Short Report

Tissue Distribution of $^{125}$I-Labeled Fetal Thyroid Hormone in Nude Mice Xenografted with Ewing Sarcoma

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Department of Orthopaedic Surgery, Tohoku University School of Medicine, Sendai 980, *University of Tennessee-Campbell Clinic Department of Orthopaedic Surgery, University of Tennessee, Memphis TN 38103, USA, and †the First Department of Surgery, Kyoto Prefectural University of Medicine, Kyoto 602

HATORI, M., DUTKOWSKY, J.P., SMITH, R.A., WILLIAMS, R.S., YOKOTA, T. and SAKURAI, M. Tissue Distribution of $^{125}$I-Labeled Fetal Thyroid Hormone in Nude Mice Xenografted with Ewing Sarcoma. Tohoku J. Exp. Med., 1992, 167 (3), 239-242. Biodistribution of fetal thyroid hormone (RT$_3$) was studied in nude mice with Ewing’s sarcoma xenografts. At 30 min and 1, 2, 4, 8, and 12 hr after injection, blood, tumor and normal organs were measured to determine the amount of radioactivity per gram of tissue. The amount of radioactivity in the blood of tumor-bearing mice decreased sharply from 7.64% of injected dose per gram of tissue (% ID/g) at 30 min after inoculation to 0.45% ID/g at 12 hr. The % of injected dose per gram for $^{125}$I-labeled RT$_3$ in the tumor reached 1.92 at 1 hr after injection and decreased to 0.22 at 12 hr. The radiolocalization indices for tumor to other organs at 12 hr ranged from 1.35 to 4.43. This study suggests increased localization of RT$_3$ in the Ewing’s sarcoma as compared to other tissues in the nude mouse. ——— Ewing’s sarcoma; nude mouse; fetal thyroid hormone; biodistribution study

Fetal thyroid hormone or 3, 3', 5' triiodothyronine (RT$_3$) is a metabolically inactive thyroid hormone after birth that exists in high concentration in the fetus (Nicod et al. 1976; Shulkin and Utiger 1984). RT$_3$ was also found in high concentration in some patients with end stage malignancy (Adami et al. 1978; Dessaint et al. 1978; Persson et al. 1985). In a virus induced erythroleukemia cell model, RT$_3$ was found to stimulate the growth of the erythroleukemia cell in culture (Gauwerky et al. 1981). Ewing’s sarcoma is one of the most lethal of the bone tumors in spite of vigorous combined treatment of irradiation, systemic chemotherapy and surgical ablation. The growth of Ewing’s sarcoma cells in culture has been shown to be stimulated by RT$_3$ (Dutkowsky et al. 1989, 1991). This implies the

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The abbreviations used are: RT$_3$, fetal thyroid hormone, 3, 3', 5' triiodothyronine; %ID/g, % of injected dose per gram of tissue; RI, radiolocalization index = %ID/g in the tumor divided by %ID/g in the tissue.
presence of a receptor to fetal thyroid hormone within these cells which may lead to potential advances in new cancer therapy (Dutkowsky et al. 1989, 1991). This study of the biodistribution of labeled RT₃ in nude mice xenografted with Ewing's sarcoma was undertaken in order to determine if the sarcoma has RT₃ specificity in vivo.

RT₃ labeled with ¹²³I was obtained from Dupont (specific activity: 1.22 mCi/ug, radiochemical purity: >99%, molecular weight: 649, N. Billerica, MA, USA). The ¹²³I-labeled RT₃ (¹²³I-RT₃) was diluted with phosphate buffered saline (PBS, 0.85% sodium chloride in 0.15 M phosphate, pH 7.2, Sigma, St. Louis, MO, USA) for this in vivo study. A cloned Ewing's sarcoma cell line (ES 1) was obtained from the solid tumor culture laboratory of St. Jude Children's Research Hospital. The Ewing's sarcoma was grown in a standard tissue culture media RPMI 1640 (Gibco, Tulsa, OK, USA), containing 5% heat-inactivated fetal calf serum (Sigma), 2 mM L-glutamine (Sigma), and 50 mg/liter of Gentamicine (Elkins-Sinn, Cherry Hill, NJ, USA). The cell line was tested for the presence of mycoplasma and was negative. The cells were subcultured weekly. The sarcoma cells were removed from culture flasks at confluence with trypsin, washed and concentrated in growth medium. Four week old female athymic mice (nu/nu) were obtained from Charles River Laboratorires (Wilmington, MA, USA). After one week acclimation the mice were given subcutaneous injections in the right hind flank with approximately 4 x 10⁶ Ewing's sarcoma cells. A total number of 18 athymic mice bearing tumors 0.3 to 1.5 cm in diameter approximately 3 weeks after inoculation were given intraperitoneal injections of 0.67 µCi of ¹²³I-RT₃ (1 x 10⁶ cpm). The mice (3 per group) were anesthetised with ketamine (67 mg/kg, Aveco Co., Inc., Fort Dodge, IA, USA) and xylazine (13 mg/kg, The Butler Co., Columbus, OH, USA) and sacrificed at 30 min, 1 hr, 2 hr, 4 hr, 8 hr, and 12 hr by exsanguination. The tumor and selected normal tissues were removed and weighed, and radioactivity was measured with a gamma counter (Micromedic, Micromedic, Horsham, PA, USA). The counts per minute per milligram (cpm/mg) were calculated. The methods for evaluating the biodistribution of ¹²³I-RT₃ were derived from the techniques used to study the localization of monoclonal antibodies in nude mice xenografted with tumor cells (Colcher et al. 1983, 1984, 1988; Brown et al. 1987).

The percentage of the injected dose per gram of tissue (%ID/g) for blood, the tumor and the selected normal tissues were determined (Table 1) and radiolocalization indices (RIs) were calculated (%ID/g in the tumor divided by the %ID/g in the tissue) (Table 2).

### Table 1. Percent injected ¹²³I-RT₃ dose per gram

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>7.64</td>
<td>5.16</td>
<td>3.06</td>
<td>1.65</td>
<td>0.78</td>
<td>0.45</td>
</tr>
<tr>
<td>Tumor</td>
<td>0.78</td>
<td>1.92</td>
<td>1.84</td>
<td>1.34</td>
<td>0.54</td>
<td>0.22</td>
</tr>
<tr>
<td>Liver</td>
<td>2.18</td>
<td>2.52</td>
<td>2.15</td>
<td>0.74</td>
<td>0.36</td>
<td>0.16</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.08</td>
<td>1.71</td>
<td>1.42</td>
<td>0.54</td>
<td>0.30</td>
<td>0.08</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.22</td>
<td>2.13</td>
<td>1.81</td>
<td>0.93</td>
<td>0.42</td>
<td>0.10</td>
</tr>
<tr>
<td>Lung</td>
<td>1.39</td>
<td>1.62</td>
<td>1.33</td>
<td>0.70</td>
<td>0.35</td>
<td>0.09</td>
</tr>
<tr>
<td>Heart</td>
<td>1.08</td>
<td>1.14</td>
<td>0.94</td>
<td>0.58</td>
<td>0.23</td>
<td>0.07</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.98</td>
<td>1.07</td>
<td>0.86</td>
<td>1.00</td>
<td>0.26</td>
<td>0.05</td>
</tr>
</tbody>
</table>
As shown in Table 1, the amount of radioactivity in the blood of tumor-bearing mice decreased sharply from 7.64 %ID/g at 30 min postinoculation to 0.45%ID/g at 12 hr. The 125I-RT3 demonstrated an extremely rapid clearance from the plasma, with most of the RT3 out of the plasma pool by 12 hr, which is probably due to its small molecular weight of 649. The %ID/g in the tumor reached a peak of 1.92 at 1 hr after injection and gradually dropped to 0.22 at 12 hr. The 125I-RT3 levels found in the selected normal organs, except the kidney and muscle, also reached their highest %ID/g at 1 hr and dropped to 0.05 to 0.16 at 12 hr. One possible explanation for the low %ID/g (0.05-2.52) of 125I-RT3 in the tissues may be due to intraperitoneal injection of 125I-RT3. It is possible to think that decrease of radioactivity in blood and organs is likely due to dehalogenation.

As shown in Table 2, RIs of the organs ranged from 0.37 to 4.43. RIs of the organs except muscle were less than 1.00 at 30 min. All organ RIs except the liver and kidney exceeded 1.00 at 1 hr and thereafter. RIs of the liver and kidney were still less than or equal to 1.00 at 2 hr. Organ RIs gradually increased to 1.35 to 4.43 at 12 hr. RIs were greater than 3 for spleen, heart, and muscle at 12 hr. The large RIs of the organs show more radioactivity present in the Ewing’s sarcoma than in the other tissues tested. The %ID/g in the tumor tended to be higher than those in the other organs, and in general RI increased with time. To check statistical difference the Student’s t-test was applied. The %ID/g in the tumor was significantly higher than those of liver (p <0.05), spleen (p <0.05), lung (p <0.05) and heart (p <0.01) at 4 hr. These results were suggestive of localization of 125I-RT3 in the Ewing’s sarcoma cloned cell line as compared to other tissues, existence of a RT3 receptor in the Ewing’s sarcoma line tested and the possibility for development of new selective chemotherapeutic agents based on this receptor. However, it is unclear at this time whether a radiolabeled RT3 will have any therapeutic applications, due to its decreased level of tumor binding as compared to radiolabeled monoclonal antibodies. On the other hand, the extremely rapid whole body clearance, potential for rapid penetration through tumors due to its decreased size may lead to consider alternative therapeutic strategies for radiolabeled RT3 such as the administration of multiple doses.

In order to confirm RT3 specificity in Ewing’s sarcoma, control study will be necessary by using non-specific hormone. And since RIs generally increased over time expanded studies using different sampling intervals and multidosing regimens (i.e., redosing at 4 hr) should improve understanding of the pharmacokinetics of RT3. Further investigations of the localization effects of RT3 on Ewing’s sarcoma and other sarcomas are indicated.
Acknowledgments

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References