In vitro and in vivo evaluation of $^{99m}$Tc - pyrophosphate capability to bind *Staphylococcus aureus*


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**SUMMARY**

**Introduction:** Scintigraphic imaging of infection and inflammation is of special interest in nuclear medicine diagnostic of infectious or inflammatory diseases. For this purpose various radiolabelled compounds have been explored.

**The aim:** The aim of this study was to find out whether $^{99m}$Tc-PYP posses capability to bind to *Staphylococcus aureus*, and possibilities for its use in bacterial infection and inflammation not only in non-specific way.

**Methodology:** $^{99m}$Tc-PYP has been used for imaging infective and non-infective skeletal diseases. Protein binding, lypophilicity measurements and in vitro binding to viable and dead bacteria of $^{99m}$Tc-PYP with 3 different concentrations of sodium pyrophosphate decahydrate were studied. Wistar rats were used in all biodistribution evaluations.

**Results:** All $^{99m}$Tc-PYP samples were on high radiochemical purity, with high protein binding and hydrophilic character. In vitro investigations have shown that the uptake of $^{99m}$Tc-PYP to *Staphylococcus aureus* was depended on concentration of pyrophosphate decahydrate in the samples. Thus the highest uptake to viable *Staphylococcus aureus* (>30 %) was obtained in the sample with 0.10 mg pyrophosphate decahydrate/1 ml. The in vivo investigation results on rats shown increased radioactivity in the infected thigh muscle (T/NT>2.3) and intensify bone uptake (5.4 ÷ 6.9 % ID/g).

**Conclusion:** Considering that the diagnosis of bone or joint infection remains a challenging problem, it is obvious how important is to investigate whether $^{99m}$Tc-PYP could be used as a specific agent for bacterial infection in the axial skeleton.

**Keywords:** $^{99m}$Tc-pyrophosphate, *Staphylococcus aureus*, infection, nuclear medicine

**INTRODUCTION**

Scintigraphic imaging of infection and inflammation is of special interest in nuclear medicine diagnostic of infectious or inflammatory diseases. For this purpose various radiolabelled compounds have been explored [1-4]. The main goal of these investigations was to find out some compound labelled with technetium which had also capability to distinguish infection from sterile inflammation. Among the labelled compounds, white blood cells (leukocytes) labelled with indium-111 or technetium-99m, by use of $^{99m}$Tc - hexam-
ethylpropylene amine oxime ($^{99m}$Tc-HMPAO) could be useful in infection diagnostic in clinical practice [5-8]. Recently, a new radiopharmaceutical $^{99m}$Tc-ciprofloxacin has been developed [9-12]. Compared with radiolabelled leukocyte scintigraphy, $^{99m}$Tc-ciprofloxacin scintigraphy supposes to be clinically more effective and specific for bacterial infection [8], but the reliability of its use is still in the phase of investigation.

Perez et al. [13] proposed $^{99m}$Tc-Sn complex of pyrophosphate ($^{99m}$Tc-PYP) for skeletal imaging soon after Subramanian et al. [14, 15] demonstrated that stable complex formed between $^{99m}$Tc and linear polyphosphates localised in skeletal lesion in higher degree than in normal bone. Some new $^{99m}$Tc-Sn complexes of polyphosphates as: ethane-1-hydroxy-1,1-diphosphonate (EHDP), methylene diphosphonate (MDP) and 2,3-dicarboxy-propane-1,1-diphosphonic acid (DPD) were proposed later as better agents for bone imaging, although the mechanisms of their localisation in skeletal was not completely understood [16]. Meanwhile, $^{99m}$Tc-PYP was used for visualization of acute myocardial infarction, for in vivo labelling of red blood cells (with $^{99m}$Tc) for radionuclide ventriculography and blood pool scintigraphy [17].

The aim of this study was to find out whether $^{99m}$Tc-PYP possesses capability to bind to Staphylococcus aureus, and possibilities for its use in bacterial infection and inflammation not only in non-specific way.

**MATERIAL AND METHODS**

$^{99m}$Tc-(Sn)-pyrophosphate was prepared by in vitro labelling with technetium. Three milliliters of $^{99m}$Tc as Na$^{99m}$TcO$_4$ elute from $^{98m}$Mo/$^{99m}$Tc generator (“Vinča” generator) were added to tin (II)-complexes of pyrophosphate obtained in lyophilised form. Pyrophosphate solutions were prepared with three different concentrations of sodium pyrophosphate decahydrate (Na$_4$P$_2$O$_7$•10 H$_2$O, p.a. Fluka, Switzerland) and stannochloride dihydrate (SnCl$_2$•2H$_2$O, p.a. Merck, Germany) in the following manner:

- PYP/1: 6.71 mg of Na$_4$P$_2$O$_7$•10 H$_2$O and 1.33 mg SnCl$_2$•2H$_2$O/1 ml;
- PYP/2: 1.02 mg of Na$_4$P$_2$O$_7$•10 H$_2$O and 0.20 mg SnCl$_2$•2H$_2$O/1 ml;
- PYP/3: 0.10 mg of Na$_4$P$_2$O$_7$•10 H$_2$O and 0.02 mg SnCl$_2$•2H$_2$O/1 ml.

The samples were prepared under the same conditions and with the same pH=5.0±5.5. A routine quality control of the radiochemical purity of all batches was done before their use. In this purpose the ascending paper chromatography (Whatman N°1) with 80% methanol and silica-gel plates (Merck) with 0.9% NaCl (p.a. Merck) were used.

**Protein binding** – The standard trichloracetic acid (TCA) precipitation method for determining the percentage of $^{99m}$Tc-PYP/1 and $^{99m}$Tc-PYP/3 bound to proteins was used [18]. 0.1 ml of the labelled compound was added to 1.5 ml of 12% human albumin (12% HA, National Blood Transfusion Institute, Belgrade) and thermostated at 37°C for different time intervals. After incubation period, 3 ml 20% w/v trichloracetic acid was added. Precipitate was separated from the solution by centrifugation (three times, 5 min, 3000 g) and rinsed with 0.9% NaCl (saline). The radioactivity of both phases (total $^{99m}$Tc) was measured separately in γ-counter with NaI (TI) detector. The radioactivity bound to HA was expressed in percent to total radioactivity of $^{99m}$Tc.

**Lipophilicity measurements** - All lipophilicity measurements for $^{99m}$Tc-PYP were done by solvent extraction method with n-octanol equilibrated with 0.15 mol dm$^{-3}$ phosphate buffers, pH=3÷7 [19]. The 50 μl samples of $^{99m}$Tc-PYP/1 and $^{99m}$Tc-PYP/3 (~74 kBq) were added to each of duplicate test tubes containing 1050 μl phosphate buffer. After mixing 100 μl was removed from the test tubes (labelled “A$_0$”). To exactly 1000 μl of l-octanol was added and after vortexing of each tube for 1 min and centrifugation at 3000 g for 5 min, 100 μl sample from aqueous phase was removed (labelled “A$'$”). Samples A$_0$ and A$'$ were counted in gamma counter and distribution coefficients were calculated using equation:

$$\frac{\text{cpm organic}}{\text{total cpm}} = \frac{A_0 - A_1}{A_0}$$

where cpm means counts per minute. All measurements were performed at room temperature.

In vitro bacteria binding assay - In vitro binding of $^{99m}$Tc-pyrophosphate to viable bacteria was investigated using S. aureus ATCC 25923 (approximately 2x10$^{10}$ CFU/ml, CFU- colony forming units). Under the same conditions in vitro binding to dead bacteria
was study. In this purpose a vial with viable S. aureus was heated in steam (autoclaving) at 120°C under 101.325 kPa (1 Atm) for thirty minutes. After that the samples were plated directly on surface of suitable growth media to confirm the dead of bacteria. The survival of bacteria was estimated by counting the colonies 48 hours and 4 days after incubation at a temperature of 37°C. As there was no grown of bacteria on incubated media, the samples were used for in vitro binding assay.

For examination the following samples were prepared:
- 0.5 ml (~74 MBq) of ⁹⁹ᵐTc-PYP (PYP/1, PYP/2 or PYP/3) with 1 ml suspension of bacteria in saline;
- 0.5 ml of ⁹⁹ᵐTc-PYP (PYP/1, PYP/2 or PYP/3) with 1 ml saline;
- 0.5 ml ⁹⁹ᵐTc⁴⁺ with 1 ml suspension of bacteria in saline;
- 0.5 ml ⁹⁹ᵐTc⁴⁺ with 1 ml saline;
- 0.5 ml ⁹⁹ᵐTc⁴⁺, 0.5 ml Sn (II) (2.66 mg/ml) with 1 ml suspension of bacteria in saline;
- 0.5 ml ⁹⁹ᵐTc⁴⁺, 0.5 ml Sn (II) (2.66 mg/ml) with 1 ml saline.

The samples were incubated one hour at 37°C and then centrifuged for 15 min at 3000 g. The supernatant was removed and the radioactivity in the tubes was determined in a gamma counter. The radioactivity related to pellets was expressed as:

\[
\text{Average} = \frac{\text{radioactivity in pellet}}{\text{Total}} \times 100
\]

where Total means total radioactivity in prepared ⁹⁹ᵐTc-PYP standards. The percentage binding of pyrophosphate to bacteria was the difference of the above: \(A_b = A_s\), where \(A_s = (\text{Average})\) was radioactivity related to bacteria and \(A_b = (\text{Average})\) radioactivity related to saline. All measurements were performed at room temperature, three times in duplicate for each value.

In vivo bacteria binding assay – Male white Wistar rats (four weeks old, 100g) were used as a model in all animal studies. The suspension containing S. aureus (0.3 ml, ~2x10⁸ CFU/ml) was injected into the right thigh muscle of the rats. Twenty four or forty eight hours later, 0.1 ml of ⁹⁹ᵐTc-PYP/3 (~74 kBq) was injected via the tail vein. 1 or 4 hours after the injection of ⁹⁹ᵐTc-PYP, the rats were sacrificed and biodistribution was determined. Samples of infected thigh muscle, contralateral normal thigh muscle, bone (femur) and blood were weighted and radioactivity was measured in gamma counter. The radioactivity of other organs was measured too. The results were expressed as the percentage uptake of injected dose per gram of tissue (% ID/g) or per organ (% ID/organ), the infected-to-normal thigh muscle ratio and the infected thigh muscle-to-blood ratio. In the same time the biodistribution of ⁹⁹ᵐTc-PYP/1 and ⁹⁹ᵐTc-PYP/3 was examined in control group of animals. Six animals were used for each of the experiment. All animal experiments were carried out in compliance with United Kingdom Biological Council’s Guidelines on the Use of Living Animals in Scientific Investigations, 2nd edn.

Statistical analysis – All data were expressed as means ± standard deviation (SD). The statistical analysis of biodistribution evaluation results was performed by One-way analysis of variance (ANOVA) and Independent t-test (at significance levels of 0.01 and 0.05) to determine the significance in the distinction between different groups.

### RESULTS

All three different preparation of PYP (PYP/1, PYP/2 and PYP/3) labelled with ⁹⁹ᵐTc were of high radiochemical purity (>95 %). Considering that in vitro bacteria binding experiments were performed with ⁹⁹ᵐTc-PYP prepared with three different concentrations of Na₄P₂O₇·10H₂O and SnCl₂·2H₂O, the influence of their concentrations on protein binding and lipophilicity results were investigated too. The results were presented in Table 1 and Table 2.

**Table 1.** The percentage of protein binding (%) of ⁹⁹ᵐTc-PYP/1 and ⁹⁹ᵐTc-PYP/3 (12% HA, TCA- method, 370)

Values represent the means±SD, (n=6)

<table>
<thead>
<tr>
<th>Radiopharmaceuticals</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>⁹⁹ᵐTc-PYP/1</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>84.88±0.23</td>
</tr>
<tr>
<td>⁹⁹ᵐTc-PYP/3</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>84.73±0.56</td>
</tr>
</tbody>
</table>

The protein binding measurements have shown that the differences in concentration of pyrophosphate did not influence on protein binding measurement results. ⁹⁹ᵐTc-PYP/1 and ⁹⁹ᵐTc-PYP/3 both exhibited high level of binding for HA, which was in accordance with literature data [20]. The lipophilicity measurement results have shown that ⁹⁹ᵐTc-PYP possessed hydrophilic behavior independent on formulations and pH.

The organ distribution results of ⁹⁹ᵐTc-PYP/1 and ⁹⁹ᵐTc-PYP/3 in control group
Table 2. n-Octanol-buffer distribution coefficients (Kd) of $^{99m}$Tc-PYP/1 and $^{99m}$Tc-PYP/3

<table>
<thead>
<tr>
<th>Kd</th>
<th>pH</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>6.0</th>
<th>7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{99m}$Tc-PYP/1</td>
<td></td>
<td>0.018±0.003</td>
<td>0.040±0.002</td>
<td>0.040±0.003</td>
<td>0.053±0.004</td>
<td>0.066±0.004</td>
</tr>
<tr>
<td>$^{99m}$Tc-PYP/3</td>
<td></td>
<td>0.087±0.006</td>
<td>0.040±0.003</td>
<td>0.060±0.004</td>
<td>0.073±0.005</td>
<td>0.063±0.004</td>
</tr>
</tbody>
</table>

Values represent the means±SD (n=6)

of animals were presented in Table 3. The influence of pyrophosphate concentration on in vivo behavior of $^{99m}$Tc-PYP in control, non-treated group of rats was confirmed. There was statistically significant difference in organ uptake for: lungs, liver, kidneys, intestine, bone, and blood. Statistical analysis confirmed these significant differences in bacteria uptake (ANOVA and independent t-test, at both level: p<0.01 and p<0.05).

In Table 4 in vitro binding of $^{99m}$Tc-PYP/1, $^{99m}$Tc-PYP/3 and $^{99m}$TcO$_4^-$ to viable S. aureus was the highest, in vivo behavior of only this formulation was examined in infected group of animals. In both in vitro and in vivo bacteria assays, the ratio between concentration of PYP or Sn (II) and bacteria number, were almost equal. The organ distribution results of $^{99m}$Tc-PYP/3 in animals infected during different time and sacrificed 60 or 240 min after injection were presented in Table 6. A statistical analysis of the results for control group of animals and animals infected 24 h and sacrificed 60 min after application showed that there was no bacteria uptake of $^{99m}$Tc-PYP to killed bacteria.

As bacteria uptake of $^{99m}$Tc-PYP/3 by S. aureus was the highest, in vivo behavior of only this formulation was examined in infected group of animals. In both in vitro and in vivo bacteria assays, the ratio between concentration of PYP or Sn (II) and bacteria number, were almost equal. The organ distribution results of $^{99m}$Tc-PYP/3 in animals infected during different time and sacrificed 60 or 240 min after injection were presented in Table 6. A statistical analysis of the results for control group of animals and animals infected 24 h and sacrificed 60 min after application showed that there was no bacteria uptake of $^{99m}$Tc-PYP to killed bacteria.

Table 3. Biodistribution results of $^{99m}$Tc-PYP/1 and $^{99m}$Tc-PYP/3 in control group of animals, sacrificed 60 min after i.v. application of radiopharmaceuticals

<table>
<thead>
<tr>
<th>Organ</th>
<th>Radiochemicals</th>
<th>$^{99m}$Tc-PYP/1</th>
<th>$^{99m}$Tc-PYP/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs</td>
<td></td>
<td>0.128±0.011</td>
<td>0.294±0.034$^{ab}$</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>22.64±2.976</td>
<td>2.06±2.73$^{b}$</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td>0.46±0.135</td>
<td>0.17±0.080$^{a}$</td>
</tr>
<tr>
<td>Kidneys</td>
<td></td>
<td>1.42±0.248</td>
<td>11.7±1.730$^{ab}$</td>
</tr>
<tr>
<td>Stomach</td>
<td></td>
<td>0.45±0.357</td>
<td>0.33±0.191</td>
</tr>
<tr>
<td>Intestines</td>
<td></td>
<td>1.96±0.545</td>
<td>5.23±1.154$^{b}$</td>
</tr>
<tr>
<td>Bone*</td>
<td></td>
<td>4.26±0.319</td>
<td>3.02±0.013$^{b}$</td>
</tr>
<tr>
<td>Thigh muscle*</td>
<td></td>
<td>0.30±0.012</td>
<td>0.08±0.009$^{b}$</td>
</tr>
<tr>
<td>Blood*</td>
<td></td>
<td>0.158±0.032</td>
<td>0.56±0.007$^{b}$</td>
</tr>
</tbody>
</table>

Values are expressed as means±SD (n=6) of the percent injected dose per organ or * per g of organ. The symbol represents statistical significance: a-represents comparison between groups $^{99m}$Tc-PYP/3 and $^{99m}$Tc-PYP/1 at the p < 0.01 level; b-represents comparison between groups $^{99m}$Tc-PYP/3 and $^{99m}$Tc-PYP/1 at the p < 0.05 level. (ANOVA and independent t-test)

Table 4. In vitro binding of $^{99m}$Tc-PYP, Sn (II) + $^{99m}$TcO$_4^-$ and $^{99m}$TcO$_4^-$ to viable S. aureus

<table>
<thead>
<tr>
<th>Samples</th>
<th>Average</th>
<th>% binding</th>
<th>$A_x-A_x$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x10$^8$ bacteria in 1 ml saline + 0.5 ml $^{99m}$TcO$_4^-$</td>
<td></td>
<td>0.01±1.12</td>
<td>0±0.42</td>
</tr>
<tr>
<td>1 ml saline + 0.5 ml $^{99m}$TcO$_4^-$</td>
<td></td>
<td>0.01±0.70</td>
<td></td>
</tr>
<tr>
<td>2x10$^9$ bacteria in 1 ml saline + 0.5 ml Sn (II) + 0.5 ml $^{99m}$TcO$_4^-$</td>
<td></td>
<td>89.89±96.40</td>
<td>0±3.64</td>
</tr>
<tr>
<td>1 ml saline + 0.5 ml Sn (II) + 0.5 ml $^{99m}$TcO$_4^-$</td>
<td></td>
<td>90.33±97.26</td>
<td></td>
</tr>
<tr>
<td>2x10$^8$ bacteria in 1 ml saline + 0.5 ml $^{99m}$Tc-PYP/1</td>
<td></td>
<td>1.26±2.89</td>
<td>0±0.88</td>
</tr>
<tr>
<td>1 ml saline + 0.5 ml $^{99m}$Tc-PYP/1</td>
<td></td>
<td>1.30±2.01</td>
<td></td>
</tr>
<tr>
<td>2x10$^9$ bacteria in 1 ml saline + 0.5 ml $^{99m}$Tc-PYP/2</td>
<td></td>
<td>10.25±13.76</td>
<td>8.28±11.02</td>
</tr>
<tr>
<td>1 ml saline + 0.5 ml $^{99m}$Tc-PYP/2</td>
<td></td>
<td>1.97±2.74</td>
<td></td>
</tr>
<tr>
<td>2x10$^8$ bacteria in 1 ml saline + 0.5 ml $^{99m}$Tc-PYP/3</td>
<td></td>
<td>37.50±51.30</td>
<td>31.15±39.33</td>
</tr>
<tr>
<td>1 ml saline + 0.5 ml $^{99m}$Tc-PYP/3</td>
<td></td>
<td>6.35±11.97</td>
<td></td>
</tr>
</tbody>
</table>

Ab = Average of bacteria = (the radioactivity of Pellet/Total) x100 for tubes with bacteria. As = Average of saline = (the radioactivity of Pellet/Total) x100 for tubes with saline. The symbol represents statistical significance: a-represents comparison between group $^{99m}$Tc-PYP/2 and its control group at the p < 0.01 and p < 0.05 levels; b-represents comparison between group $^{99m}$Tc-PYP/3 and its control group at the p < 0.01 and p < 0.05 levels. (ANOVA and independent t-test)
of $^{99m}$Tc-PYP/3 has shown that there was significant statistical difference at both levels of significance for spleen, kidneys, stomach, intestine, bone, infected thigh muscle and blood. There was no difference for lungs, liver as well as non-infected thigh muscle. The statistical analysis of the results for control group of animals and animals infected 24 h, but sacrificed 240 min after application of $^{99m}$Tc-PYP/3 has shown that there was significant statistical difference at both levels of significance for lungs, liver, kidneys, bone, infected thigh muscle and blood, but there was no statistical difference for spleen, stomach, intestine and non-infected thigh muscle. If two groups of animals infected 24 h, but sacrificed 48 h was done, the statistical comparison of the uptake of 99mTc-PYP/3, for infected animals and non-infected animals (control group) were of special interest. The results point at significant difference at both levels of significance for lungs, spleen, kidney, bone muscle and blood was confirmed.

The comparisons of the thigh muscle and bone uptake for infected and non-infected animals (control group) were of special interest. The results point at significant difference at both levels of significance for lungs, spleen, kidney, bone muscle and blood was confirmed.

Values are expressed as means SD (n=6) of the percent of injected dose per organ or tissue or * per g of organ or tissue

The symbol represents statistical significance:

- A-represents comparison between group 48h (240 min) and control group;
- B-represents comparison between group 24h (240 min) and control group;
- C-represents comparison between groups 24h (60 min) and 24h (240 min);
- D-represents comparison between groups 48h (60 min) and 48h (240 min) at the p < 0.01 and p < 0.05 levels

(ANOVA and independent t-test)

### Table 5. In vitro binding of $^{99m}$Tc-PYP and $^{99m}$TcO$_4$- to dead S. aureus (2x10$^8$ CFU/ml)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Average</th>
<th>% binding</th>
<th>A$_b$</th>
<th>A$_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead bacteria in 1 ml saline + 0.5 ml $^{99m}$TcO$_4$</td>
<td>A$^b$</td>
<td>0.01±0.56</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1 ml saline + 0.5 ml $^{99m}$TcO$_4$</td>
<td>A$_a$</td>
<td>0.01±0.70</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dead bacteria in 1 ml saline + 0.5 ml $^{99m}$Tc-PYP/1</td>
<td>A$^b$</td>
<td>1.40±2.70</td>
<td>0.10±0.69</td>
<td>-</td>
</tr>
<tr>
<td>1 ml saline + 0.5 ml $^{99m}$Tc-PYP/1</td>
<td>A$_a$</td>
<td>1.30±2.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dead bacteria in 1 ml saline + 0.5 ml $^{99m}$Tc-PYP/3</td>
<td>A$^b$</td>
<td>6.54±7.63</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1 ml saline + 0.5 ml $^{99m}$Tc-PYP/3</td>
<td>A$_a$</td>
<td>6.35±11.97</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 6. Biodistribution results of $^{99m}$Tc-PYP/3 in infected animals

The symbol represents statistical significance:

- A-represents comparison between group 48h (240 min) and control group;
- B-represents comparison between group 24h (240 min) and control group;
- C-represents comparison between groups 24h (60 min) and 24h (240 min);
- D-represents comparison between groups 48h (60 min) and 48h (240 min) at the p < 0.01 and p < 0.05 levels

(ANOVA and independent t-test)

### Table 7. T/NT and T/B ratios of $^{99m}$Tc-PYP/3, for infected animals, sacrificed 60 and 240 min after administration of $^{99m}$Tc-PYP/3

<table>
<thead>
<tr>
<th>Time after infection (h)</th>
<th>Time after administration of $^{99m}$Tc-PYP/3 (min)</th>
<th>T/NT ratio</th>
<th>T/B ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>2.529</td>
<td>0.618</td>
<td>4.177</td>
</tr>
</tbody>
</table>

The higher percentage of radioactivity in the bone tissue (femur) for infected animals than in control group of animals could be the consequence of infection site on bone.
There was significant difference at significance levels of 0.01 and 0.05 (ANOVA and independent t-test), between the blood uptake for infected and control group of animals. Also there was statistically significantly difference between the blood uptakes in animals infected for different time.

The infected-to-normal thigh muscle ratio and the infected thigh muscle-to-blood ratio were shown in Table 7. As it could be seen, the obtained results point at increased radioactivity in infected thigh muscle (T/NT>2.3). There was some difference in thigh muscle uptake in dependence on the time after infection. At significance levels of 0.01 and 0.05 (ANOVA and independent t-test), the means were statistically significantly different for all treated animals, but T/NT ratio was higher in 48 hours-infection animals. The ratio between the target and non-target thigh muscle obtained 24 h after infection was higher in animals sacrificed 4 hour after administration of $^{99m}$Tc-PYP than for animals sacrificed 1 hour after administration. The same results were obtained for 48-hours infection animals. At the 0.05 as well as 0.01 level, there was statistically significant difference in thigh muscle uptake between two groups of treated animals. There were no significant differences at the level 0.05 or 0.01 between contra lateral normal tight in infected animals and tight in control group of animals.

**DISCUSSION**

$^{99m}$Tc-PYP has been used successfully for imaging skeletal diseases, infective and non-infective, like osteomyelitis, bone tumors or metabolic bone diseases [22, 23]. It was used in radionuclide scanning joint imaging and evaluation of articular disease [24]. There were also some attempts to use $^{99m}$Tc-PYP in staphylococcal sepsis in whole pelvic osteomyelitis [25] and staphylococcus aureus meningitis associated with pyogenic infection of the sacroiliac joint [26]. These results have shown that radionuclid scanning with $^{99m}$Tc-PYP could be helpful.

As a bone seeking imaging agent $^{99m}$Tc-PYP is used in imaging infection and inflammation as non-specific radiopharmaceuticals. The main uptake mechanisms of this agent could be increased vascular permeability and increased bone metabolisms [27]. Besides this, the use of $^{99m}$Tc-PYP for in vitro labelling of human leukocyte is well known. In infection and inflammation diseases the response of leukocytes occurs rapidly. They migrate to sites of infection by chemotaxis and might be used to transport radiotracers to the infected area [27]. As their functional behaviour did not seem affected by labelling, such $^{99m}$Tc-labelled leukocytes might be used for specific infection and inflammation imaging [28].

In this study we didn't investigate in what extent $^{99m}$Tc-PYP bound to leukocytes. If it was assumed that some percentage of leukocytes was labelled through $^{99m}$Tc-PYP in vivo, such labelled leukocytes could enhance the uptake of radiotracer in thigh muscle and bone of infected animals.

On the other hand, in this study the capability of $^{99m}$Tc-PYP to bind viable S. aureus was estimated. It is well known that staphylococcus aureus is the leading cause of osteomyelitis and the presence of staphylococcus aureus in bone morrow cultures in a rabbit model for S. aureus osteomyelitis was found out earlier [29]. Therefore it could be presumed that the increased bone uptake in osteomyelitis was the consequence of $^{99m}$Tc-PYP uptake to S. aureus in bone morrow.

**CONCLUSION**

Considering that the diagnosis of bone or joint infection remains a challenging problem, it is obvious how important it is to investigate whether $^{99m}$Tc-PYP could be used as a specific agent for bacterial infection in the axial skeleton. The attempts to use $^{99m}$Tc-PYP in staphylococcal infection have to be continued and comprehensive clinical studies of this well-known radiopharmaceutical in specific infection and inflammation imaging ought to confirm its diagnostic capability.

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In vitro i in vivo ispitivanja mogućnosti primene 99mTc-pirofosfata u detekciji bakterijskih infekcija izazvanih Staphilococcus aureusom

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KRATAK SADRŽAJ

Uvod: Jedna od najvažnijih savremenih primena nuklearne medicine je detekcija infektivnih žarišta. 99mTc-pirofosfat je prvobitno napravljen i primenjivan za scintigrafiju kostiju (metastaze, primarni tumor, vaskularne bolesti, inflamatorne bolesti, metaboličke bolesti i dr).

Cilj: Cilj ovog rada je procena da li 99mTc-PYP ima sposobnost vezivanja za Staphilococcus aureus, te mogućnost upotrebe za detekciju i evaluaciju infektivnih žarišta izazvanih Staphilococcus aureusom na modelu pacova.


Rezultati: Sva tri uzorka 99mTc-pirofosfata koji su korišćeni u ovim ispitivanjima su imali radiohemijsku čistoću veću od 95%, visok procenat vezivanja za protein, kao i hidrofilni karakter. In vitro istraživanja su pokazala da vezivanje 99mTc-pirofosfata za bakteriju je zavisilo od koncentracije pirofosfat dekahidrata u samim uzorcima. Tako je najveći procenat vezivanja za vijabilni S. aureus (> 30%) dobijeno u uzorku sa 0.10 mg pirofosfat dekahidrata/1 ml (99mTc-PYP/3). In vivo rezultati na pacovima pokazali su povećano nakupljanje 99mTc-PYP/3 u inficiranom butinom mišiću (T/NT>2.3) kao i povećano nakupljanje u butnoj kosti (5.4±6.9 % ID/g).

Zaključak: S obzirom da dijagnoza bakterijskih infekcija u kostima i zglobovima i dalje ostaje problem u medicini veoma je važno da se nastave ova ispitivanja da bi se potvrdilo da li 99mTc-PYP/3 bi mogao da se koristi kao specifičan agens za bakterijske infekcije u aksijalnom skeletu.

Ključne reči: 99mTc-pirofosfat, Staphilococcus aureus, infekcija, nuklearna medicina

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