BIOLOGICAL BEHAVIOR OF $^{99m}$Tc(v)DMSA IN MICE

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ABSTRACT

The $^{99m}$Tc(v)DMSA is a tumor-seeking agent which has been reported in bone metastasis and others tumors scintigraphies. The bone affinity and tumor uptake has been researched to elucidate its mechanism and biological behavior. The aim of this study was to obtain $^{99m}$Tc(v)DMSA from a conventional DMSA kit, to evaluate its biodistribution in an animal model, and to verify the efficiency of this method based on literature data. DMSA kit (produced by IPEN) were used to prepare $^{99m}$Tc(v)DMSA by reconstituting the lyophilized kit with 0.2mL of 3.5% NaHCO$_3$ and addition of $^{99m}$TcO$_4^-$. The biodistribution assays were made with mice (130, 115 and 70 days old), males and females. To each assay, the control group (n=5) received intravenous $^{99m}$Tc-DMSA and the experimental group (n=5) received $^{99m}$Tc(v)DMSA. After 30 min or 1h, the animals were sacrificed, the organs excised and the activity measured by a gamma counter. The uptake percentage per gram (% uptake/g), tissue/blood ratio (kidney/blood - K/BL) and kidney/bone ratio (K/BO) were evaluated. In all assays there were different significant biodistribution (p≤0.05) between control and experimental groups, the results showed the less kidney uptake of $^{99m}$Tc(v)DMSA while increase bone affinity as young as be the animal. The $^{99m}$Tc(v)DMSA arising from DMSA kit by modified binding method was compatible with literature data.

1. INTRODUCTION

The advanced in nuclear medicine is due to new diagnosis techniques and development of radiopharmaceuticals more specifics. Radiopharmaceuticals are drugs with radionuclide inside their structure which are used in diagnosis and therapeutic ways in nuclear medicine. However, more than 95% of them are used for diagnostic purposes. In this respect, the $^{99m}$Tc has become the mainstay radionuclide due its favorable physical chemistry properties: emission of gamma ray of optimal energy (140 keV), a suitable half-life (6h), availability from $^{99}$Mo-$^{99m}$Tc generator systems and possibility labeling reactions [1].

The nuclear medicine techniques are important instruments within oncology to diagnosis, staging and choice of therapeutic strategies. Tumor-seeking agents has been researched and
Yokoyama (1985) reported that $^{99m}$Tc dimercaptosuccinic acid with the Tc-atom in the 5+ oxidation state [$^{99m}$Tc(v)-DMSA] had affinity for Ehrlich ascites cells, then it might be of considerable value in the detection of various tumors and their metastases [2].

Metastases are about 90% morbidity cause in oncological patients. Bone metastasis is more common in mama, prostate and lung cancer [3,4]. Conventional bone scanning is highly sensitive but not specific for the detection of secondary bone lesions.

The $^{99m}$Tc(v)-DMSA scintigraphy has a well established role in medullary thyroid carcinoma and some other soft tissue tumors [5,6]. On the other hand, it holds considerable promise is for seeking of bone metastasis. A similarity has been observed between $^{99m}$Tc(v)-DMSA and $^{99m}$Tc-MDP uptake for osseous and nonosseuos lesions after bone scan [7].

Has been reported that $^{99m}$Tc(v)-DMSA didn’t fix in normal physiologic bone [8] and showed specificity for some brain and lung cancer [9,10]. The $^{99m}$Tc(v)-DMSA uptake mechanism is unknown, wherever recent studies about molecular biology has elucidated its bone and tumoral affinity [11].

The aim of this study was to obtain $^{99m}$Tc(v)DMSA from a conventional DMSA kit, to evaluate its biodistribution in an animal model, and to verify the efficiency of method comparing with literature data.

2. MATERIALS AND METHODS

2.1. Ethics Aspects

The follow procedures were submitted and approved by Animal Experimentation Ethics Committee of Biological Sciences Center of Federal University of Pernambuco (Comitê de Ética em Experimentação Animal / Centro de Ciências Biológicas / Universidade Federal de Pernambuco - CEEA-UFPE, of. 017/06).

2.2. Animals

Mice (Mus musculus ‘Swiss’) were used for the biodistribution assays. The animals received a standard pelleted mouse diet and water *ad libitum*, and were maintained under environmental conditions (25 ± 3 °C, 12h of light/dark cycles). The assays were made with 130, 115 and 70 days old mice (30-40g), both males and females.

2.3 Radiopharmaceuticals Prepare

Commercial DMSA kits (IPEN-Brazil) were utilized. The $^{99m}$Tc-DMSA was prepared under standard method and the $^{99m}$Tc(v)-DMSA was made by lyophilized kit reconstituting with
0.2mL of 3.5% NaHCO₃ following TcO₄⁻ elute addition. Each of them received 5.55 MBq (150µCi) activities (⁹⁹Mo-⁹⁹mTc generator, IPEN-Brazil).

2.4 Biodistribution Assay

To each assay, the control group (n=5) received ⁹⁹mTc-DMSA and the experimental group (n=5) received ⁹⁹mTc(v)DMSA, both them 74-111kBq (2-3µCi) administered intravenous (tail vein). After determinate time (30 min or 1h), the animals were anesthetized (50mg/kg thiopental) sacrificed, their organs excised and weighed, and the activity measured by a NaI(Tl) well scintillation counter (NZ-138 Shielded Counter and ND-302/E photomultiplier tube – GAMA; 2012 amplifier, 2030 pulse height analyzer and 1772 counter time - CANBERRA). Uptake percentage per gram (% uptake/g), tissue/blood ratio (kidney/blood - K/BL) and kidney/bone ratio (K/BO) were evaluated, and it was considered significance level p≤0.05 (test-T).

3. RESULTS AND DISCUSSION

The experiment was made in three assays. In the first one, the mice, 115 days old, were sacrificed 30 min after radiopharmaceuticals injection. There was significant difference in behavior. In the % uptake/g there was a reduction in blood and kidney of experimental group compared to control, although the uptake in bone increased (p≤ 0.02) as well the bone/blood ratio (p≤ 0.01) [Tab.1]. The results showed the difference between DMSA and (v)DMSA, indicating that the second one has not affinity for kidney tissue and uptakes in more proportion in bone, which is evidenced by reduction of kidney/bone ratio (p≤ 0.02).

Table 1: ⁹⁹mTc-DMSA and ⁹⁹mTc(v)DMSA biodistribution 30min and 1h after injection.

<table>
<thead>
<tr>
<th></th>
<th>30min after injection</th>
<th>1h after injection</th>
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<tbody>
<tr>
<td></td>
<td>tissue/blood</td>
<td>% uptake/g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99mTc-DMSA</td>
</tr>
<tr>
<td>blood</td>
<td></td>
<td>5.8 ± 2.3</td>
</tr>
<tr>
<td>kidney</td>
<td>3.0 ± 1.2</td>
<td>15.5 ± 4.8</td>
</tr>
<tr>
<td>Bone (femur)</td>
<td>0.52 ± 0.16</td>
<td>2.81 ± 0.71</td>
</tr>
<tr>
<td>kidney/bone</td>
<td>6.1 ± 2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>99mTc-(v)DMSA</td>
</tr>
<tr>
<td>blood</td>
<td></td>
<td>1.69 ± 0.40</td>
</tr>
<tr>
<td>kidney</td>
<td>4.2 ± 3.2</td>
<td>6.5 ± 3.3</td>
</tr>
<tr>
<td>Bone (femur)</td>
<td>2.65 ± 0.88</td>
<td>4.27 ± 0.72</td>
</tr>
<tr>
<td>kidney/bone</td>
<td>1.48 ± 0.59</td>
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</table>

Average ± standard deviation (n=5)
In the second assay, the time between radiopharmaceutical injection and sacrifice was longer: the mice (130 days old) were sacrificed 1h after the administration. The results showed the less kidney uptake of $^{99m}$Tc(v)DMSA. The % uptake/g of kidney decreased from $26.00 \pm 7.32\%$ to $3.6 \pm 0.99\%$ ($p \leq 0.02$) and kidney/blood ratio from $10.66 \pm 2.34$ to $4.22 \pm 1.22$ ($p \leq 0.03$) related control to experimental groups, respectively, whereas kidney/bone ratio decreased of $11.61 \pm 1.74$ to $1.12 \pm 0.62$ ($p \leq 5 \times 10^{-6}$).

In this case, the difference between experimental and control groups was more expressive. At 1 hour after injection, the DMSA fixed more in the kidney but the (v)DMSA decreased its kidney uptake and didn’t change the kidney/bone ratio. This experimental condition let available better the in vivo radiopharmaceutical affinity. The kidney/bone ratios are particularity expressive, because they indicate less kidney uptake in (v)DMSA administration related to bone uptake. These data are significant as to 30 min as well to 1h, but more accentuate in the second case.

In the last one assay, mice 70 days old were used in biodistribution. The % uptake/g to the blood decreased from $3.24 \pm 0.76$ to $0.82 \pm 0.23$ and to the kidney it decreased from $34.55 \pm 6.01$ to $4.77 \pm 1.22$ related control to experimental groups ($p \leq 0.03$). The kidney/bone ratio decreased from $16.30 \pm 2.24$ (control) to $0.77 \pm 0.23$ (experimental). These data are in agreement with Yokoyama et al (1985) [Tab. 2].

**Table 2: Comparison of literature data about tissue % uptake/g related to administered activity**

<table>
<thead>
<tr>
<th></th>
<th>Mice biodistribution</th>
<th>Rats biodistribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Data presents*</td>
<td>Yokoyama et al, 1985</td>
</tr>
<tr>
<td>blood</td>
<td>0.82 ± 0.23</td>
<td>0.86 ± 0.17</td>
</tr>
<tr>
<td>kidney</td>
<td>4.77 ± 1.22</td>
<td>3.8 ± 0.47</td>
</tr>
<tr>
<td>bone</td>
<td>6.23 ± 0.52</td>
<td>7.66 ± 0.35</td>
</tr>
</tbody>
</table>

Average ± standard deviation; * 70 days old animals (n=4)

Westera et al (1985) and Wasburn et al (1995) published data of rats biodistribution (Wistar) [12,13]. The data show that there are differences between mice and rats biodistribution, specially related to bone uptake. Yokoyama [3], however, reported that mice have immature state bone; maybe this can explain the difference of (v)DMSA bone uptake observed between mice and rats.

The $^{99m}$Tc(v)DMSA obtained was compatible with literature data, without kidney affinity and with more uptake in ossification processes (osteoblastic activity). The results concerning kidney are the most representative in biological behavior, because they express the difference in molecules obtained from binding process. If the binding origins $^{99m}$Tc(v)DMSA, this molecule won’t have affinity for kidney tissue.
4. CONCLUSIONS

The binding method employed in this work was efficient, the $^{99m}$Tc(v)DMSA arising from a DMSA kit (IPEN) showed the same biological behavior reported in literature.

ACKNOWLEDGMENTS

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REFERENCES