Direct delivery of anticancer agents: experimental treatment of intraocular malignancy

Hsiao S. Liu, Miguel F. Refojo, Henry D. Perry, and Daniel M. Albert

Two anticancer agents, one lipophilic, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), and one hydrophilic, 5-(3,3-dimethyl-1-triazeno) imidazol-4-carboxamide (DTIC), were used to treat Brown-Pearce epithelioma in the anterior chamber of rabbit eyes. The BCNU test animals were divided into three groups: one treated by direct injection of the drug into the subconjunctival space or the anterior chamber, the second by both direct injection and intravenous administration, and the third by intravenous injection alone. The DTIC test animals were treated with only local injection into the subconjunctival space or anterior chamber. Dosage, delivery system, and effectiveness were compared following clinical observation and histopathologic examination. Direct delivery of BCNU or DTIC in subconjunctival space or anterior chamber delayed the growth of Brown-Pearce epithelioma in rabbit eye. The effectiveness of this treatment was significantly enhanced by combining direct injection with systemic administration of a lower dose of BCNU.

Key words: direct delivery, BCNU, DTIC, Brown-Pearce epithelioma in rabbit eye, intraocular malignancy, combined treatment, anticancer agents

In recent years, significant advances have been made in anticancer chemotherapy; among these has been the introduction of nitrosourea compounds such as the lipophilic drug 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and the hydrophilic drug 5-(3,3-dimethyl-1-triazeno) imidazol-4-carboxamide (DTIC). Ideally anticancer agents should be delivered directly to the tumor so that a therapeutic dose to the neoplasm can be achieved without systemic toxicity. This concept is in large measure utilized with radiotherapy such as insertion of radon seeds into the tumor itself or the suture of a radon shell to the sclera adjacent to the tumor. The object of the experiments reported here is the determination of a therapeutic intraocular level of directly delivered chemotherapeutic agents, one lipophilic and the other hydrophilic, that will control the growth of intraocular tumor. That such treatment may be practical is suggested by the successful control of severe intraocular infections by direct injection of antibiotics.

Our previous studies and Wilczek's report indicate that repeated periocular injections of DTIC in limited dosages produced no clinical or histological changes; this is also true for BCNU (our unpublished data).
Fig. 1. O.U., 14 days following tumor implantation. Top, O.D., treated with BCNU by repeated subconjunctival injections; O.S., control. Lower left, O.D., closeup view. Normal size and shape retained. Lower right, O.S., closeup view. Distortion is caused by tumor expansion. Arrow points to corneal opacity.

Higher concentrations of these drugs in aqueous humor and vitreous can be achieved with periocular administration than with intravenous injections of the same dosage. In the present experiments three modes of chemotherapy were studied both singly and in combination: (1) injection directly into the anterior chamber of the eye, (2) subconjunctival injection, and (3) intravenous injection. The tumor model employed was the Brown-Pearce epithelioma growing within the rabbit anterior chamber. We selected this model for our initial experiments because Brown-Pearce epithelioma grows in a predictable manner in the rabbit eye and because the eye is of a more suitable size for the required manipulation than are eyes of smaller animals.

Materials and methods

Preoperative ocular examination of 120 eyes in 60 New Zealand female albino rabbits, weighing 2.0 to 2.5 kg, included biomicroscopy, tonometry, and indirect ophthalmoscopy. All animals were found to have normal eyes. Both eyes of each animal were inoculated with approximately 6 x 10^5 cells of Brown-Pearce epithelioma tissue (previously grown in a rabbit eye), which was obtained by emulsification of about 1 mm^3 of tumor tissue in the culture medium prior to injection into the anterior chamber through the limbus with a 25-
Fig. 2. Pupil-optic section, untreated control, 20 days after implantation. Two main cell types are seen—one, round to ovoid, and the other, tending to be spindle shaped. Both types showed marked mitotic activity. (HE and PAS; ×485.)

gauge needle. The method has been previously described.15 These animal models were randomized and divided into two groups. Group I animals were treated with DTIC injected locally into the subconjunctival space or anterior chamber in one eye; the fellow eye served as untreated control. Group II animals were treated with BCNU. Subgroup II-A received the drug by local (subconjunctival or intracameral) injection, subgroup II-B by both local (subconjunctival) and intravenous administration, and subgroup II-C by intravenous administration only.

Animals to be treated with intracameral injection were anesthetized with sodium pentobarbital administered intravenously, 24 mg/kg body weight; those to be treated with subconjunctival injection were anesthetized by local instillation of 0.5% proparacaine hydrochloride ophthalmic solution (Ophthaine). Treatment was started 3 days after tumor tissue implantation; the schedules are shown in Tables I, II, and III.

Complete ocular examinations were repeated on alternate days during the follow-up period. Both treated and control eyes were enucleated on the twentieth day after tumor implantation, except for eyes in two animals in the subgroup II-B, which were followed for 4 months. The effectiveness of treatment was measured by comparing (1) the extent of tumor growth on the iris surface and in the anterior chamber of treated and control eyes, (2) the occurrence of distortion and perforation of the globe due to tumor growth, and (3) the histopathologic changes in the eye.

**Group I. Local injection of DTIC**

**Intracameral.** After routine preparation of the rabbit and the eyes, a tuberculin syringe with a 30-gauge needle was inserted into the anterior chamber at the limbus; 0.05 ml of aqueous humor was aspirated, and the same amount of DTIC solution injected. Treatment was started on the third day following tumor tissue implantation, and the same dose was repeated every three days. The total course was six injections (3, 6, 9, 12, 15, and 18 days after tumor implantation) completed
within 15 days. Intracameral injections consisted of DTIC, 0.6 mg/0.05 ml, in four eyes. One eye of each animal was treated; the fellow control eye was injected with an equal volume of drug diluent (distilled water) (Tables I and II).

Subconjunctival. The schedule of injections was the same as for the intracameral group. Six eyes were injected with DTIC, 1.5 mg/0.1 ml. One eye of each animal was treated; the fellow control eye was injected with an equal volume of drug diluent (Tables I and II).

Group II. Treatment with BCNU

Subgroup II-A. Local injection. The schedule of injections was the same as for the local injection of DTIC. Ten eyes were injected with BCNU, 0.2 mg/0.05 ml intracamerally, and 20 eyes with BCNU, 0.5 mg/0.1 ml subconjunctivally. One eye of each animal was treated; the fellow control eye was injected with an equal volume of drug diluent (10% ethanol) (Tables I and II).

Subgroup II-B. Combined local and systemic treatment. Twenty eyes of 10 animals were treated with repeated local (subconjunctival) injections of BCNU. All 10 animals also received systemic (intravenous) administration of BCNU. The preparation, intervals, and total number of injections for direct delivery were the same as for the animals injected locally with DTIC. Intravenous injections of BCNU, 2.25 mg/kg body weight, were given at 3, 6, 9, and 12 days after tumor implantation, for a total dose of 9.0 mg/kg body weight (Table III).

Subgroup II-C. Systemic treatment. Ten animals were treated systemically by intravenous injections of BCNU, 3.13 mg/kg body weight, at 3, 6, 9, and 12 days after tumor implantation, for a total dose of 12.5 mg/kg body weight (Table III).

Preparations for histopathologic examination. After the observation period (20 days for 58 animals, 4 months for two animals), the eyes were enucleated and fixed in 10% buffered formalin. A sagittal section on either side of the optic nerve was made, and pupil–optic nerve sections and two calottes were processed routinely for histologic

Fig. 3. Sclera and episcleral tissues of control eye are diffusely infiltrated by tumor tissue. (HE and PAS; ×4.)
Table I. Administration of DTIC or BCNU to tumor model rabbits

<table>
<thead>
<tr>
<th>Group, Drug</th>
<th>Route</th>
<th>No. of rabbits</th>
<th>No. of eyes or animals treated</th>
<th>No. of controls</th>
<th>Injection site</th>
</tr>
</thead>
<tbody>
<tr>
<td>I, DTIC</td>
<td>Local</td>
<td>10</td>
<td>10 eyes</td>
<td>10 eyes</td>
<td>Intracameral (4) Subconjunctival (6)</td>
</tr>
<tr>
<td>II, BCNU:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-A</td>
<td>Local</td>
<td>30</td>
<td>30 eyes</td>
<td>30 eyes</td>
<td>Intracameral (10) Subconjunctival (20)</td>
</tr>
<tr>
<td>II-B</td>
<td>Local</td>
<td>10</td>
<td>20 eyes</td>
<td>—</td>
<td>Subconjunctival +</td>
</tr>
<tr>
<td>Systemic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-C</td>
<td>Systemic</td>
<td>10</td>
<td>(10 rabbits)</td>
<td>10 rabbits</td>
<td>Intravenous</td>
</tr>
</tbody>
</table>

Table II. Schedule for direct delivery of drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Injection site</th>
<th>Treated eyes</th>
<th>Control eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Dosage*</td>
<td>No.</td>
</tr>
<tr>
<td>DTIC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracameral</td>
<td>4</td>
<td>0.6 mg/0.05 ml</td>
<td>4</td>
</tr>
<tr>
<td>Subconjunctival</td>
<td>6</td>
<td>1.5 mg/0.1 ml</td>
<td>6</td>
</tr>
<tr>
<td>BCNU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracameral</td>
<td>10</td>
<td>0.2 mg/0.05 ml</td>
<td>10</td>
</tr>
<tr>
<td>Subconjunctival</td>
<td>20</td>
<td>0.5 mg/0.1 ml</td>
<td>20</td>
</tr>
</tbody>
</table>

*Injections started third day after tumor implantation and given subsequently every third day for a total of 6 injections.

Table III. Schedules for combined (direct and systemic) delivery of BCNU in the same animals (Subgroup II-B) and for intravenous delivery alone (Subgroup II-C)

<table>
<thead>
<tr>
<th>Injection site</th>
<th>No. of eyes or animals treated</th>
<th>Dosage*</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined delivery: Subconjunctival</td>
<td>20 eyes</td>
<td>0.5 mg/0.1 ml</td>
<td>Every third day for a total of 6 injections</td>
</tr>
<tr>
<td>Intravenous</td>
<td>10 animals</td>
<td>2.25 mg/kg body weight</td>
<td>Every third day for a total of 4 injections</td>
</tr>
<tr>
<td>Intravenous delivery</td>
<td>10 animals</td>
<td>3.13 mg/kg body weight</td>
<td>Every third day for a total of 4 injections</td>
</tr>
</tbody>
</table>

*Injections started third day after tumor implantation.

Table IV. Clinical results in drug-treated animals on day 14 after tumor implantation

<table>
<thead>
<tr>
<th>Group, Drug</th>
<th>Route</th>
<th>Injection site</th>
<th>No. of eyes</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>I, DTIC</td>
<td>Local</td>
<td>Intracameral</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Subconjunctival</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>II, BCNU:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-A</td>
<td>Local</td>
<td>Intracameral</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>II-B</td>
<td>Local</td>
<td>Subconjunctival</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>Systemic</td>
<td></td>
<td>Subconjunctival</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>II-C</td>
<td>Systemic</td>
<td>Intravenous</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>
evaluation. Hematoxylin and eosin (HE) and periodic acid–Schiff (PAS) stains were used. The eyes were embedded in paraffin and sectioned for light microscopy. Representative sections, 10 of every 50 sections, were evaluated from each globe.

Results

Clinical observations. The clinical results at day 14 after tumor implantation are shown in Table IV. This day was chosen because it seemed a critical time in the control eyes.

Control eyes. In untreated eyes, the implanted Brown-Pearce epithelioma usually filled the anterior chamber within 10 days. All 40 control eyes were distorted and ruptured at 12 to 14 days by tumor growth (Fig. 1).

Group I. Direct delivery of DTIC. At 14 days following tumor implantation, all 10 eyes treated directly with DTIC retained normal shape and size, and all control eyes were distorted and ruptured. Growth of the implanted tumor in the anterior chamber was slowed in three of the four eyes that received DTIC intracamerally and in four of the six eyes that received the drug subconjunctivally (Table IV).

Group II. Animals treated with BCNU

Subgroup II-A. Direct delivery. Growth of the implanted tumor in the anterior chamber was slowed in 21 out of 30 eyes (70%). In two eyes that had received BCNU intracamerally, arrest of the tumor was evidenced by the normal pattern in three-fourths of the iris surface 12 days following the first drug injection. Eleven days after the first treatment (14 days following tumor implantation), the other 19 eyes retained normal shape and size (Fig. 1 and Table IV).

Subgroup II-B. Combined subconjunctival and intravenous administration. All 20 eyes of the 10 animals showed good response. On
day 14 following tumor implantation, tumor tissue growing in the anterior chamber was completely confined to the iris (Table IV). On day 20 after tumor implantation, all eyes retained normal shape and size. Four eyes (two animals) were followed for 4 months and retained normal shape and size. No corneal or lens opacity was seen.

Subgroup II-C. Intravenous administration. On day 14 following tumor implantation, 12 of the 20 eyes in this group were distorted and ruptured by tumor expansion; in the remaining 8 eyes, tumor growth was slightly delayed, and there was no distortion or rupture (Table IV).

Histopathologic observations
Control eyes. Tumor growth usually caused rupture of globes in 12 to 14 days after implantation. The site of rupture was usually anterior between the limbus and ora. Tumor was often seen extending through the globes into the subconjunctival space or orbit. In these eyes the intraocular contents were completely replaced by tumor.

Two main cell types were noted in the tumor (Fig. 2). One type was round to ovoid, with ill-defined cell margins and scant eosinophilic cytoplasm, giving the so-called epithelial appearance. The nuclei were large (nucleus-cytoplasm ratio, approximately 3:1), with prominent nucleoli and chromatin clumping. The second type of cells was less common and tended to be spindle-shaped with large nuclei and several prominent nucleoli. Their cytoplasm was also often ill-defined and showed mild basophilia. Both cell types showed marked mitotic activity. Necrosis was present in areas not surrounding blood ves-
sels. The sclera and episcleral tissues were diffusely infiltrated by tumor cells (Fig. 3).

**Group I and Subgroup II-A eyes.** Histologic examination of eyes receiving intracameral or subconjunctival injection of DTIC or BCNU showed large areas of necrosis (Fig. 4). Amid the necrosis were islands of viable tumor cells (Fig. 5). Less mitotic activity was seen than in the untreated controls. There was a greater variability in histologic appearance than in the control eyes. A distinct tendency toward replacement of intraocular structures was found, with only two instances of maintenance of intraocular architecture (Fig. 6). In addition, there were usually signs of infiltration of the ciliary body, inner scleral lamellae, and emissary canals by tumor cells (Fig. 7); in five eyes the vitreous cavity, retina, or choroid were infiltrated by a few tumor cells. The cells remaining viable were epithelial in character (Fig. 5).

There was no significant difference in the histologic appearance between the intracameral and subconjunctivally treated eyes among the BCNU-treated animals. Nor did these alternative routes of direct delivery result in histologic differences among the DTIC-treated animals in Group I.

**Subgroup II-B eyes.** Eyes of animals that had received combined subconjunctival and intravenous treatment with BCNU exhibited the most significant eradication of tumor tissue. In each eye studied histologically, there was almost complete necrosis of tumor. Considerably more maintenance of normal in-

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**Fig. 6. Intraocular architecture was maintained in this eye (and in one other) that received local BCNU injections only. (HE and PAS; x4.)**
traocular architecture was seen (Fig. 8) than in the locally treated group. Only rare foci of viable tumor cells were found (Fig. 9). With almost no evidence of mitosis, there was no infiltration of the inner scleral lamellae or emissary canals by tumor cells. Only occasional or few tumor cells were seen in the vitreous cavity, retina, or choroid in a few cases.

Subgroup H-C eyes. Intravenous administration of BCNU alone did not control tumor growth significantly. Tumor infiltrated the sclera diffusely, replaced the intraocular contents, and often resulted in perforation. This group showed slightly decreased mitosis in comparison with the control eyes.

Discussion

Direct delivery systems for the injection of drugs into the eye have received a great deal of attention in recent years in attempts to optimize the ocular bioavailability of chemotherapy for serious eye diseases.\textsuperscript{10–14} However, such a therapy system must be approached with great caution because of its possible complications—perforation of the eye being one of the most serious of these.\textsuperscript{15–18} It is even possible to cause occlusion of the central retinal artery following retrobulbar injection.\textsuperscript{19}

In the present study, the effectiveness of DTIC administered by intracameral or subconjunctival injection in treating an experimental intraocular malignancy was studied. This was compared with the effectiveness of BCNU by similar delivery. Our long-range goal is to find a cancer chemotherapeutic agent that will be able to be delivered through an episclerally implanted device in a sustained-release system. BCNU is lipophilic and known to diffuse from silicone devices. Therefore we concentrated on BCNU and extended our evaluation of this drug to combined local and intravenous administration and to intravenous injection only.

Treatment with BCNU by intracameral or
Fig. 8. Almost complete necrosis of tumor and good maintenance of normal intraocular architecture is seen in eye that received combined subconjunctival and intravenous BCNU treatment. (HE and PAS; ×4.)

Subconjunctival injection [total dose, 1.2 mg (0.2 mg × 6 intracameral) to 3.0 mg (0.5 mg × 6 subconjunctival)] produced significant response in 70% of the treated eyes. However, BCNU by intravenous injection alone [total dose, 25 to 31.25 mg (3.13 mg/kg body weight × 4)] produced no definitive response. Combined local and intravenous treatment with BCNU [total dose, 18 to 22.5 mg (2.25 mg/kg body weight × 4 intravenously + 3.0 mg subconjunctivally)] greatly inhibited the growth of implanted tumor tissue in the anterior chamber. On day 14 after implantation, these rabbits retained little or no visible living tumor tissue on the iris. Four eyes (two animals) retained normal shape and size after 4 months, and there was no visible abnormality. All control eyes on day 14 were destroyed by invasion of tumor into the eyes.

Histologic examination of the various treatment groups confirmed the effectiveness and the superiority of the combined treatment with BCNU over the other means. This was the only group to show uniformity in histologic appearance with some preservation of normal architecture and no tendency toward infiltration of the inner scleral lamellae or emissary canals. Even in this group, however, viable tumor cells persisted.

Experimental reports have documented higher ocular tissue and fluid levels of antibiotics following direct injection than after intravenous or intramuscular injection. Therefore a higher concentration of anticancer agent in ocular tissue will be obtained
Fig. 9. Rare foci of viable tumor cells with almost no evidence of mitosis in eye that received combined subconjunctival and intravenous BCNU treatment. (HE and PAS; x56.)

when the drug is delivered directly to the eye. The objective of this technique would be to achieve a therapeutic concentration of the drug in or around the tumor tissue in order to control ocular malignancies without reaching a level of systemic toxicity. It would appear from the present experiments that administration of BCNU or DTIC may hold promise for future therapy.

Whether this technique may eventually be applicable to treatment of malignant melanoma of the uveal tract and retinoblastoma cannot be determined at this time. Our laboratory is currently conducting experiments on the treatment of Greene melanoma in the anterior chamber of rabbit eyes and on the detection of the amount of drug released in the eye from the implanted silicone device by using labeled nitrosourea.

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REFERENCES
4. Schabel, F. M., Jr.: Nitrosoureas: a review of ex-