Distribution of hematoporphyrin derivative in canine glioma following intraneoplastic and intraperitoneal injection

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Hematoporphyrin derivative (HPD) is a photosensitizing agent that has been used to locate and kill tumors. The distribution of tritiated (3H)-HPD was studied in a transplantable canine glioma model following intraperitoneal or direct intraneoplastic injection. Compared to intraperitoneal administration of 3H-HPD, direct injection resulted in levels that were more than 2.5 times higher in tumor tissue and approximately 10 times lower in skin. Dose-corrected analysis of the data indicated that outside the central nervous system (CNS) the distribution of 3H-HPD is dose-related, regardless of route of injection. Within the CNS, direct injection leads to more efficient uptake of 3H-HPD, especially at the tumor periphery. Fluorescence microscopy confirmed the selective biodistribution of HPD fluorescence within the cytoplasm of tumor cells.

KEY WORDS: hematoporphyrin derivative • glioma • photoradiotherapy • drug delivery • dog

The selective biodistribution of the photosensitizer hematoporphyrin derivative (HPD) is well known in a wide range of neoplastic, embryonic, and regenerating tissues. Intracellular HPD can be activated by light of the appropriate wavelength (630 nm), yielding a cytotoxic effect mediated by the production of singlet oxygen, hydroxyl radicals, and superoxide. This process has been termed "photoradiotherapy." Clinical trials utilizing intravenously administered HPD have been tried in the treatment of human malignant brain tumors. Photodynamic killing, as evidenced by tumor-cell necrosis, has been seen, albeit in the absence of improved survival. Cutaneous phototoxicity has been the major side effect limiting the use of higher HPD doses. To obtain increased tumor concentrations of HPD and to minimize systemic toxicity following parenteral administration of the dye, Kostron, et al., attempted direct intraneoplastic injection into subcutaneous and intracranial tumors in the rat glioma model. The results indicated that intraneoplastic injection was superior to intraperitoneal injection for increasing tumor concentrations of HPD, and also decreased HPD concentrations in surrounding brain, in the skin, and in other normal tissues. Furthermore, the elevated HPD concentrations in tumor were found to correlate with an increased cytotoxic effect, both in vitro and in vivo.

To study both the qualitative and quantitative aspects of HPD concentrations in a tumor system more closely resembling human malignant brain tumors, we utilized the modified canine glioma model of Salcman, et al., in our present investigation. The canine glioma is a fatal, rapidly growing tumor with many of the histological characteristics of human glioblastoma, including capillary proliferation, pseudopallisading, frequent mitotic figures, and multinucleated giant cells. In addition, the tumor system lends itself well to radiological evaluation and surgical manipulation, thus rendering it an appropriate model for studying HPD photoradiotherapy as a potential treatment of malignant brain tumors.

Materials and Methods

The modified transplantable canine glioma model was used in this investigation. Frozen tumor brei* was thawed and grown in tissue culture. At passage 6, the cells of three confluent tissue culture flasks were concentrated into an injectable 1-cc tumor cell suspension of $3.1 \times 10^7$ cells/cc. The 0.1-cc cell suspension followed by 0.1 cc of air was injected to a depth of 0.8 cm through the left lateral corner of the anterior fontanel.

* Frozen tumor brei obtained from Michael Salcman, M.D., University of Maryland School of Medicine, Baltimore, Maryland.
of each puppy in a litter of neonatal beagles (12 to 48 hours old). All injections were performed under sterile conditions. After injection, the animals remained active and healthy and were returned to their mother.

On postinjection Days 10 to 14, the animals were sacrificed and tumor brei was harvested for frozen storage, tissue culture, and histological verification. Tumor was prepared for frozen storage by mincing it into submillimeter pieces, followed by fine grinding in a tissue-grinding tube containing culture medium. After coarse straining through a gauze sponge, viability was assessed by exclusion of trypan blue, and 2-cc aliquots were frozen at -60°C. For subsequent injections into neonatal beagles, a vial of the frozen tumor brei was thawed in a 35°C water bath and immediately injected through the left lateral corner of the anterior fontanel.

Quantitative analysis of 3H-HPD distribution was studied in both tumor and normal tissues. After rinsing with normal saline, the tissue samples were weighed on an analytical balance, dissolved in Protosol, and bleached with H2O2. Econofluor (10 cc) was added to the completely dissolved samples, which were counted in a Beckman LS 230 scintillation counter. The 3H-HPD content was calculated by converting counts per minute/gm wet tissue weight to mCi/gm. Data are reported both in absolute terms (mCi/gm) and in relative terms (mCi/gm tissue/mCi 3H-HPD injected), thereby correcting for the dosage variation between direct and intraperitoneal routes of administration. All data are expressed as the mean value and standard deviation, with significance being determined by the paired Student t-test.

Coronal brain sections through the frontotemporal tumor were photographed with Kodak ASA 400 daylight film before being prepared by conventional histological methods. Some of the preparations were stained for histological examination, while unstained samples were used for fluorescence microscopy. For fluorescence microscopy, sections 5-μ thick were examined with a Zeiss Orthoplan microscope using a 405-nm excitation beam (G405 filter) with a red-fluorescence detection filter (LP590).

Results

Tumor growth was evident in specimens from all animals, and gross visualization of HPD content and distribution was possible with a Wood’s lamp (Fig. 1). In both intraperitoneally and directly injected animals, fluorescence was seen in the choroid plexus as well as in the tumor.

The six animals with intracerebral tumors that were directly injected with 1.75 mg (0.098 mCi) of 3H-HPD exhibited no apparent adverse effects (Table 1). In tissue samples from these dogs the average content of 3H-HPD was 1.84 mCi/gm wet tissue weight at the tumor center and 6.72 mCi/gm at the tumor periphery. The edematous brain adjacent to the tumor contained 0.98 mCi/gm, a value comparable to that of the normal ipsilateral and contralateral brain tissue. The mean 3H-HPD content in the skin of these six animals was 0.08 mCi/gm. In the one sample of choroid plexus studied, the 3H-HPD value was 18.21 mCi/gm.

The five animals that were injected intraperitoneally with 25 mg 3H-HPD/kg body weight demonstrated an average 3H-HPD value of 1.90 mCi/gm wet tissue weight at the tumor center and 2.55 mCi/gm at the tumor periphery. The edematous brain contained 1.06 mCi/gm.

† Hematoporphyrin-diacetate obtained from Photofrin Medical, Inc., Cheektowaga, New York; catalytic exchange labeling performed by New England Nuclear, Boston, Massachusetts.

‡ Protosol manufactured by New England Nuclear, Boston, Massachusetts.
§ Econofluor manufactured by New England Nuclear, Boston, Massachusetts; scintillation counter manufactured by Beckman Instruments, Palo Alto, California.
¶ Orthoplan microscope manufactured by Carl Zeiss, Inc., Thornwood, New York.
while normal brain tissues had comparable values. The skin contained 0.91 mCi/gm. In the one choroid plexus sample, the 3H-HPD value was 4.97 mCi/gm. For the doses tested, the 3H-HPD levels in edematous and normal brain were not significantly different between the two routes of administration. In contrast, the 3H-HPD content was significantly higher (p < 0.05) in the periphery of tumors injected directly than in the periphery of tumors injected intraperitoneally.

To correct for variations in the original dosages administered to each group, mCi/gm wet tissue weight values were divided by 0.098 mCi and an average of 1.12 mCi for animals undergoing direct or intraperitoneal (IP) injection. Derived from data in Table 1.

<table>
<thead>
<tr>
<th>Sample Location</th>
<th>Direct Injection</th>
<th>IP Injection</th>
<th>Direct/IP Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>tumor center</td>
<td>1.84 ± 1.51</td>
<td>1.90 ± 0.68</td>
<td>0.97</td>
</tr>
<tr>
<td>tumor periphery</td>
<td>6.72 ± 2.68</td>
<td>2.55 ± 1.05</td>
<td>2.64</td>
</tr>
<tr>
<td>edematous brain</td>
<td>0.98 ± 0.62</td>
<td>1.06 ± 0.49</td>
<td>0.92</td>
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<tr>
<td>choroid plexus</td>
<td>18.21</td>
<td>4.97</td>
<td>3.66</td>
</tr>
<tr>
<td>rt occipital lobe</td>
<td>0.93 ± 0.74</td>
<td>0.81 ± 0.66</td>
<td>1.15</td>
</tr>
<tr>
<td>lt occipital lobe</td>
<td>0.94 ± 0.46</td>
<td>0.83 ± 0.56</td>
<td>1.13</td>
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<tr>
<td>rt cerebellum</td>
<td>1.32 ± 0.69</td>
<td>0.75 ± 0.20</td>
<td>1.76</td>
</tr>
<tr>
<td>lt cerebellum</td>
<td>1.35 ± 0.57</td>
<td>0.98 ± 0.33</td>
<td>1.38</td>
</tr>
<tr>
<td>lumbar cord</td>
<td>4.21 ± 1.37</td>
<td>0.83 ± 0.17</td>
<td>5.07</td>
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<tr>
<td>skin</td>
<td>0.08 ± 0.03</td>
<td>0.91 ± 0.35</td>
<td>0.09</td>
</tr>
<tr>
<td>muscle</td>
<td>0.14 ± 0.03</td>
<td>1.10 ± 0.33</td>
<td>0.13</td>
</tr>
<tr>
<td>liver†</td>
<td>0.33 ± 0.06</td>
<td>5.68 ± 1.38</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* Values are in mCi/gm wet tissue weight (means ± standard deviations) for six animals receiving direct intraneoplastic injection of tritiated hematoporphyrin derivative (3H-HPD, 1 mg/cu cm tumor volume) and five animals receiving intraperitoneal (IP) injection of 3H-HPD (25 mg/kg body weight).
† Differences between directly and intraperitoneally injected groups are significant at p < 0.05.
‡ Value obtained from only one animal in each group.
Distribution of hematoporphyrin in canine glioma

**TABLE 3**

<table>
<thead>
<tr>
<th>Sample Location</th>
<th>Direct Injection</th>
<th>IP Injection</th>
<th>Direct/IP Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>tumor periphery:</td>
<td>6.86:1</td>
<td>2.43:1</td>
<td>2.82:1</td>
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<tr>
<td>edematous brain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tumor periphery:</td>
<td>84.00:1</td>
<td>2.80:1</td>
<td>30.00:1</td>
</tr>
<tr>
<td>skin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*[^3H]-HPD = tritiated hematoporphyrin derivative; IP = intraperitoneal. For a description of the method of determining uptake see Table 2.*

Fluorescence microscopy confirmed the selective biodistribution of fluorescence within tumor tissue. Not only was fluorescence clearly localized within tumor-cell cytoplasm and possibly the cell membranes (Fig. 2), but it also revealed clearly demarcated borders between normal and tumor tissue and demonstrated small tumor "fingers" within normal brain tissue in both the intraperitoneal and direct injection groups.

**Discussion**

The selective biodistribution of HPD in malignant tumors, coupled with the photodynamic cytotoxic effect, offers a unique approach to the therapy of malignant brain tumors. To date, all human studies have utilized intravenously administered HPD. Although these clinical studies have been promising in terms of computerized tomography evidence for tumor shrinkage and histological evidence for tumor necrosis, there has been no improvement in survival time. Our hypothesis has been that direct intraneoplastic injection can increase the HPD concentration within the tumor while decreasing it systemically. The idea of direct injection is not new. It has been used with such compounds as radioisotopes and various cytostatic agents in an attempt to circumvent the blood-brain barrier, minimize systemic toxicities, and increase tumor drug concentrations. What makes HPD different from these other compounds is its selective binding activity. Thus, the theoretical advantage of maximizing the HPD concentration in the immediate tumor environment is to alter the equilibrium between bound and unbound forms of HPD, thereby favoring increased tissue binding. The increased tissue concentrations of HPD would then result in increased light-activated cytotoxicity. Prior studies of HPD distribution and cytotoxicity in a rat tumor model lend support to this idea.

The objective of the current study was to examine the distribution characteristics of HPD in an experimental brain-tumor model that not only more closely resembles human malignant brain tumors than does the rat tumor model but also allows for future radiological and surgical experiments. The canine glioma model is ideal for this purpose.

Tritiated-HPD and scintillation counting were used to quantify HPD tissue concentrations in our present experiment. In 1979, Gomer and Dougherty reported the use of tritium ([^3H]) and carbon-14 ([^14C])-labeled HPD for studying the distribution of porphyrins in tissues. Incorporation of [^14C]-glycine into the stable heme ring structure and[^3H] labeling of HPD by the technique of catalytic exchange allowed these investi-
Intraperitoneal injection of HPD produces higher levels of HPD at the tumor periphery relative to brain, skin, and other organs when compared with parenteral administration. Because intraneoplastic injection was well tolerated and produced maximal HPD concentration in the infiltrating islands of tumor, we believe that this technique should be further evaluated as a therapeutic modality for malignant gliomas.

Summary

We have shown in a canine glioma model that direct intraneoplastic injection of HPD produces higher levels of HPD at the tumor periphery relative to brain, skin, and other organs when compared with parenteral administration. Because intraneoplastic injection was well tolerated and produced maximal HPD concentration in the infiltrating islands of tumor, we believe that this technique should be further evaluated as a therapeutic modality for malignant gliomas.

Acknowledgments

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