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# Clinical Pharmacology of 2,5′-Diaziridinyl-3,6-biscarboethoxyamino-1,4-benzoquinone (AZQ)*

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**Abstract**—*2,5′-Diaziridinyl-3,6-biscarboethoxyamino-1,4-benzoquinone (AZQ) is an alkylating compound which has exhibited a broad spectrum of antitumor activity against a variety of experimental tumors, particularly those implanted intracranially. We have studied the clinical pharmacology of AZQ in 11 patients with various types of tumors. AZQ was administered at 1–12 mg/m² daily for 5 days by i.v. infusion in 10–30 min. $^{14}$C-labelled AZQ was given on day 1 only. Blood and urine specimens were analyzed radiochemically and chromatographically. The plasma disappearance of unchanged AZQ was essentially biphasic, with an initial plasma $t_{1/2}$ of 1.4 ± 0.4 hr and a terminal $t_{1/2}$ of 45.5 ± 3.1 hr. The apparent volume of distribution was 14.2 ± 3.0 l/kg. The cumulative urinary excretion of unchanged AZQ was 4.3% in 24 hr and 8.6% in 96 hr. The total clearance of the drug was 200 ml/kg/hr. Cerebrospinal fluid AZQ concentration peaked 45–90 min after drug administration, reaching about 70% of that in plasma, and then declined at nearly the same rate.*

## INTRODUCTION

THE AZIRIDINYLBENZOQUINONES constitute a class of quinones that have exhibited significant activity against the intraperitoneally (i.p.) implanted L1210 lymphoid leukemia as well as other tumor models [1]. These compounds possess physiochemical properties associated with significant penetration into the central nervous system (CNS) [2], and subsequently many were found to be effective against the intracerebrally (i.c.) implanted L1210 leukemia [3]. A number of derivatives, including 2,5′-diaziridinyl-3,6-biscarboethoxyamino-1,4-benzoquinine (AZQ, NSC-182986) (Fig. 1), were synthesized by Driscoll *et al.* in an attempt to improve their water solubility while maintaining intracerebral antitumor activity [4]. A comparison of 31 aziridinylbenzoquinones in two i.c. and three i.p. implanted murine tumor systems showed that AZQ was the superior analogue [4]. On the basis of significant experimental antitumor activity and its ability to cross the blood–brain barrier, the agent was selected for clinical trial. In conjunction with our phase I clinical trial, we have studied the clinical pharmacology of AZQ in patients with various types of tumors.

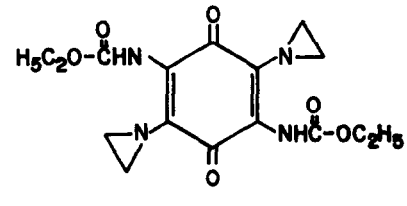


*Fig. 1. Structure of 2,5′-diaziridinyl-3,6-biscarboethoxyamino-1,4-benzoquinone (AZQ NSC-182986).*

## MATERIALS AND METHODS

### *Patient selection*

Informed consent was obtained from all patients in this study. The patient characteristics are shown in Table 1. Eleven patients receiving 100–200 μCi [$^{14}$C]-AZQ at doses ranging from 1 to 12 mg/m² were studied. Prior to initiation of AZQ therapy all patients had normal renal and liver function, and during the treatment with AZQ all other medications were suspended if possible.

AZQ concentrations were measured in plasma, urine and cerebrospinal fluid (CSF), radio-

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*Table 1. Patient characteristic*

| Patient No. | Sex | Tumor type | Site of metastasis |
|---|---|---|---|
| 1* | F | squamous cell carcinoma of esophagus | none |
| 2 | M | astrocytoma | none |
| 3 | M | astrocytoma | none |
| 4 | F | large cell carcinoma of lung | brain |
| 5* | F | adenocarcinoma of colon | liver |
| 6 | M | squamous cell carcinoma of mouth | none |
| 7 | M | malignant fibrohistiocytoma | lung |
| 8 | F | melanoma | lung |
| 9 | F | large cell undifferentiated carcinoma of lung | bone |
| 10 | F | astrocytoma | none |
| 11* | F | carcinoma of gall bladder | liver |

*Abnormal liver function.

chemically and chromatographically. Samples of venous blood were drawn from the opposite injection site into a 10-ml heparinized tube before drug administration and at predetermined times after completion of the infusion. The blood samples were placed on ice until centrifugation for 10 min at 12,000 *g* in a Sorall RC2-B centrifuge (Dupont Instrument, Wilmington, DL). The plasma was separated from the RBCs and kept frozen until analysis.

If possible 1 ml of CSF was obtained from patients with an indwelling Ommaya reservoir at a predetermined time. All samples were kept frozen.

Urine samples were collected at 6-hr intervals for 24 hr and at 24-hr intervals for 72 hr.

*Drug-formulation and administration*

Ring-labelled [$^{14}$C]-AZQ (sp. act. 8.8 mCi/mol) was supplied by the National Cancer Institute, Bethesda, MD. Its chemical and radiochemical purity was 95% as determined by radiochemical and chromatographic techniques. The drug was formulated in 0.5 ml of *N,N*-dimethylacetamide and 9.5 ml of sterile 0.01 M phosphate buffer, pH 6.5. The radioactive AZQ was then filtered through a 0.45-$\mu$m millipore filter and shown to be sterile and pyrogen-free. It was then combined with the non-labelled drug, further diluted in normal saline to a final concentration of 0.5 mg/ml and administered intravenously (i.v.) over 10–30 min on day 1 of the 5-day cycle. All drugs were freshly prepared before administration.

*Radiochemical technique*

Radioactivity was determined with a Packard Tricarb liquid scintillation spectrometer model 2650; quenching was corrected by the external standard channels ratio method; for $^{14}$C the counting efficiency was about 90%. Plasma or urine (0.2 ml) was counted in 11 ml of PCS, a commercial phase-combining counting solution available from Amersham, Arlington Heights, IL. Analyses were carried out with a Waters Associates model 204 chromatograph equipped with a $\mu$ Bondapak $C_{18}$ reverse-phase column (30 cm × 4.0 mm) eluted isocratically with 20% acetonitrile (v/v) in distilled water at a flow rate of 1.5 ml/min, with the u.v. monitor set at 341 nm. The output signals were recorded with a Brinkmann model 2544 strip-chart recorder. The location of unchanged AZQ was ascertained by running unlabelled AZQ through the column. The retention time of AZQ was 7 min. The eluent was collected at 1-min intervals for 30 fractions; each fraction was mixed with 15 ml of PCS and the total radioactivity determined. The recovery of the radioactivity was 90% or better.

*Computation of the results*

Non-linear regression analysis of the results was performed with the aid of the PROPHET program. Best fit was based on an open 2-compartment model.

## RESULTS AND DISCUSSION

In the present study the AZQ pharmacokinetic parameter was determined in eleven patients at drug doses ranging from 1.0–12.0 mg/m$^2$, as shown in Table 2. The plasma disappearance of AZQ could fit into a two-compartment open model for all patients. Figure 2 depicts a typical plasma disappearance curve in a patient (patient 7) who received 4 mg/m$^2$ of AZQ. The harmonic

*Table 2. Pharmacokinetic parameters of AZQ in patients*

| Pt | Dose mg/m² | $t_{½}$, hr Int. | $t_{½}$, hr Term | VD, l/kg | Cxt, ng/ml/hr | Cl, ml/kg/hr | U. Ex. % of dose 24 hr | U. Ex. % of dose 96 hr |
|---|---|---|---|---|---|---|---|---|
| 1 | 1.0 | 1.3 | 43.9 | 11.1 | 153.7 | 175.9 | 2.5 | 4.6 |
| 2 | 1.5 | 1.1 | 56.3 | 9.3 | 355.2 | 114.1 | 2.7 | NC† |
| 3 | 1.5 | 2.4 | 56.4 | 18.0 | 182.9 | 221.7 | 0.4 | NC |
| 4 | 2.0 | 1.9 | 43.6 | 10.3 | 329.6 | 164.0 | 3.9 | 8.2 |
| 5 | 2.0 | 1.7 | 49.5 | 13.5 | 285.6 | 189.3 | 4.4 | 7.4 |
| 6 | 4.0 | 1.9 | 64.8 | 41.9 | 241.1 | 448.4 | 1.3 | 16.6 |
| 7 | 4.0 | 1.7 | 29.7 | 8.8 | 523.8 | 206.4 | 9.1 | 9.6 |
| 8 | 6.0 | 5.1 | 55.5 | 11.7 | 1113.4 | 145.7 | 12.5 | 13.5 |
| 9 | 8.0 | 1.8 | 45.0 | 18.5 | 757.4 | 285.5 | 2.5 | 3.5 |
| 10 | 12.0 | 0.9 | 38.3 | 9.4 | 1900.4 | 170.7 | 3.2 | 6.8 |
| 11 | 12.0 | 0.8 | 38.9 | 4.2 | 4354.4 | 74.5 | 5.4 | 6.8 |
| Mean ± S.E. | | 1.4 ± 0.4* | 45.5 ± 3.1* | 14.2 ± 3.0 | | 199.6 ± 3.0 | 4.3 ± 1.0 | 8.6 ± 1.4 |

†NC, Not collected.
*Harmonic mean.

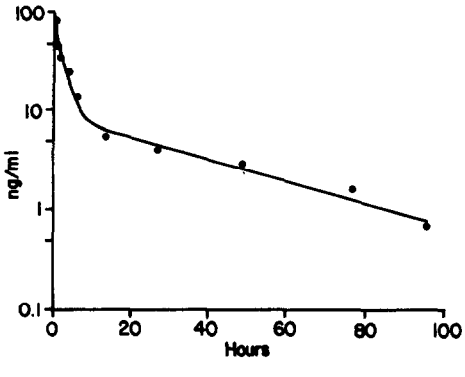


*Fig. 2. Plasma clearance of a patient after receiving 4 mg/m² of AZQ.*

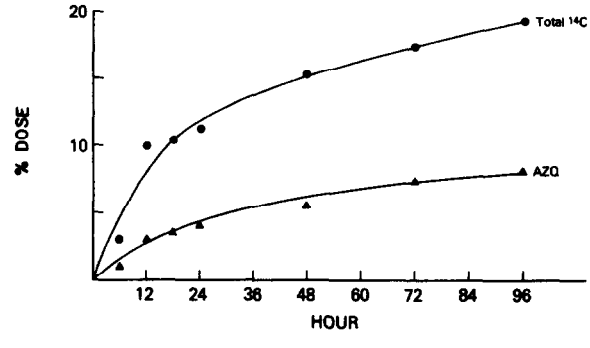


*Fig. 3. Cumulative urinary excretion of a patient after receiving 2 mg/m² of AZQ.*

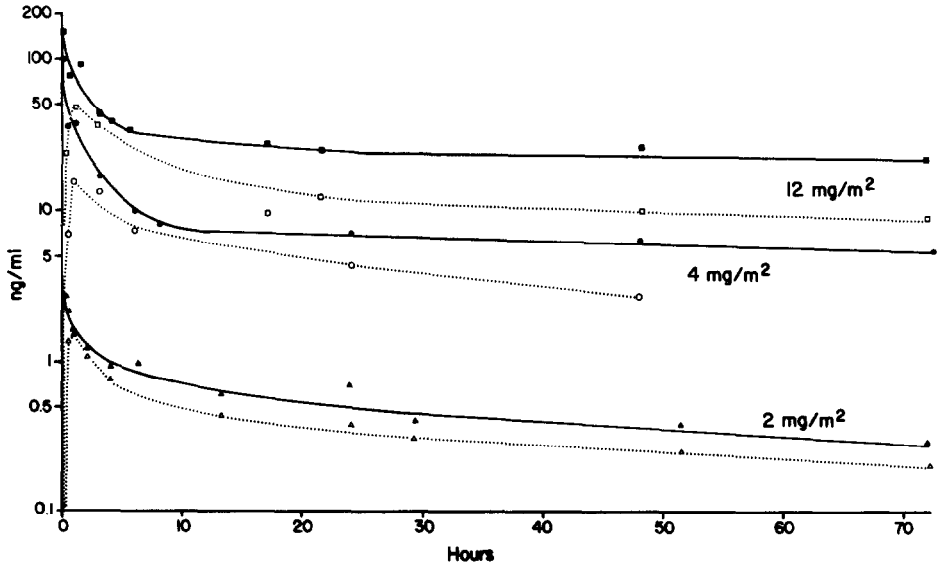


*Fig. 4. Plasma and CSF concentrations of patients after receiving AZQ. ———— Plasma; ———— CSF.*

means of the initial half-life was 1.4 ± 0.4 hr and the terminal half-life was 45.5 ± 3.1 hr. This persistence of AZQ in the plasma is most probably caused by its extensively binding to erythrocytes followed by slow release into the plasma. The clearance ranged from 75 to 448/ml/kg/hr, with a mean of 200 ± 3.0 ml/kg/hr, twice the renal creatinine clearance in humans. The apparent volume of distribution was 14.2 ± 3.0 l/kg, suggesting that AZQ is extensively taken up by tissues.

The average urinary excretion of unchanged AZQ was 4.3 ± 1.0% in 24 hr and 8.6 ± 1.4% in 96 hr. A typical urinary excretion curve was shown in Fig. 3. It clearly shows that AZQ was only responsible for one-fourth of the total excreted radioactivity. These results suggest that AZQ either underwent extensive metabolism or extensive spontaneous drug decomposition and very little of the administered dose remained as intact drug. There were at least two unidentified metabolites in the urine.

The ability of AZQ to penetrate into the CNS was confirmed in three patients with Ommaya reservoirs (Fig. 4). AZQ concentration peaked at 45–90 min after drug administration, reaching about 70% of the plasma drug concentration. It also persisted in the CSF at least 96 hr after drug administration. Previously we also demonstrated that AZQ is capable of penetrating into human brain tissue as well as intracerebral tumors [5]. Our results provide further support that AZQ is capable of crossing the brain capillaries with ease and thus may be a potentially useful agent for the treatment of brain tumors.

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