Subconjunctival Versus Intravenous Cytosine Arabinoside: Effect of Route of Administration and Ocular Toxicity

Jack Rootman,* Gedy Gudauskas,† and Chris Kumi‡

The ocular penetration, elimination, and toxicity of equidose cytosine arabinoside (Ara-C) (37.5 mg/kg) following subconjunctival versus intravenous injection were compared in rabbits. Drug levels were measured at 0.5, 1, 2, 4, 8, and 12 hours in serum, urine, and ocular compartments. Following subconjunctival administration, the peak concentration of Ara-C in the anterior chamber was 15 times and in the vitreous twice that obtained following intravenous administration. Drug levels obtained were considered high enough to be effective in inhibiting the growth of selected tumor lines. The cumulative urinary excretion over 12 hours was 67% and 70% for the subconjunctivally and intravenously injected animals, respectively, and peak serum levels were 0.061 mM and 0.170 mM, respectively. Clinical and histologic toxicity following subconjunctival injection was minimal, and no bone marrow suppression was detected at the dose level administered.


Cytosine arabinoside (Ara-C, Cytarabine, 1-B-arabinofuranosylcytosine) is a pyrimidine analogue that has proved to be effective in the treatment of acute leukemias and lymphomas in humans.1–3 In addition, it is an effective antiviral for the treatment of herpes simplex keratitis.4,5 Ara-C inhibits DNA biosynthesis at the polymerase level.6,7 It can be given intravenously, intrathecally, and topically to the eye.4,8,9 When administered systemically, dose-dependent bone marrow suppression occurs.10,11 Also, a dose-related corneal toxicity (punctate keratitis) has been noted both after repeated topical application and high-dose systemic administration in the rabbit and humans.12–14 These local effects are reversible when therapy is discontinued.12–14 We have studied the effect of route of administration on ocular absorption following local and systemic administration of Ara-C.

Materials and Methods

Ocular Absorption

Locally supplied, New Zealand white female rabbits weighing between 2.20 ± 0.03 kg (SD) were tranquilized by im injection of ketamine: acepromazine (100 mg/ml) (10:1), approximately 0.2 ml/kg per half hour. Normal saline was infused into the left marginal ear vein at a rate of 30 to 40 ml/hour for one hour to establish urine outflow prior to drug administration. Ara-C14 labeled in the number 2 position was obtained from Moravek Biochemicals (City of Industry, CA). This was checked for purity using a high pressure liquid chromatograph (HPLC) system and was found to be 98% pure. Commercial Ara-C was obtained from the Upjohn Company of Canada Ltd. (Don Mills, Ontario, Canada). Ara-U was obtained from the National Cancer Institute (Bethesda, MD). All other chemicals used were of reagent grade and were purchased from the Fisher Scientific Company (Vancouver, B.C.).

Unlabeled drug was mixed with Ara-C14 specific activity (55 mCi/mMol) in saline to achieve a radioactivity of approximately 60 × 106 dpm per 150 mg/ml of solution. The resulting solution was clear, thus indicating solubility at this concentration. A dose of 0.5 ml of this solution (equal to 75 mg and 30 × 106 dpm) was administered either by subconjunctival or by intravenous routes. The subconjunctival injection was given posterior to the superior limbus in the right eye using a 30-gauge needle raising a similar volume bleb for each experimental animal. The intravenous injection was made into the left marginal vein. One-milliliter blood samples were drawn into heparinized syringes from the medial artery, placed in micro-test tubes and centrifuged for 5 minutes. The plasma was collected by decantation and frozen at –4°C. Urine was drained continuously into disposable containers through an 8-gauge Foley pediatric catheter and volumes at 0.5, 1, 2, 3, 4, 8, and 12 hours were recorded. Samples (1.5
Table 1. Peak concentration of drug (Ara-C) in ocular compartment, urine, and serum (mM)

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Subconjunctival</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAC</td>
<td>1.305 ± 0.092 mM</td>
<td>0.090 ± 0.014 mM</td>
</tr>
<tr>
<td>LAC</td>
<td>0.053 ± 0.003 mM</td>
<td></td>
</tr>
<tr>
<td>RV</td>
<td>0.082 ± 0.011 mM</td>
<td>0.044 ± 0.002 mM</td>
</tr>
<tr>
<td>LV</td>
<td>0.026 ± 0.0004 mM</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>0.061 mM</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>25.19 mM</td>
<td>8.9 mM</td>
</tr>
</tbody>
</table>

ml) were centrifuged for 5 minutes, frozen, and stored. For both iv and subconjunctival experiments, three rabbits were sacrificed at 0.5, 1, 2, 4, 8, and 12 hours. Immediately after death, the rabbits' eyes were proptosed, the corneas rinsed with tap water, blotted dry, and an anterior chamber paracentesis performed through the inferior limbus. A volume of about 0.1 ml was obtained, placed in micro-test tube, and frozen.

After paracentesis, each eye was enucleated and the adherent episclera cleaned off and discarded. The globe was rinsed with tap water, blotted dry, incised with scissors, and the vitreous expressed. The vitreous was disrupted with a sonicator using a microtip for 30 seconds, centrifuged for 5 minutes, and the supernatant collected and frozen.

With the exception of the anterior chamber samples, all other samples, urine, vitreous, and serum were prepared by extracting them in 12% trichloroacetic acid (1 part sample: 3 parts trichloroacetic acid) in order to deproteinize them. Aliquots (100 µl) of the resulting supernatant solution were used for our assays except for anterior chamber samples where 50 µl aliquots were used.

The Radio High-Pressure Liquid Chromatograph, (Radio-HPLC) Analysis.

In order to trace the metabolic fate of Ara-C, we analyzed samples from serum, urine, and ocular compartments at all time periods on the HPLC. This was carried out using a Beckman® 110A pump/Rheodyne 7120 injector connected to a Partisil 10/25 ODS column (Reverse Phase), Whatman Industries; precolumn was packed with 10 µl pellicular ODS material. The solvent system was ammonium formate buffer 0.01 M, pH = 4.8. Flow rate was 2 ml per minute and column temperature was ambient. One hundred microliters of prepared samples were injected onto the HPLC column and peaks eluting from the system were monitored by means of an U.V. detector—Hitachi Spectrophotometer Model 100-40 at 254 nm.

The HPLC system was sensitive enough to detect one nanogram Ara-C or Ara-U. In each sample we found only one peak corresponding to Ara-C. Ara-U was not detected, probably because of the lack of cytidine deaminase activity in the rabbit.15

Liquid Scintillation Counting

As a result of being able to account for all the radioactivity in the form of Ara-C, we decided to use the Searle® Mark III Liquid Scintillation Counter using a wide 14C window and 10 ml PSC (Phase Combining System) Amersham/Searle as a scintillation cocktail to determine the concentration of Ara-C. The corrected disintegrations per minute were expressed in mM.

Ocular Toxicity Studies: Clinical and Histopathological

Five rabbits (2.2 kg) were injected subconjunctivally with 37.5 mg/kg Ara-C in 0.5 ml normal saline, pH 6.7, posterior to the superior limbus on the right eyes. The left eyes were injected with normal saline, pH 7.2, and served as controls. All animals were examined clinically with a slit lamp and fluorescein throughout the experiment and eyes were photographed serially.
The rabbits were sacrificed at 2, 4, 12, 24, and one week, respectively. The eyes were enucleated, cleansed of blood and adherent episclera, marked at the point of injection on the superior sclera to maintain geographical orientation, embedded in paraffin, sectioned, and stained with hematoxylin-eosin for histologic examination.

Bone Marrow Toxicity Studies

Two groups of two rabbits each (2.2 kg) were injected subconjunctivally and intravenously with 37.5 mg/kg Ara-C in 0.5 ml normal saline. Peripheral white blood cells were monitored on the first, third, and seventh day after injection to determine the extent of bone marrow toxicity.

Results

Subconjunctival Experiments (Table 1; Figs. 1-3)

The anterior chamber attained an average peak concentration of 1.305 ± 0.092 mM at 1 hour decaying to 0.145 ± 0.04 mM at 12 hours. The left anterior chamber reached a peak of 0.053 ± 0.003 mM at 4 hours and leveled off to 0.035 ± 0.006 mM at 12 hours. The right vitreous reached a peak of 0.082 ± 0.011 mM at 1 hour and decayed to 0.044 ± 0.006 mM at 12 hours. The left vitreous reached a peak of 0.026 ± 0.0004 mM at 8 hours. The average peak serum level reached with subconjunctival injections was 0.061 mM at 1 hour. The average peak urine concentration was 8.9 mM at 2 hours.

Intravenous Experiments (Table 1; Figs. 1-3)

In a paired t-test, no significant difference was found between values for the right and left ocular compartments; therefore, the data were pooled (P = 0.05). The peak concentration of drug in the anterior chamber was 0.090 ± 0.014 mM at 1 hour and diminished to 0.019 ± 0.002 mM at 12 hours. The peak concentration in the vitreous was 0.044 ± 0.002 mM at 4 hours. This leveled off to 0.023 ± 0.001 mM at 12 hours. The highest average drug level measured in the vascular compartment was at 0.5 hour (0.170 mM) and decreased to 0.009 mM at 12 hours. The peak urine concentration was 25.19 mM at 1 hour. Bioavailabilities of Ara-C in the ocular compartments were cal-

![Graph 1](image1)

Fig. 1. Concentration of Ara-C in serum and urine following intravenous and subconjunctival administration. *Vertical bars indicate range of the standard error of the mean, and the central point represents the mean.

![Graph 2](image2)

Fig. 2. Concentration of Ara-C entering the vitreous following intravenous and subconjunctival administration. *Vertical bars indicate range of the standard error of the mean, and the central point represents the mean.

![Graph 3](image3)

Fig. 3. Concentration of Ara-C in serum and urine following intravenous and subconjunctival administration. *Vertical bars indicate range of the standard error of the mean, and the central point represents the mean.
Table 2. Bioavailabilities of drug in ocular compartments (μg·hr)

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Subconjunctival</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAC</td>
<td>254</td>
<td>30.4</td>
</tr>
<tr>
<td>LAC</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>RV</td>
<td>206</td>
<td>130</td>
</tr>
<tr>
<td>LV</td>
<td>91</td>
<td></td>
</tr>
</tbody>
</table>

calculated using the Area Under the Curve rule (See Table 2).

Ocular Toxicity Studies: Clinical

Using 37.5 mg/kg, response to injections was mild in all animals throughout the experiment. At 1–2 hours slight conjunctival injections were noted on the bleb. By 12 hours the bleb was flat, leaving a slight conjunctival hyperemia around the superior limbus. By 24 hours no local side effect was visible. All other ocular tissue appeared normal. The control left eye injected with saline produced a small bleb that had flattened by 4 hours.

Histopathologic Findings

The eyes injected with Ara-C showed a sparse subconjunctival infiltrate of polymorphonuclear cells and lymphocytes that increased over the first 12 hours and diminished thereafter. The cornea, anterior chamber, iris, and ciliary body remained normal throughout. Focal areas of conjunctival erosion were noted at 12 hours and 24 hours, with normal lining thereafter. There was slight subconjunctival infiltrate of polymorphonuclear cells noted at 12 hours in the control left eyes. This had diminished by 24 hours. All other ocular tissue appeared normal.

The control left eye injected with saline showed a sparse subconjunctival infiltrate of polymorphonuclear cells at 12 hours. The conjunctiva was swollen but this had disappeared by 12 hours.

Bone Marrow Toxicity

There were no clinical signs of bone marrow toxicity as monitored by peripheral counts of leukocytes over one week. The average leukocyte count before the subconjunctival injection was 6,700; it was 6,300 on the third day and 7,400 on the seventh day after injection. For the intravenous injections, the average leukocyte count was 4,800 before the injection, 5,300 on the third day, and 5,200 on the seventh day.

Discussion

The peak drug concentration and bioavailability in the anterior chamber of the subconjunctivally injected eye was 14.5 and 8.7 times, respectively, that obtained following intravenous injection. Similarly, the peak and bioavailability obtained in the vitreous were 2 and 1.6 times as much when given subconjunctivally versus intravenously. In humans, following systemic administration, Ara-C is metabolized to Ara-U (1-B-D-ara- binofuranosyluracil), a therapeutically inactive metabolite via a reaction catalyzed by cytidine deaminase in liver and kidney. Our study and others suggest this reaction is limited in the rabbit. It can be assumed, therefore, that in humans, the ocular absorption would be even less than noted in the rabbit following systemic administration. Further, paraocular administration leads to direct absorption thus circumvents cytidine deaminase.

Barza et al in studying the ocular penetration of gentamicin have suggested that subconjunctivally administered antibiotic penetrates the anterior segments more readily than systemically administered drug. Our study supports this contention. Further, they indicate that in the case of gentamicin administered to an uninflamed eye, the penetration into the vitreous is not substantially different when comparing these routes. In contrast, we have shown that Ara-C penetrates the vitreous, both following intravenous and subconjunctival administration, and with subconjunctival administration, the bioavailability in the vitreous was twice that seen with the intravenous administration.

The serum pharmacokinetics of Ara-C following subconjunctival versus iv administration differs insofar as the serum curve reflects distribution and elimination while the subconjunctival curve includes an absorption phase (Fig. 3). Ocular absorption following intravenous administration would reflect the different pharmacokinetics. When comparing the peak vitreous time and concentration in the subconjunctivally injected eye and contralateral eye, we note an earlier and higher level in the vitreous following subconjunctival administration (Fig. 2). We note the same when comparing anterior chamber concentration following subconjunctival versus iv administration. The differing pharmacokinetics, in this study and previous ones that we have published, in our opinion reflect a contribution from direct local absorption following subconjunctival injection, although there may indeed be some contribution to the vitreous component from systemic absorption.

Some of the observed differences may be a result of the physiochemical properties of the compounds. The
Relative paucity of gentamicin absorption versus that of Ara-C could be related to the larger molecular size and different charge distribution at physiologic pH of gentamicin.

The suggested level of Ara-C necessary for cell kill is dependent on cell line, concentration of drug, and duration of exposure. For example, the minimum cytototoxic concentration of Ara-C is between 10 and 36 micromolar for L-5178Y, L-cells, and L1210 for an exposure up to two hours.22 Longer exposures (up to 24 hours) require cytotoxic concentrations of 0.3 to 3.6 micromolar for L-1210, V-794, HeLa, L-5178Y and L-cells.22 Our study suggests that following subconjunctival administration, the anterior chamber concentrations were at least 36 times those necessary for cell kill within two hours for selected tumor lines. Further, the concentration achieved in the vitreous was 2.3 times that required for cell kill. This contrasts to 2.5 times and 1.2 times the concentration necessary for cell kill in the anterior chamber and vitreous, respectively, following equidose intravenous administration. As noted, in humans, the concentration of active Ara-C following systemic administration would be negligible, since in humans Ara-C is metabolized rapidly and almost completely (within 5–15 minutes following injection of a single high dose intravenously) to Ara-U, an inactive metabolite.§ Thus, our study supports the contention previously suggested by ourselves that local administration of antineoplastics would afford the opportunity to use lower levels of drugs and may circumvent the metabolic pathways of systemic administration.20,21,23

Key words: ocular penetration, cytosine arabinoside, subconjunctival, intravenous, rabbits, ocular toxicity

References