Responsiveness of Retinoblastoma to Local Diaziquone

Studies in a Xenograft Model

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The benefits of chemotherapy in the management of early or bilateral retinoblastoma are doubtful and are difficult to study. A xenograft model has been developed in which the therapeutic response of retinoblastoma heterotransplanted to the anterior chambers of nude mouse eyes can be evaluated. Cyclophosphamide has been shown to be the most effective of the conventional agents. The new drug diaziquone was tested in this model against five patient-derived xenografted cell lines, using both systemic (intraperitoneal) and local (eye drops) methods of administration. A total of 359 xenograft tumors in 229 experimental animals were monitored after treatment with intraperitoneal cyclophosphamide, intraperitoneal diaziquone, or local diaziquone. Responses to all three regimens were demonstrated in each of the five xenograft lines. Diaziquone compared favorably with cyclophosphamide as systemically administered chemotherapy. Local diaziquone was as effective as intraperitoneal injection in producing tumor responses. It is suggested that methods for local administration of diaziquone may be adapted to the clinical setting, and that a role for this modality may be found in a combination of nonoperative approaches to the management of small, intraocular tumors.

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The role of chemotherapy in the treatment of retinoblastoma (Rb) remains poorly defined and difficult to evaluate in the clinical setting. Development of a multi-modality program incorporating effective chemotherapeutic agents may reduce the need for enucleation in the management of early or bilateral intraocular disease.

In order to study the efficacy of conventional or newly developed agents, a model for intraocular heterotransplantation of human Rb in nude mice has been adapted to the sequential evaluation of therapeutic response. The results in the model were found to correlate with limited clinical data, and confirmed cyclophosphamide (CPM) to be the most effective of the conventional chemotherapeutic agents.

Materials and Methods

Nude mice (nu,nu) of BALB-c background, aged 4–6 weeks, were supplied by the Australian Nuclear Science and Technology Organization (Sydney). The experimental animals were bred and maintained in a protected and controlled environment according to previously published methods. Intraperitoneal anesthesia (pentobarbitone, 40 mg/kg; Abbott Australasia) was used for potentially painful procedures, and inhalational (enflurane; Abbott Australasia Pty Ltd) anesthesia was used for stereoscopic observation of
mouse eyes. All protocols were conducted in accordance with permits issued by the Committee on the Use of Animals in Research or Teaching of the University of New South Wales, and with the ARVO Resolution on the Use of Animals in Research.

Five Rb xenograft lines were established by the heterotransplantation of cells obtained at enucleation of the respective patients (Table 1). The cells were suspended in RPMI-1640 culture medium at a concentration of between $1 \times 10^6$ and $5 \times 10^7$ cells/ml and injected into the anterior chambers of nude mouse eyes with a 30-gauge needle, under stereoscopic control, as reported previously.\textsuperscript{4,5} Allowing for variable reflux through the perforation created by the needle, an estimated 4–8 µl of fluid were retained, representing a calculated tumor load of between $4 \times 10^3$ and $4 \times 10^5$ cells. Serial passaging of xenografted tumours was performed by enucleation of affected eyes, resuspension of cells, and injection into the anterior chambers as described.

The experimental animals received bilateral injections into the anterior chambers of their eyes with cell suspensions from one of five passaged Rb xenograft lines (Tables 1, 2). Tumor size was monitored by weekly observation of eyes proptosed under stereoscopic control. Visible growth was documented in a majority of injected eyes after a latency of 4–6 weeks. A grading system has been developed whereby tumor progression (weeks) with systemically administered chemotherapy, the number of treatment courses ($n$) exceeded the number of evaluable tumors, including 8 controls; and JGRB-1 (passage 2): 40 mice, 62 tumors, including 9 controls. Since many tumors were treated sequentially with more than one course of chemotherapy, the number of treatment courses ($n$) exceeded the number of evaluable tumors. The mean and standard deviation of delay in tumor progression was calculated for all courses of a particular treatment in each of the five xenograft lines.

### Table 1. Comparison of delay in tumor progression (weeks) with systemically administered chemotherapy

<table>
<thead>
<tr>
<th>Rb cells</th>
<th>AZQ-treated</th>
<th>CPM-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>Mean</td>
</tr>
<tr>
<td>LARB-69</td>
<td>18</td>
<td>5.4</td>
</tr>
<tr>
<td>LARB-109</td>
<td>82</td>
<td>2.2</td>
</tr>
<tr>
<td>EPRB-2</td>
<td>71</td>
<td>4.3</td>
</tr>
<tr>
<td>KDRB-3</td>
<td>49</td>
<td>4.3</td>
</tr>
<tr>
<td>JGRB-1</td>
<td>31</td>
<td>3.0</td>
</tr>
</tbody>
</table>

$n =$ number of evaluable courses administered.

### Table 2. Effectiveness of systemic vs local AZQ in the model: Tumor progression delay in weeks

<table>
<thead>
<tr>
<th>Rb cells</th>
<th>Systemic AZQ</th>
<th>Local AZQ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>Mean</td>
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Control (saline-treated) tumors grew in a predictable and fairly constant manner, averaging 1 week or less to progress from one grade to the next. Therefore, the number of weeks (mean ± standard deviation) required to progress from grade 1 to 4 was as follows: LARB-69, 2.3 ± 0.9; LARB-109, 4.1 ± 0.6; EPRB-2,
3.0 ± 1.1; KDRB-3, 3.2 ± 0.7; and JGRB-1, 2.7 ± 0.4. Tumor response to treatment was measured as regression according to grades or delay in progression. Results were expressed as the number of weeks required for each tumor to progress to the next grade and were compared with the figure of 1 week for control tumors. The most frequent pattern was tumor shrinkage 1 or more weeks after administration of effective treatment, followed by regrowth at the same rate as control tumors. When documentation was complete, it was possible to administer further courses of therapy to some tumors.

The results of comparisons between intraperitoneally administered AZQ and CPM are shown in Table 1. The comparison of delay in tumor progression achieved by systemic versus locally administered AZQ is given in Table 2. All of the five xenograft cell lines were demonstrated to respond to each of the three regimens of chemotherapy, by comparison with control tumors. Furthermore, no significant differences were demonstrable between the two systemic regimens (Table 1) or between local AZQ and systemic dosage (Table 2).

**Discussion**

Contrary to its role in most pediatric neoplasia, chemotherapy has yet to find an established place in the treatment of nonmetastatic Rb.¹ The possibility of a multi-modality therapy program incorporating locally effective regimens such as cryotherapy, laser therapy, radioactive plaque, external beam radiation, or photodynamic therapy, in combination with effective systemically or locally administered chemotherapy, is very appealing. Sporadic reports of chemotherapy-induced responses in intraocular Rb have
The possibility of local therapy has arisen because AZQ, unlike CPM, does not require metabolic activation and therefore may be effective by direct application to tumors in situ. This strategy has been attempted in various malignancies with intraarterial perfusion, intrathecal injection (Poplack D: personal communication), topical cream, and subconjunctival injection (Murphree AL: personal communication). The pharmacokinetics of the subconjunctival route of administration have been demonstrated convincingly with a number of other drugs in rabbit models. Cytotoxic agents also have been shown to be effective by episcleral delivery. Successful local therapy of intraocular leukemia or lymphoma in patients, using methotrexate, cytarabine, and steroids, has been described. The potential benefits of regional or local administration are maximal tumor concentrations of the drug with minimization of myelosuppression and other systemic toxicities.

In these experiments, the five xenografted Rb lines were shown to be highly responsive to systemically administered CPM or AZQ, consistent with prior studies. Furthermore, in each of the five patient-derived xenografts, local therapy with AZQ was as effective as systematic dosage. The cell line relatively most sensitive to all three regimens was LARB-69, which, according to control data, appeared to grow fast, whereas LARB-109 was slowest-growing and least sensitive to therapy. Despite some variations among the five xenografts, the pattern of untreated growth allowed for the approximation of 1 week as the interval required for progression from one grade to the next, in all five. Previous data in the model have shown no significant differences in progression or responsiveness according to the starting grade of therapy (up to grade 3) or in the number of sequential courses administered (up to four courses).

The chosen method of local administration, namely eye drops, is considered the safest, easiest, and least invasive in the current model. It is not suggested that intraocular Rb in patients would respond to the same method. The obvious anatomic and physiologic differences in the clinical setting require that other methods of local drug application be considered. In this context, preliminary work at the Ocular Oncology Center of the Children’s Hospital of Los Angeles, California, with a rabbit model has demonstrated markedly elevated levels of AZQ in the retina after subconjunctival versus intravenous injection (Murphree AL: personal communication). For optimal clinical use, further work, including the possible development of sustained-release local applications, needs to be considered.
AZQ has been demonstrated to be a chemotherapeutic agent with activity against Rb. Due to its pharmacology and efficacy, local AZQ therapy with reduction in systemic toxicity is feasible. Furthermore, systemic CPM may contribute to the risk of secondary neoplasia in Rb patients. It is suggested that for early intraocular Rb, a multi-modality treatment program incorporating local AZQ may be developed, thereby reducing the need for enucleation of affected eyes.

Key words: retinoblastoma, xenograft, chemotherapy, cyclophosphamide, diaziquone

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References