Cerebrospinal Fluid Pharmacokinetics of Intraventricular and Intravenous Aziridinylbenzoquinone

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ABSTRACT

The cerebrospinal fluid (CSF) pharmacokinetics of aziridinylbenzoquinone (AZQ) was studied following i.v. and intraventricular drug administration. Initial studies were performed in six rhesus monkeys with chronic indwelling Ommaya reservoirs. Following intraventricular administration of 0.2 mg of AZQ, elimination was monoeponential with half-lives of 32 and 39 min in ventricular and lumbar CSF, respectively. AZQ clearance (0.2 ml/min) was 5-fold greater than estimated CSF bulk flow, indicating that transcapillary passage and/or metabolism may be important clearance mechanisms for this drug. In spite of its rapid clearance from ventricular CSF, a substantial peak AZQ concentration was achieved in lumbar CSF (12 μM), which was 7 times higher than the peak ventricular CSF level (1.7 μM) achieved following i.v. AZQ administration (16 mg/sq m). Moreover, the mean area under the CSF concentration-time curve in ventricular CSF was 20-fold greater following intraventricular versus i.v. AZQ dosing, despite an 80-fold-lower dose. AZQ was not detectable in plasma (<0.06 μM) following intraventricular administration. No animals demonstrated clinical evidence of acute neurotoxicity.

Subsequently, intraventricular AZQ was administered to a patient with refractory meningeal leukemia. Intraventricular AZQ (0.5 mg) resulted in a peak ventricular (56 μM) CSF level which was 80-fold higher than ventricular CSF levels achieved following systemic AZQ administration of a dose of 24 mg/sq m in humans. Moreover, intraventricular AZQ yielded substantial CSF levels without detectable plasma concentrations. These data suggest that intraventricular administration of AZQ is feasible and may have pharmacological advantages over systemic administration for the treatment of meningeal neoplasia.

INTRODUCTION

AZQ is a new antitumor agent of the aziridinylbenzoquinone class which has a high degree of lipid solubility and was designed for enhanced penetration of the blood-brain barrier (4, 11). This agent is currently undergoing Phase II testing and has demonstrated antineoplastic activity in a variety of intracranial animal and human tumors (4, 5, 8, 9, 11). Following i.v. infusion, AZQ has been shown to enter the CNS to a significant degree over, the mean area under the CSF concentration-time curve in lumbar CSF (12 μM) was achieved following i.v. AZQ administration (16 mg/sq m). More recently, the mean area under the CSF concentration-time curve in ventricular CSF was 20-fold greater following intraventricular administration versus i.v. AZQ dosing, despite an 80-fold-lower dose. AZQ was not detectable in plasma (<0.06 μM) following intraventricular administration. No animals demonstrated clinical evidence of acute neurotoxicity.

Subsequently, intraventricular AZQ was administered to a patient with refractory meningeal leukemia. Intraventricular AZQ (0.5 mg) resulted in a peak ventricular (56 μM) CSF level which was 80-fold higher than ventricular CSF levels achieved following systemic AZQ administration of a dose of 24 mg/sq m in humans. Moreover, intraventricular AZQ yielded substantial CSF levels without detectable plasma concentrations. These data suggest that intraventricular administration of AZQ is feasible and may have pharmacological advantages over systemic administration for the treatment of meningeal neoplasia.

Materials and Methods

Animals. Adult male rhesus monkeys (Macaca mulatta) weighing 8 to 10 kg were obtained from the NIH Primate Center. Each animal was kept in a separate cage and fed Purina monkey chow and water ad libitum. A silicone Pudenz catheter was surgically placed into the fourth ventricle and attached to an Ommaya CSF reservoir implanted s.c. as described previously (16). This system permits repeated sampling of CSF in the unanesthetized state and has been shown to provide mixing of administered drugs with ventricular CSF (16). Lumbar CSF samples were obtained utilizing an indwelling lumbar catheter. After sterile preparation of the lumbar sacral region, an 18-gauge Hustead needle was inserted into the subarachnoid space at the L5-L6 intervertebral space. Polyethylene tubing (PE 50) was then threaded into the subarachnoid space to permit repeated sampling of CSF. To evaluate the possible neurotoxicity of intraventricular AZQ, 2 rhesus monkeys were given an intraventricular dose of 0.5 mg twice weekly for 1 month. In these animals, CSF was obtained twice weekly, and the animals were closely observed for clinical symptoms. Systemic toxicity was monitored on a weekly basis and included determination of plasma hemoglobin, platelet count, white blood count, blood urea nitrogen, creatinine, serum glutamic oxaloacetic acid transaminase, serum glutamic pyruvic transaminase, and total bilirubin.

Drug Formulation and Administration. AZQ was obtained from the Pharmaceutical Resources Branch, National Cancer Institute, and was supplied in 10-ml vials, each of which contained 10 mg of drug. This was initially dissolved in 0.5 ml of N,N-dimethylacetamide and then diluted with 9.5 ml of 0.01 M phosphate buffer, pH 6.5, to a final concentration of 1 mg/ml. For intraventricular administration in monkeys, 0.2 ml of the AZQ solution was further diluted to a final volume of 1.0 ml with sterile Elliott's B solution. This volume was then injected into the Ommaya reservoir, after which the reservoir was pumped 4 to 6 times to ensure adequate mixing throughout the ventricular system. For i.v. dosing, the AZQ drug solution was diluted to a final volume of 30 ml with 0.9% NaCl solution and administered over 15 min.

Assay and Sampling Times. Plasma and CSF samples were measured by high-pressure liquid chromatography using the method of Kelley and Chong (10) (lower limit of assay sensitivity, 0.06 μM). Ventricular CSF, lumbar CSF, and plasma samples were obtained immediately prior to and at 15 min, 30 min, 1 hr, 1.5 hr, 2 hr, 2.5 hr, 3 hr, 4 hr, and 5 hr following intraventricular AZQ administration (0.2 mg) to 6 rhesus monkeys. Plasma and ventricular CSF samples were obtained at identical time points following a 15-min i.v. infusion of AZQ (16 mg/sq m) to 3 rhesus monkeys. In one patient with refractory meningeal leukemia, a
dose of 0.5 mg of AZQ was administered via Ommaya reservoir after obtaining informed consent. Multiple CSF and plasma samples were obtained at regular intervals for 7 hr.

Pharmacokinetic Calculations. CSF disappearance curves following intraventricular AZQ administration were fit to a monoeponential function \( C = C_0 e^{-K_e t} \), using MLAB, a weighted, nonlinear, least-squares regression program (12). The elimination \( t_{1/2} \) was then calculated from 0.693/Kel. The AUC was determined by the linear trapezoidal rule up to the final measured CSF or plasma concentration \( C_{\text{meas}} \) and extrapolated to infinity by addition of the term: \( C_{\text{meas}}/\text{Kel} \). AZQ clearance from ventricular CSF following intraventricular administration was calculated by dividing the dose of AZQ administered by the ventricular CSF AUC. Following i.v. administration, the total-body clearance was calculated by dividing the dose of AZQ administered by the plasma AUC, and \( V_{\text{dss}} \) was calculated by the model-independent formula (14)

\[
V_{\text{dss}} = \frac{\text{Dose} \times AUMC}{(AUC)^2} \times \frac{T_{\text{dose}}}{2 \times AUC}
\]

where \( AUMC \) is area under the moment curve, and \( T \) is the infusion duration. The CSF:plasma ratio for systemic AZQ was calculated by dividing the \( AUC_{\text{CSF}} \) by the \( AUC_{\text{plasma}} \). The volume of distribution in CSF following intraventricular administration was calculated by dividing the dose administered by the extrapolated CSF AZQ concentration at time 0.

Plasma Protein Binding. Pooled, fresh rhesus monkey plasma was obtained, and AZQ was added to a concentration of 1 \( \mu \)g/ml. AZQ was then added to 0.2 M phosphate buffer, pH 7.4, to a concentration of 2.8 \( \mu \)M. Three ml of buffer and 3 ml of plasma were then added to separate Centrifi membrane cones (CF50A; Amicon) and centrifuged for 10 min. The concentration of AZQ was then determined in the protein-free filtrate. The percentage of unbound (free) AZQ was then determined by dividing the AZQ concentration in the plasma filtrate by the AZQ concentration in the buffer filtrate. Experiments were carried out in triplicate.

RESULTS

Following intraventricular administration of 0.2 mg of AZQ to 6 monkeys, the drug disappeared rapidly from ventricular CSF. As shown in Chart 1, although initial ventricular AZQ levels exceeded 56 \( \mu \)M, by 5 hr after dosing, AZQ levels were less than 0.2 \( \mu \)M. The major pharmacokinetic parameters of AZQ following intraventricular administration are listed in Table 1. AZQ was eliminated from ventricular CSF with a mean \( t_{1/2} \) of 32 min. The mean AZQ clearance from CSF was 0.2 ml/min. Despite its rapid clearance from ventricular CSF, substantial AZQ concentrations were achieved in lumbar CSF following intraventricular dosing with a mean peak AZQ level in the lumbar CSF of 12 \( \mu \)M, which occurred at a mean of 1.3 hr following AZQ administration. The mean ratio of the AUC of AZQ in lumbar CSF to the AUC in ventricular CSF was 0.38. The disappearance rate of AZQ from the lumbar CSF following intraventricular administration was rapid, paralleling that from ventricular CSF with a \( t_{1/2} \) of 39 min. In contrast to the high CSF levels achieved following intraventricular dosing of AZQ, the drug was not detectable in plasma within the limits of assay sensitivity (<0.06 \( \mu \)M).

No animal demonstrated evidence of acute neurotoxicity or systemic toxicity following intraventricular AZQ administration. In the 2 animals that received AZQ (0.5 mg) twice weekly for one month via the Ommaya reservoir, there was no evidence of neurotoxicity, myelosuppression, or renal or hepatic injury.

In order to evaluate the extent of CNS penetration of systematically administered AZQ, 3 monkeys each received a 15-min i.v. infusion of AZQ (16 mg/sq m). The concentration-time course of AZQ in CSF and plasma is shown in Chart 2. AZQ disappeared rapidly from plasma, falling from a mean peak plasma concentration of 39 \( \mu \)M to 0.1 \( \mu \)M in less than 3 hr with a mean terminal \( t_{1/2} \).

![Chart 1](image-url)

**Table 1.** Pharmacokinetics of intraventricular AZQ, 0.2 mg, in monkeys

<table>
<thead>
<tr>
<th>Monkey</th>
<th>( t_{1/2} ) (min)</th>
<th>( t_{1/2} ) (min)</th>
<th>( C_{\text{V}} ) (ng/ml)</th>
<th>( V_{\text{CSF}} ) (ml)</th>
<th>( C_{\text{meas}} ) (( \mu )M)</th>
<th>( AUC_{\text{V}} ) (m( \mu )M-min)</th>
<th>( AUC_{\text{L}} ) (m( \mu )M-min)</th>
<th>( \frac{AUC_{\text{L}}}{AUC_{\text{V}}} ) ratio</th>
<th>( T_{\text{peak}} ) (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>804-F</td>
<td>32.0</td>
<td>27</td>
<td>0.125</td>
<td>5.7</td>
<td>14</td>
<td>4.4</td>
<td>1.1</td>
<td>0.25</td>
<td>1.0</td>
</tr>
<tr>
<td>470-J</td>
<td>30.3</td>
<td>18</td>
<td>0.200</td>
<td>11.8</td>
<td>31</td>
<td>2.8</td>
<td>1.6</td>
<td>0.58</td>
<td>0.5</td>
</tr>
<tr>
<td>255-G</td>
<td>35.7</td>
<td>71</td>
<td>0.530</td>
<td>20.0</td>
<td>11</td>
<td>1.1</td>
<td>0.9</td>
<td>0.89</td>
<td>0.5</td>
</tr>
<tr>
<td>793-F</td>
<td>35.0</td>
<td>18</td>
<td>0.095</td>
<td>5.8</td>
<td>1.7</td>
<td>6.2</td>
<td>0.2</td>
<td>0.04</td>
<td>3.0</td>
</tr>
<tr>
<td>928-E</td>
<td>31.0</td>
<td>48</td>
<td>0.089</td>
<td>4.3</td>
<td>5.0</td>
<td>3.8</td>
<td>0.5</td>
<td>0.13</td>
<td>1.5</td>
</tr>
<tr>
<td>802-F</td>
<td>29.0</td>
<td>30</td>
<td>0.150</td>
<td>6.4</td>
<td>12</td>
<td>4.0</td>
<td>0.8</td>
<td>0.38 ± 0.15</td>
<td>1.3 ± 0.04</td>
</tr>
</tbody>
</table>

\( t_{1/2} \), elimination half-life in ventricular CSF; \( t_{1/2} \), elimination half-life in lumbar CSF; \( C_{\text{V}} \), clearance in ventricular CSF; \( V_{\text{CSF}} \), CSF volume of distribution; \( C_{\text{meas}} \), peak lumbar concentration; \( AUC_{\text{V}} \), ventricular AUC; \( AUC_{\text{L}} \), lumbar AUC; \( T_{\text{peak}} \), time to peak concentration.

Mean ± S.E.
of 36 min. The major pharmacokinetic parameter values for i.v. AZQ are listed in Table 2. The mean clearance of AZQ from plasma was 319 ml/min/sq m (26 ml/min/kg), and the mean Vdss was 1.1 liter/kg. The mean peak AZQ level in CSF following i.v. dosing was 1.7 μM. The ratio of the mean AZQ AUC in CSF to the mean AUC in plasma was 1.27. As shown in Chart 3, there was a marked difference in the CSF AZQ levels achieved following intraventricular versus systemic AZQ administration. The AUC in ventricular CSF was 20 times greater and in lumbar CSF over 4 times greater than the AUC of AZQ in CSF following intraventricular compared with i.v. administration of AZQ. Plasma protein binding studies revealed that AZQ was 18% protein bound.

In one patient with refractory meningeal leukemia, AZQ (0.5 mg) was administered via an Ommaya reservoir. As shown in Chart 4, initial ventricular CSF levels of AZQ exceeded 56 μM. The terminal t1/2 of AZQ in ventricular CSF was 1.65 hr. A lumbar CSF sample obtained 2 hr after AZQ administration demonstrated an AZQ level which exceeded 1.4 μM.

**DISCUSSION**

In the present study, we have demonstrated that direct intraventricular administration of AZQ is feasible and has a number of pharmacological advantages. Intraventricular AZQ administration would provide an advantage in treating meningeal neoplasia by producing high drug concentrations in the CSF bathing the meninges. In addition, systemic exposure to AZQ would be greatly reduced, since much smaller doses would be required to achieve high CSF levels with i.t. administration. This would minimize the likelihood of severe bone marrow toxicity, a side effect that has been reported in the clinical trials of systemic AZQ (5, 7, 15).

This study demonstrated that AZQ had a short t1/2 and was cleared rapidly from CSF following intraventricular administration. The clearance rate of AZQ from CSF, 0.2 ml/min, exceeded the rate of CSF bulk flow in the rhesus monkey by approximately 5-fold (13), suggesting that the clearance of AZQ results from not only bulk flow of entrained drug but also transcapillary passage through the CSF barrier.
of drug. It is possible that metabolism or chemical degradation contributes to AZQ clearance from CSF. However, extensive metabolism in the CSF is not likely, since the CSF:plasma ratio is very high following systemic administration of AZQ. If CNS metabolism were extensive, less drug would be detectable in the CSF.

Despite rapid clearance of AZQ from CSF following intraventricular administration, high concentrations of drug were achieved in lumbar CSF as manifested by a ratio of AUC in lumbar CSF to AUC in ventricular CSF of 0.38. This suggests that, following intraventricular AZQ administration, distal sites, such as the lumbar region, are exposed to substantial concentrations of AZQ. There was substantial variability in the amount of AZQ reaching the lumbar region following intraventricular dosing, as manifested by the ratio of the AUC in lumbar CSF to the AUC in ventricular CSF ranging from 0.04 to 0.89. The AUCs of AZQ in ventricular CSF and lumbar CSF were 20-fold and 4-fold higher, respectively, following intraventricular compared to i.v. administration of AZQ. These differences were particularly striking, since the dose of AZQ given intraventricularly was only 2% of the dose administered i.v., which indicates that there is a 1000-fold advantage for intraventricular compared with i.v. administration of AZQ.

In this study, it was also observed that, in the doses utilized, AZQ could be administered intraventricularly to monkeys without evidence of neurotoxicity or systemic toxicity.

Administration of AZQ i.v. resulted in a higher CSF:plasma ratio and volume of distribution in monkeys than had been reported previously in humans (7). This may be due to the lower plasma protein binding of AZQ in monkeys (18%) than that reported in humans (79%) (1), which would permit AZQ to more readily diffuse out of the intravascular space.

The pharmacokinetics of intraventricular AZQ was also examined in a single patient, and the results were similar to those obtained in rhesus monkeys. With a dose of only 0.5 mg, ventricular and lumbar CSF AZQ levels were higher than those reported previously following i.v. administration of much larger doses of AZQ (1). The AUC in ventricular CSF following intraventricular AZQ was approximately 35 times greater than that obtained in patients given i.v. AZQ (1). It is interesting to note that this patient achieved a partial (>90% reduction in CSF blast count) response to intraventricular AZQ.

In summary, the results of this study indicate that there is a pharmacological advantage for intraventricular administration of AZQ. Although it is probable that intraventricular therapy may not be as appropriate as systemic administration to treat deep parenchymal disease (2, 3), intraventricular administration of AZQ may be of value in the treatment of meningeal neoplasia. A Phase I–II study utilizing this approach is currently in progress.

REFERENCES


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