Sustained release of BCNU for the treatment of intraocular malignancies in animal models

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Sustained release of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) via an episcleral implanted silicone device was used to treat Brown-Pearce epithelioma in the anterior chamber of rabbit eyes. One group of test eyes received BCNU dissolved in sesame oil; a second group received BCNU in pure ethanol. Control eyes received only the diluent, sesame oil or pure ethanol. The effectiveness of the various dosages and diluents was compared by clinical observation, by weight of the enucleated eyes, and by histopathologic examination. Sustained release of BCNU via an episcleral implanted silicone device delayed the growth of Brown-Pearce epithelioma in rabbit eyes of both test groups. The most effective action resulted from administration of BCNU in pure ethanol.

Key words: sustained release, BCNU, episcleral implanted silicone device, Brown-Pearce epithelioma, sesame oil, pure ethanol, intraocular malignancy

Significant advances have been made in the technology of drug delivery systems by sustained release of anticancer agents and ocular therapeutic agents. Cancer chemotherapy with nitrosourea compounds has been reported to be effective to some degree, but the action of these compounds is not specific for cancer cells and often results in significant systemic toxicity. Laboratory experiments involving sustained release of nitrosourea from an implanted (either peritoneal or subcutaneous) silicone capsule in mice carrying L1210 leukemic cells showed good results. Ideally this technique should be used to deliver 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) directly to the area in or around the solid tumor site, as was done by intraneoplastic injection of another nitrosourea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), in an experimental mouse brain tumor model, or by insertion of radon seed into the tumor itself, or by suture of a radon shell to the sclera adjacent to the tumor. The aim was to achieve a therapeutic concentration of radiation dose at the tumor site without systemic toxicity.

The object of the experiments reported here is the determination of therapeutic response to the sustained release of BCNU to control the growth of an experimental intraocular tumor.

Maximum tolerable doses of BCNU were previously established in non-tumor-bearing rabbits for intraocular and periocular routes of drug administration. These maximum tolerable doses were then given by the in-
tracameral or subconjunctival routes to rabbits bearing Brown-Pearce epithelioma in the anterior chamber with encouraging results. 13 We selected this model for our initial experiments because this malignancy grows in a predictable manner in the rabbit eye and because the rabbit eye is of more suitable size for the required manipulations than are eyes of smaller animals. Future studies will employ a more relevant model: Greene melanoma in the rabbit eye.

Materials and methods

The silicone device is made of two sheets of silicone rubber. The sheets are glued to each other at their periphery with silicone adhesive, except where a small unidirectional silicone tube, permitting the injection of drugs into the center of the device without returning, is sandwiched between them. When fluid is forced into the center, the two sheets of silicone rubber expand (Fig. 1).

A detailed description of the silicone device and of the release rate in vitro of BCNU in sesame oil and in ethanol has been reported. 14 The rate of release of BCNU from sesame oil solution in the device into water is not constant. BCNU in absolute ethanol in the device resulted in rapid diffusion of the alcohol, leaving essentially pure BCNU in the device; consequently, the release rate of BCNU is essentially constant for a period of time that depends on the amount of BCNU present. If the diffusion and decomposition of the drug deplete the drug depot earlier than desired, more drug can be injected into the device to replenish it.

Preoperative examination of 28 eyes in 14 New Zealand female albino rabbits, weighing 2.0 to 2.5
kg, included biomicroscopy, tonometry, and indirect ophthalmoscopy. All animals had normal eyes. Both eyes of each animal were inoculated intracameraly with Brown-Pearce epithelioma tissue as previously described. 13

Three days after tumor tissue implantation, a silicone device was implanted episclerally in each eye. Animals were anesthetized with sodium pentobarbital administered intravenously, 24 mg/kg body weight. After routine preparation of the rabbit and the eyes, a pocket beneath the conjunctiva and Tenon’s capsule was made in the upper temporal quadrant of the eye. The silicone device was inserted into the pocket but the small tube remained to permit injection. Two 7-0 silk sutures were applied on both shoulders of the silicone device to the episcleral tissue at the pars plana to hold the device in position, and the conjunctiva was closed with two 7-0 silk sutures. Then 0.15 ml of the BCNU solution or the diluent (control) was injected into the device with a tuberculin syringe and a 30-gauge needle through the tube, which was outside the closed conjunctiva (Fig. 2), and the injecting tube was fixed on the upper fornix.

Seven animals (Group IA) received 3.0 mg of BCNU in 0.15 ml of sesame oil on one eye on days 3, 6, and 9 after tumor implantation (total dose 9.0 mg). Three animals (Group IB) received 12.0 mg of BCNU in 0.15 ml of sesame oil on the same schedule (total dose 36.0 mg). Four animals (Group II) received 13.0 mg of BCNU in pure ethanol on days 3 and 6 after tumor implantation (total dose 26.0 mg). The fellow eyes served as controls and received diluent only.

Ocular examinations were repeated on alternate days during the follow-up period of 20 days after tumor implantation; at that time all eyes were enucleated. The effectiveness of treatment was measured by (1) comparing the extent of tumor growth in the anterior chamber of treated and control eyes, (2) noting the occurrence of distortion and perforation of the globe due to tumor growth, (3) weighing all eyes following enucleation, and (4) comparing the histopathologic changes in the eyes.

In preparation for histopathologic examination, the enucleated eyes were fixed in 10% buffered formalin. A sagittal section on either side of the optic nerve was made and the pupil–optic nerve sections and the two calottes were processed routinely for histologic evaluation. Hematoxylin and eosin (H&E) stain was used. The eyes were embedded in paraffin and sectioned for light microscopy. Sections 6 μm thick were cut and every fifth section was stained. About 50 sections were examined from each eye.
Fig. 3. By day 15 in this control eye, intraocular contents were completely replaced by tumor rupturing the globe at the limbus. (H&E and periodic acid–Schiff; ×4.)

Results

Clinical observations. The clinical results are shown in Table I.

Control eyes. In untreated eyes, the implanted epithelioma usually filled the anterior chamber within 10 to 12 days after tumor tissue implantation. Of the 14 control eyes, 13 were distorted and ruptured due to tumor expansion within 15 days and the last one was ruptured at 16 days. The average weight of the globe following enucleation was 6.5 gm (Table I). (Normal weight is 3.182 gm ± 0.506.)

Group I: Eyes treated with BCNU in sesame oil. Growth of the implanted tumor tissue in the anterior chamber was slowed in all 10 eyes. In six eyes of the seven that had received 3.0 mg of BCNU in sesame oil three times (Group IA), delay of the tumor growth was evidenced by retention of normal shape and size of the eye without distortion 19 days following tumor implantation. In two eyes of the three that had received 12.0 mg of BCNU in sesame oil three times (Group IB), delay of the tumor growth was evidenced by retention of normal shape and size of the eye without distortion 19 days following tumor implantation. The average weight of the 10 treated eyes after enucleation was 5.2 gm (5.7 gm in Group IA eyes and 4.1 gm in Group IB eyes).

Group II; Eyes treated with BCNU in pure ethanol. All four eyes showed good response. On day 15 following tumor implantation, tumor tissue growing in the anterior chamber was almost completely arrested in three out of four treated eyes. On day 20 after tumor implantation, all four treated eyes retained normal shape and size. The average weight of the four treated eyes after enucleation was 3.1 gm; the average weight of the fellow control eyes was 5.4 gm (Table I).

Histopathology observations

Control eyes. Tumor was often seen filling the globe and extending into the subconjunctival space or orbit. In these eyes the intraocular contents were completely infiltrated and replaced by tumor (Fig. 3). The
Fig. 4. Eye that had been treated with BCNU in pure ethanol, on day 20. Tumor distends the entire iris stroma and iris root to the base of the ciliary body but there is relatively good maintenance of the normal intraocular architecture. (H&E and periodic acid-Schiff; ×4.)

Two main cell types were noted in the tumor. Most cells were round to ovoid, with ill-defined cell margins and scant eosinophilic cytoplasm, giving an epithelioid appearance. The nuclei were large, with prominent nucleoli and chromatin clumping. Other cells tended to be spindle-shaped, but they also had large nuclei and prominent nucleoli. Their cytoplasm was also ill defined and they showed mild basophilia. Both types of cells showed marked mitotic activity (6 to 8 per high-power field). Necrosis was common in all areas not immediately adjacent to blood vessels. The sclera and episcleral tissues were diffusely infiltrated by tumor cells.

Group I: Eyes treated with BCNU in sesame oil. Tumor growth tended to be limited to the anterior segment but in some instances replaced the iris and ciliary body. The inner scleral and corneal lamellae were infiltrated with tumor; no extrascleral extension was noted. The mitotic index remained high and necrosis was prominent. There was no observable difference histologically between Group IA eyes, which had received a total of 9.0 mg of BCNU, and Group IB eyes, which had received a total of 36.0 mg.

Group II: Eyes treated with BCNU in pure ethanol. These eyes exhibited histologically the most significant reduction of tumor growth; only small foci of tumor, limited to the anterior segment, were observed. Typically, small nodules of tumor were found in the iris stroma and iris root, with occasional involvement of the ciliary body. The mitotic index remained high, 6 to 8 per high-power field. However, necrosis was less prominent than in control eyes. There was good maintenance of normal intraocular architecture, with no evidence of invasion of the cornea or sclera (Fig. 4).

Summary of results. At day 12 after tumor tissue implantation, the tumor occupied the entire anterior chamber in four of 14 BCNU-treated eyes and in 13 out of 14 fellow control eyes. The average weight of the 14 treated eyes after enucleation was 4.6 gm; the av-
average weight of the 14 fellow control eyes was 6.5 gm. As determined by Student's t test, the weight difference between treated and untreated eyes is highly significant (p < 0.01).

Although treatment with BCNU in sesame oil via the silicone device (Group I) delayed growth of the implanted tumor in comparison with untreated eyes, the response was less marked—by all our criteria of effectiveness—than in eyes treated with BCNU in pure ethanol (Group II). There was no difference in drug effectiveness, either clinically or histologically, between BCNU in sesame oil administered in doses of 3.0 mg (Group IA) or 12.0 mg (Group IB).

Discussion

BCNU has previously been shown to be active against Brown-Pearce epithelioma in rabbit eye by direct intracameral or subconjunctival injection. In the work reported here, another modality of BCNU delivery—sustained release from a refillable device implanted episclerally—was also found effective against this type of intraocular tumor in the rabbit. The mode of action of BCNU is not well understood. However, it seems that BCNU and/or some of its active metabolites can penetrate into the eye and be effective therapy against a fast-growing tumor.

The quantity of BCNU that diffuses through an implanted silicone device is influenced by many factors, including solubility of BCNU in the solvent, nature of the solvent, and surface area and thickness of the device, but the most important determinant is the instability of BCNU.14

Experiments in vitro have indicated a release rate of BCNU of 200 to 300 µg/hr to stirred water for a length of time that varies with the amount of BCNU in ethanol solution that has been injected into the silicone balloon. These experiments do not take into account the metabolic breakdown products of BCNU, such as the alkylation and carboxymethylating moieties responsible for the drug's antitumor activity. The assay of BCNU-containing balloons in agar plates seeded with Escherichia coli presumably responds also to these metabolic products of BCNU breakdown. The antibacterial activity emanating from the balloons in these assays was found to be equivalent to 900 to 2500 µg of BCNU for each overnight incubation.14 The results of these experiments are not exactly applicable to the in vivo situation reported in this paper. The nonstirred boundary layers' effect upon a drug released from an implant in vivo, the ocular penetration of BCNU and its active metabolites, and the dissipation of the drug and its decomposition products away from the tumor site are not predicted by the in vitro studies. Obviously, the in vitro experiments serve only as a guideline for the in vivo application of the drug-releasing device. Thus, sustained release at a constant rate of about 200 to 300 µg/hr is expected from a silicone balloon implanted episclerally and injected with a BCNU solution in absolute ethanol. We have determined the response of an intraocular tumor to this arbitrary dose of BCNU supplied to the eye from an implanted episcleral balloon filled with the drug in ethanol solution. Poorer response was obtained with BCNU in sesame oil, also delivered from a silicone device, probably due to the decreasing release rates of the drug under these conditions.14 The arbitrary dose of BCNU given to the rabbit eyes from the silicone implants has not produced any untoward clinical effects on the animals. However, further research is warranted on animal tolerance of increased amounts of BCNU sustained-delivered from silicone rubber implants, as well as on the response of experimental intraocular tumors to these increased amounts of BCNU.

There are commercially available osmotic minipumps that will deliver a drug at a predetermined constant rate for up to 1 week. However, the relatively large size of these pumps (2.6 cm long and 0.6 cm in diameter) and their rigid cylindrical shape obviate their use as episcleral implants. Furthermore, silicone balloons can be easily made in the laboratory at a fraction of the cost of commercial osmotic minipumps, and are easy to implant and fix episclerally. These balloons
are expandable and have a small indirectional tube allowing reinjection of drug into the implant. The tissue tolerance of silicone episcleral and intrascleral implants is well documented. However, an implant is still a foreign body in the eye which may cause inflammation or contribute to infection. The manipulations must be performed with proper precautions.

The authors thank Dr. Charles L. Schepens for his generous encouragement and advice throughout this project. Drs. Harry B. Wood, Jr., of the Drug Development Branch, and John S. Penta, of the Drug Liaison and Distribution Section, Division of Cancer Treatment, National Cancer Institute, provided the experimental drug BCNU (NSC-409962).

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