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Pharmacokinetics of 2,5-Diaziridinyl-3,6-bis(2-hydroxyethylamino)-1,4-benzoquinone (BZQ, NSC 224070) During a Phase I Clinical Trial

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Abstract—*Plasma levels of 2,5-diaziridinyl-3,6-bis(2-hydroxyethylamino)-1,4-benzoquinone (BZQ, NSC 224070) were measured in nine patients after i.v. administration of the drug during a Phase I trial. Our own isocratic high performance liquid chromatographic (HPLC) method with a sensitivity of 3 ng/ml was used to quantify BZQ. Patients receiving 18–60 mg BZQ i.v. showed α and β plasma decays with half-lives of 6.2 ± 1.5 (mean ± S.D.) and 24 ± 4 min respectively. The apparent volume of the central compartment was 12.2 ± 4.6 l, and the total volume of distribution was 33.6 ± 11.3 l. The calculated plasma AUCs were linearly related to dose. A marked similarity in kinetic parameters was found for BZQ and diaziquone (AZQ, NSC 182986), another diaziridinylbenzoquinone that has recently completed phase II clinical trials.*

INTRODUCTION

As PART of a systematic search for new central nervous system (CNS) antitumour agents, a rationally designed group of diaziridinylbenzoquinones have shown good activity against murine L1210 cells [1]. The compounds possess good lipid solubility and a low degree of ionization, both of which are claimed to be required for CNS penetration [1]. 2,5-Diaziridinyl-3,6-bis(carboethoxy-amino)-1,4-benzoquinone (AZQ, NSC 182986, Fig. 1) was eventually selected as the first of these compounds for clinical trial [2]. Studies *in vitro* have shown that the AZQ aziridinyl groups readily alkylate other molecules and its bifunctionality allows cross-linking of DNA [3]. However, the possibility of free radical formation of the quinone group is also important [4, 5] and the combination of this and the alkylating ability may be synergistically cytotoxic [6].

In the next series of diaziridinylbenzoquinones to be synthesized and tested 2,5-diaziridinyl-3,6-bis (2-hydroxyethylamino)-1,4-benzoquinone (BZQ, NSC 224070, Fig. 1) was identified as having good activity against intraperitoneal L1210 tumour. However the drug's activity in the intracranial models was not as good, probably due to its decreased lipophilicity [7].

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During Phase I and II clinical trials of AZQ some encouraging activity against brain tumours was noted [3]. This prompted the NCI to further investigate BZQ as a possible CNS antitumour agent, and the drug has recently completed a Phase I trial here in Bath, U.K. In this report we present pharmacokinetic data from nine patients, determined using our own sensitive HPLC method for the measurement of plasma concentrations of BZQ [8].

MATERIALS AND METHODS

Drugs and reagents

BZQ was synthesized by Dr D.E.V. Wilman of The Institute of Cancer Research (Sutton, U.K.), under the auspices of the Cancer Research Campaign Phase I/II Trials Committee. The drug was dissolved in 1.26% w/v sodium bicarbonate (Boots Hospital Products, Nottingham, U.K.) at 1 mg/ml (with ultrasonication) for maximum stability [9]. HPLC grade methanol was obtained from Rathburn Chemicals Ltd. (Walkerburn, U.K.).

Patients

All patients gave informed consent before undergoing the studies. The characteristics of each patient are shown in Table 1. Drug doses of 9.3, 13, 18 and 33 mg/m^2 were administered to five patients, the remaining four all received 25 mg/m^2.

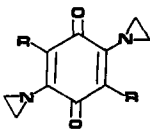

Fig. 1. Structures of AZQ (R = $-NHCO_2CH_2CH_3$) and BZQ (R = $NHCH_2CH_2OH$).

Investigation design

A baseline blood sample was taken before administration of the drug. BZQ was administered i.v. over 1–3 min via a butterfly needle pre and post flushed with sodium bicarbonate. Blood samples were taken at between 3 min and 3 h after injection, the first five samples being taken within 30 min. The blood, collected in heparinized tubes, was cooled on ice until centrifugation at 1720 ***g*** for 7.5 min at 4°C. The plasma thus obtained was stored on ice for analysis the same day.

Plasma analysis

BZQ levels in plasma were measured by our own HPLC method described elsewhere [8]. Briefly, BZQ was extracted from 3 ml of plasma onto an activated C18 solid phase extraction cartridge (Sep-Pak, Millipore, Harrow, U.K.). The cartridge was then washed with water (pH 8.5) and air dried. The drug was eluted from the cartridge with cold methanol. The eluate was then diluted with ammonium acetate (0.0525 M) and samples of this injected onto the HPLC column.

The HPLC system consisted of a Waters 600E pumping system and a Hewlett Packard 1040A diode array detector monitoring at 385 nm with a bandwidth of 20 nm. Samples were injected via a Rheodyne 7125 valve fitted with a 200 μl loop. A reversed phase HPLC column (Spherisorb ODS1 4.6 × 250 mm, 5 μm) maintained at 40°C by a column heating block was used with an eluting solvent of methanol–0.05 M ammonium acetate (40—60 v/v) at a flow rate of 1 ml/min.

Data analysis

Analysis of pharmacokinetic data was conducted using the STATGRAPHICS least squares non-linear regression analysis program on an IBM PC XT computer. The biexponential decline in plasma concentration of BZQ was fitted to the following equation

$$C = Ae^{-\alpha t} + Be^{\beta t} \quad (1)$$

where C is the concentration of BZQ in plasma at time t (min), A and B are intercepts measured in ng/ml, and α and β (min^{-1}) are the apparent first-order distribution rate constants. A weighting was applied to both sides of the equation by taking natural logs thus:

$$\ln C = \ln(Ae^{-\alpha t} + Be^{-\beta t}). \quad (2)$$

Plasma half-lives were calculated from the parameter estimates and the area under the curve (AUC) was calculated using the equation

$$\text{AUC} = \frac{A}{\alpha} + \frac{B}{\beta}. \quad (3)$$

The apparent volume of the central compartment V_c (l) and the apparent volume of distribution V_d (l) were calculated using equations (4) and (5) respectively:

$$V_c = \frac{10^3.\text{dose}}{A + B} \quad (4)$$

$$V_d = \frac{\text{dose}}{\text{AUC}.\beta}. \quad (5)$$

Total body clearance was calculated from the following relationship:

Table 1. Patient characterization

Patient No.	Age	Sex	Surface area	Diagnosis
1	52	M	1.94	Carcinoma of stomach
2	66	M	1.70	Carcinoma of liver
3	66	M	1.85	Myeloma
4	58	M	1.75	Carcinoma of kidney
5	61	F	1.30	Carcinoma of rectum
6	65	M	1.90	Chronic lymphocytic leukaemia
7	62	M	1.90	Large cell follicular lymphoma
8	67	F	1.40	Unknown primary
9	67	M	1.85	Carcinoma of stomach

$$C_b = \frac{10^3.\text{dose}}{\text{AUC}}. \quad (6)$$

The rate of elimination K_{elim} was calculated as:

$$K_{elim} = \frac{A + B}{10^3.\text{AUC}}. \quad (7)$$

RESULTS

Thirty-five patients were entered into the Phase I trial, and of these, nine agreed to allow blood sampling for the pharmacokinetic study. The average age of this study group was 63 and consisted of seven men and two women. With one patient (No. 5) we had technical problems with the analysis system leading to poor results. There was not enough plasma left to allow a repeat analysis, thus all mean pharmacokinetic parameters exclude the data from this patient.

Figure 2 shows a typical plasma decay curve for BZQ from one of the patients studied. The results of fitting the concentration versus time data to a biexponential equation for each patient are shown in Table 2. All data fitted the two compartment model well with good correlations ($r^2 > 0.995$).

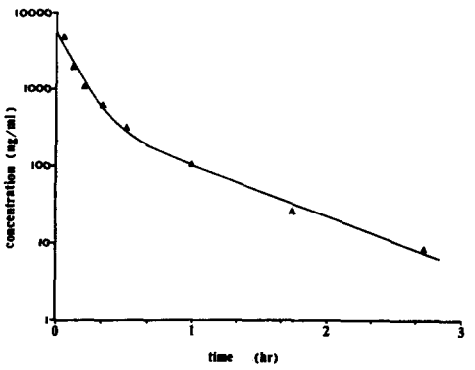

Fig. 2. Typical BZQ plasma decay curve (patient 7).

The α and β slopes for nine patients studied show a great similarity with mean values (± S.D.) of 0.117 ± 0.026 min^{-1} and 0.029 ± 0.005 min^{-1} respectively. Initial BZQ plasma concentrations of 0.7–5.1 μg/ml were measured immediately following injection. These fell to 0.1–0.9 μg/ml by 30 min and were below 20 ng/ml after 2 h.

Table 3 lists the calculated kinetic parameters for BZQ. We have observed rapid removal of the drug from plasma with mean values for $t_{1/2}\alpha$ and $t_{1/2}\beta$ of 6.2 ± 1.5 and 24 ± 4 min respectively. The mean value for K_{elim} was 0.08 ± 0.02 min^{-1} and was not affected by the drug dose.

Preliminary pharmacokinetic results on a small patient sample showed a possible non-linear relationship between AUC and dose [8], however this was not supported by our larger patient sample, the AUC being linearly related to dose of drug. The total body clearance averaged 541 ± 166 ml/min/m^2. The apparent volume of distribution for the eight patients averaged 33.6 ± 11.3 l.

No BZQ was detected in the 24 h urine samples from three patients. This may be attributable to the drug's poor aqueous stability at acid pH [9].

DISCUSSION

BZQ is the second of a group of rationally designed diaziridinylbenzoquinones to be entered into clinical trial. The first, AZQ, while structurally similar to BZQ, exhibits some very different physical properties. In Table 4 we see that the aqueous stability (pH 7, room temp.) of AZQ is much greater than BZQ with $t_{0.95}$ of 73 h [10] and 0.91 h [9] respectively. Aqueous solubility is also quite different with the more hydrophilic BZQ [7] showing approximately 10-fold greater solubility than AZQ

Table 2. Pharmacokinetic parameters of BZQ calculated from the plasma concentration versus time data

Patient No.	*A* (ng/ml)	*B* (ng/ml)	α (min^{-1})	β (min^{-1})	r^2
1	1076.89	43.83	0.0905	0.0245	0.998
2	1966.13	207.40	0.1219	0.0274	0.997
3	3600.80	532.37	0.1393	0.0306	0.998
4	2567.49	1673.24	0.0995	0.0356	0.999
5*	7000.63	399.80	0.0680	0.0170	0.999
6	1837.31	472.21	0.1105	0.0370	0.999
7	5501.30	446.17	0.1452	0.0254	0.995
8	3684.38	313.56	0.0794	0.0228	0.999
9	2802.13	1190.79	0.1480	0.0322	0.997
Mean†	2879.55	609.95	0.1168	0.0294	
± S.D.	1379.19	546.44	0.0260	0.0053	

*Data for this patient unreliable due to analytical problems.
†Excluding patient 5.

Table 3. Pharmacokinetic parameters of BZQ calculated from those listed in Table 2

Patient No.	Dose (mg)	Dose (mg/m²)	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (min)	AUC (min.μg/ml)	AUC/dose [min.μg/ml/(mg/m²)]	V_c (l)	V_{ss} (l)	K_{elim} (min^{-1})	Clearance (ml/min)	Clearance (ml/min/m²)
1	18	9	7.7	28	13.7	1.52	16.1	53.7	0.082	1314.2	677.4
2	22	13	5.7	25	23.7	1.82	10.1	33.9	0.092	927.8	545.8
3	33.3	18	5.0	23	43.2	2.40	8.1	25.2	0.096	770.5	416.5
4	43.8	25	7.0	19	72.8	2.91	10.3	16.9	0.058	601.4	343.7
5	32	25	10.2	41	126.6	5.06	4.3	14.9	0.059	252.8	194.5
6	48	25	6.3	19	29.4	1.18	20.8	44.1	0.079	1633.2	859.6
7	47.5	25	4.8	27	55.5	2.22	8.0	33.7	0.107	856.4	450.7
8	38	27	8.7	30	60.1	2.23	9.5	27.7	0.067	632.1	451.5
9	60	32	4.7	22	56.0	1.75	15.0	33.3	0.071	1071.8	579.4
Mean*			6.2	24	44.2	2.00	12.2	33.6	0.081	975.9	540.6
± S.D.			1.5	4	20.4	0.55	4.6	11.3	0.016	353.1	165.7

*Excluding patient 5.

Table 4. *Comparison of AZQ and BZQ physical properties*

	AZQ	BZQ
$t_{1/2}$ (h)*	73	0.91
Aqueous solubility (mg/ml)	0.2	2.0
Protein bound (%)	79	<2

*pH 7, room temp.

[10]. Ultrafiltration experiments have shown minimal protein binding (<2%) for BZQ [8] whereas in a single experiment with AZQ only 21 ± 1% was able to be filtered [11].

AZQ has recently completed both Phase I and II trials [3, 12, 13]. Comparison of pharmacokinetic parameters calculated for AZQ [13] and BZQ (Table 5) shows surprising similarities considering the marked differences in the physical properties discussed above. Both the drugs show rapid removal from plasma with α and β half-lives for AZQ of 4.8 and 31 min respectively and for BZQ a $t_{1/2}\alpha$ of 6.2 min and a $t_{1/2}\beta$ of 24 min. Total AUC/dose of 2.36 ± 1.00 (calculated from [13]) and 2.00 ± 0.55 μg.min/ml per mg/m^2 were found for AZQ and BZQ respectively. The total body clearance for each drug was also similar with 467 ± 57 ml/min/m^2 [13] for AZQ and 541 ± 166 ml/min/m^2 for BZQ.

One major difference found between AZQ and BZQ was in their apparent volumes of distribution with values of 277 ± 61 ml/kg [12] and 503 ± 158 ml/kg respectively. Although the AZQ V_d was calculated using the steady state method [13], comparison with the BZQ V_d calculated by the area method is said to be possible [14]. Plasma protein binding can restrict drug distribution and, as discussed above, AZQ shows much greater binding than BZQ. This may explain the almost two-fold increase in V_d found for BZQ over AZQ. However the increased hydrophilicity exhibited by BZQ should not be ignored as this may confer both minimal protein binding and increased distribution.

Table 5. *Comparison of AZQ and BZQ pharmacokinetic parameters*

	AZQ	BZQ
$t_{1/2}\alpha$ (min)	4.8 ± 1.4*	6.2 ± 1.5
$t_{1/2}\beta$ (min)	31 ± 4	24 ± 4
AUC (μg.min/ml)	2.36 ± 1.00	2.00 ± 0.55
Clearance (ml/min/m^2)	467 ± 57	541 ± 166
V_d (ml/kg)	277 ± 61†	503 ± 158‡

*Mean ± S.D.
†V_d (steady state).
‡V_d (area).

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