Tissue distribution of 3H-hematoporphyrin derivative in athymic "nude" mice hetero-transplanted with human retinoblastoma.

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Quantitative uptake levels of tritium-labeled hematoporphyrin derivative (3H-HpD) have been obtained for normal and malignant tissue of athymic "nude" mice hetero-transplanted intraocularly with human retinoblastoma. It was determined that eyes with tumor accumulated higher levels of 3H-HpD than those in control eyes. In addition, an improved therapeutic retention ratio of the drug (eyes with tumor compared with control eyes) was obtained at extended time intervals after administration.

The generalized procedure for photoradiation therapy (PRT) is the parenteral administration of hematoporphyrin derivative (HpD) followed several days later by localized illumination of a malignant lesion with visible red light. This therapy, currently under clinical investigation as a primary modality for the treatment of solid tumors, involves both the preferential retention of HpD in malignant tissue and the in situ destruction of the tumor by HpD-induced photosensitization. Photodynamic action of visible light–activated HpD results in the production of cytotoxic singlet oxygen, which induces rapid tumor necrosis.

The potential utilization of HpD PRT for the treatment of intraocular tumors appears promising because of the accessibility and excellent optical properties of the eye. However, information regarding either the uptake characteristics of HpD in ocular tumors or the photosensitized inactivation of intraocular tumor tissue by HpD is limited. Preferential localization of HpD has been observed in transplanted melanoma grown in the anterior chamber of hamsters and in transplanted VX-2 carcinoma grown in the anterior chamber of rabbits. Enhanced localization of HpD has also been demonstrated in an amelanotic hamster melanoma transplanted in the choroid segment of the rabbit eye. In these previous investigations, fluorescence was used as the sole indication of HpD presence, and in neither case was quantitative documentation possible.

In the present report, we describe the quantitative uptake of 3H-HpD in nude mice hetero-transplanted with human retinoblastoma.

Materials and methods. Human retinoblastoma was originally obtained from a freshly enucleated surgical specimen and has subsequently been maintained in serial passage in the eyes of athymic "nude" mice. The procedure utilized for transplanting the retinoblastoma has previously been reported. Only mice having passage number 5 of tumor specimen CL-6 were used in this study. Tritiated HpD (50 mCi/mM) was obtained from New England Nuclear, Boston, Mass. This material was combined with nonradioactive HpD to obtain a working solution of 3H-HpD at a concentration of 1 mg/ml with a specific activity of 12 mCi/mM or 4.4 × 10⁴ disintegrations per minute/μg. Tissue distributions were previously determined to be the same for 3H-HpD and 14C-HpD, indicating that in vivo tritium exchange did not occur with 3H-HpD. For the distribution study, mice received a single intraperitoneal injection of the working solution of 3H-HpD (20 mg/kg, 10 to 12 μCi/mouse) when the anterior chamber of the eye was approximately 95% filled with tumor. In addition, control mice (not heterotransplanted with retinoblastoma) were also injected with 3H-HpD. The mice were then placed in a darkened room to prevent photosensitization. At various times after injection (1 to 72 hr), the animals were sacrificed and whole blood (0.2 ml) and samples (15 to 300 mg) of lung, skin, muscle, spleen, kidney, liver, brain, feces, and whole eyes were collected. The tissue samples were rinsed in saline and blotted with surgical gauze. The wet weight of each sample was recorded and the specimens were then individually oxidized with a Packard Tri-Carb Model 306 Oxidizer. The 3H content of each sample was determined by standard liquid scintillation counting with a Beckman Model LS-3155 Liquid Scintillation Counter. Tissue samples from mice injected with nonradioactive HpD were also combusted, and the counts obtained served as background measurements. The method of external standard channel ratios was used to correct for quenching of the scintillation fluid and to deter-
Table I. Tissue and blood distribution of $^3$H-HpD in athymic mice at various times after injection*

<table>
<thead>
<tr>
<th>Time after injection (hr)</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eye (tumor)</td>
</tr>
<tr>
<td>1</td>
<td>1.84 ± 0.31</td>
</tr>
<tr>
<td>6</td>
<td>3.66 ± 0.53</td>
</tr>
<tr>
<td>24</td>
<td>2.47 ± 0.35</td>
</tr>
<tr>
<td>48</td>
<td>1.65 ± 0.25</td>
</tr>
<tr>
<td>72</td>
<td>1.34 ± 0.09</td>
</tr>
</tbody>
</table>

*Data expressed as mean µg $^3$H-HpD/gm of tissue ± S.D.; numbers in parentheses are the number of samples analyzed (one sample per mouse).
reasons vs. control eyes) was obtained at prolonged intervals after administration. This coincides with the theory that the preferential localization of HpD in malignant tissue is related to the enhanced retention of the drug in tumor tissue compared with surrounding normal tissue.  

It is unclear as to why HpD is preferentially retained in tumor tissue, but it has been suggested that the high vascular permeability together with the lack of adequate lymphatic drainage of tumors may contribute to this localization.

Although several of the characteristics of the human retinoblastoma are retained when grown in the nude mouse, it is necessary to emphasize that this tumor model differs from the original both in its environment (anterior chamber) and presumably in its growth rate. Therefore the uptake kinetics of HpD in the clinical case of retinoblastoma may differ from that observed in the nude mouse model.

Current treatment modalities for intraocular tumors offer only limited success and in many patients enucleation is necessary. A treatment such as HpD PRT (which has been reported to be beneficial either as a primary treatment modality or as a follow-up treatment in conjunction with radiation therapy) may prove to be useful in selectively destroying intraocular tumors. In addition to the localized uptake of HpD in ocular tumors, it has been reported that HpD PRT can produce marked tumor toxicity in the mouse such as human retinoblastoma model and can be lethal to retinoblastoma cells (Y-79 and WERI-Rbl) in vitro.  

Preclinical evaluation (ocular tissue toxicity, lightdelivery development, and tumor treatment) of ocular HpD PRT is currently being pursued with the pigmented rabbit and the Greene melanoma tumor model. It is hoped that these studies will lead to the safe and effective use of HpD PRT in the clinical treatment of intraocular tumors.

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