other had been given 36 mg/m^2 (nadir, 58×10^9 platelets/l). Falls in haemoglobin were not thought to relate significantly to biantrazole treatment. Nausea and vomiting was generally absent or mild but was seen at the higher doses (Table 4).

Three patients complained of a metallic taste following doses of 2 and 15 mg/m^2. Alopecia was observed in seven subjects at doses of >18 mg/m^2; in five cases it manifested as WHO grade 1 and in 2 cases, as WHO grade 2. Mild elevations (WHO grade 1) in serum creatinine and urea levels in nine patients were not believed to be attributable to biantrazole administration. Abnormal liver-function tests above WHO grade 1 were observed only in patients with pre-existing hepatic metastases and were thought to be related to carcinoma. The one exception involved increases in gamma-glutamyl transferase levels (WHO grade 2) in six subjects, but these increases were again not clearly related to biantrazole.

One woman aged 51 years, who had renal carcinoma extensively metastatic to the lung and no prior history of cardiac disease, developed apparent left ventricular failure 6 weeks after completing one course of biantrazole at 8 mg/m^2. Her ECG, which was normal at presentation, revealed non-specific T-wave flattening. She responded well to diuretics but subsequently died. An autopsy was refused and we remain uncertain as to the potential role of biantrazole in the causation. No further cardiac or ECG abnormalities attributable to biantrazole were detected.

Pharmacokinetics

According to the hypothesis that mouse pharmacokinetics predict for human toxicity, an AUC of 277 μM × min would be expected to represent the maximal tolerated dose of biantrazole in man (derived from the AUC calculated for mice given the LD_{10}). Table 5 demonstrates the AUC observed at various doses, and a value of 277 μM × min

Table 5. Pharmacokinetics of biantrazole

Patient	Dose (mg/m^2)	PC[a] (μM)	AUC (μM × min)	*C* (ml min^{-1} m^{-2})	$t_{1/2}$(h)		
					a	b	c
1	4	3.84	43	218	0.1	2.37	–
2	6	4.99	125	112	0.15	1.07	29.9
3	6	2.32	33	428	0.07	0.27	8.5
4	8	5.2	68	276	0.10	0.71	19.8
5	8	13.66	96	196	0.05	0.32	9.4
6	8	4.13	71	264	0.03	0.20	7.1
7	12	23.34	168	167	0.05	0.22	8.2
8	12	16.54	162	174	0.04	0.25	16.1
9	24	33.0	399	130	0.05	0.51	8.1
10	24	31.2	151	343	0.02	0.16	7.1
11	36	44.2	508	154	0.02	0.3	23
12	36	50.2	410	191	0.02	0.2	9.8
13	36	15.1	381	205	0.14	1.04	21.7

[a] PC, Peak plasma concentration extrapolated back to time zero. All other pharmacokinetic parameters are explained in Materials and methods

Table 6. Mean plasma biantrazole concentrations at weeks 1–3

Dose (mg/m^2)	Patients (n)	1-h concentration (ng/ml)			6-h concentration (ng/ml)		
		Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
6	1	14	27	21	3	3.2	3.2
8	3	63	72	46	11	6.8	–
12	2	73	147	83	11.6	17.7	14
24	1	379	176	204	55	52	28
32	3	124	88	121	24	21	26
36	1	282	270	387	38	28	46

was achieved at doses of $\geqslant 24$ mg/m^2. Although large inter-patient variations were observed, there was a good correlation between dose and AUC ($r = 0.924$). Biantrazole has a long terminal half-life (7.1–29.9 h) after two phases of rapid distribution and in this respect is similar to doxorubicin [1].

Drug accumulation

On this weekly schedule there was no evidence of drug accumulation on the spot samples taken at 1 h and 6 h after biantrazole administration at weeks 2 or 3 (see Table 6), nor was there evidence of circulating metabolites.

Urinary excretion

Examination of 24-h urine samples from 2 patients revealed that 7.9%–15.7% of the delivered dose of biantrazole appeared in the urine as unchanged drug. There was no evidence of circulating metabolites, but two products were found that were more water-soluble than the parent drug.

Discussion

Biantrazole was well tolerated at most doses given on this schedule. Due to the large inter-patient variations in pharmacokinetics, we could not accurately use the AUC to target the limiting dose as was originally planned. The dose-limiting toxicity was myelosuppression, with leucopenia predominating. Biantrazole demonstrated linear kinetics over the dose range studied, displaying two phases of rapid distribution followed by a long terminal half-life. The excretion of unchanged drug in urine was higher than that seen with doxorubicin, and two water-soluble metabolites were observed in urine but not in plasma. Because there was little evidence of circulating metabolites, metabolism is not thought to be an important factor in the pharmacology of biantrazole. The drug concentrations measured on administration of the 2nd and 3rd weekly doses of biantrazole failed to demonstrate any drug accumulation between courses. The recommended dose of biantrazole for use on this schedule is 24 mg/m^2, which consistently resulted in the development of leucopenia of WHO grade 2–3 by week 3 of treatment in moderately pre-treated patients. Whether this weekly dose enables (a) the administration of a higher dose of biantrazole per unit of time and (b) less toxicity, as previously reported for low-dose doxorubicin [12] is unclear at present. In our group of pre-treated patients and in subjects with chemo-resistant tumour types, no responses to biantrazole were observed.

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