A comparative analysis of pharmacokinetics properties of diagnostic bone-seeking radiopharmaceuticals on the basis of phosphonic acids and technetium-99m

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Abstract. This work is devoted to comparative research of pharmacokinetics properties of four bone-seeking radiopharmaceuticals (RPP) on the basis of bi-, tetra- and penta-phosphonic acids. Biodistribution studies were performed in intact rats after intravenous injections of 99mTc-hydroxyethylidenediphosphonic acid (99mTc-HEDP), 99mTc-oxabiphor (99mTc-OXB), 99mTc-ethylenediaminetetramethylene-phosphonic acid (99mTc-EDTMP) or 99mTc-diethylenetriaminopentakis(methylphosphonic acid. In the structure of the HEDP contains two phosphonic groups, OENTMP and EDTMP – five phosphonic groups. Radiochemical yield of labeled 99mTc HEDP, OENTMP, EDTMP, PPA is not less than 95%, the radiochemical impurities does not exceed 5%. The investigated compounds have high stability in vivo and selective accumulation in osseous tissue. The highest concentrations of labeled compounds is reached in 3–24 hours after their intravenous injections. The investigated compounds are rapidly excreted from blood and soft organs and tissues mainly through the urinary routes. So present study has showed that these RPP have properties, which making them promising candidates as a diagnostic pharmaceuticals of bone metastases.

1. Introduction
The success of cancer treatment depends on exact and well-timed tumor detection, and also on the evaluation of its dissimination in the body. A serious complication of many solid and hematological cancers is the development of bone metastases. The primary method of screening metastatic bone lesions is scintigraphy of skeleton using phosphonates labeled with technetium-99m (99mTc). To date, 99mTc-MDP represents the working horse for diagnostic bone scanning in conventional nuclear medicine due to the appropriate nuclear and physical characteristics of 99mTc (half-life 6 h, gamma-energy 140 keV). Various derivatives of phosphonates, containing from one to six phosphonic groups, are ideal ligands to react with 99mTc. Phosphonates have high affinity for hydroxyapatite crystals of
bone and accumulate predominantly in inflammatory, destructive and metastatic sites of skeleton so these regions can be imaged as “warm foci”. In addition to bone metastases imaging scintigraphy can be used to assess the efficacy of chemotherapy.

The mechanism of action of phosphonates depends on their structure. So, nonnitrogen-containing bisphosphonates can be metabolically incorporated into nonhydrolyzable analogues of adenosine triphosphate (ATP) [1 – 3]. Nitrogen-containing phosphonates inhibit enzymes of the mevalonate pathway, e.g. the farnesyl diphosphate synthase and geranylgeranyl diphosphate synthase [1 – 4]. They are necessary for posttranslational modification (prenylation) of small GTPases. As a result, this leads to the loss of osteoclast activity as well as the induction of tumor cells apoptosis [4, 5].

There are a lot of data of experimental researches of different phosphonates labeled with $^{99m}$Tc in scientific literature [6 – 8]. For example, $^{99m}$Tc-EDTMP had high affinity for bone tissue, but also accumulated in the kidneys: up to 2% of the injected dose at 90 min after intravenous injection [9]. In another study the possibility of bone metastases imaging with $^{99m}$Tc-EDTMP and dicarboxypropane-diphosphonate labeled with $^{99m}$Tc in patients clinical was assessed [10]. The results showed that the ratio of activity in metastatic sites of bone and soft tissue was higher for dicarboxypropane-diphosphonate labeled with $^{99m}$Tc (p < 0.05), whereas the ratio of activity in metastatic and healthy areas of the skeleton for both drugs had no significant differences [10]. Láznicek M et al. [11] synthesized calcium salt of diethylenetriaminopentamethylphosphonic acid (PAA) labeled with $^{99m}$Tc and compared its pharmacokinetic properties with $^{99m}$Tc-MDP. Stability of $^{99m}$Tc-PAA was higher than $^{99m}$Tc-MDP. The levels of accumulation $^{99m}$Tc-MDP and $^{99m}$Tc-PAA in osseous tissue were of nearly equal.

The purpose of this work is to perform the comparative studies of pharmacokinetic properties of four radiopharmaceuticals based on phosphonic acids with two, four and five phosphonic groups in intact rats after intravenous injection. $^{99m}$Tc-1-hydroxyethylidene-1,1-diphosphonic acid ($^{99m}$Tc-HEDP) and $^{99m}$Tc-oxybis(ethylenedinitrilo)tetramethyleneephosphonic acid ($^{99m}$Tc-OENTMF) have two phosphonic groups, $^{99m}$Tc-N,N,N',N'-ethylenediaminetetras(O-methyleneephosphonic acid) ($^{99m}$Tc-EDTMP) has four phosphonic groups and $^{99m}$Tc-diethylenetriaminopentakil(methyleneephosphonic acid) ($^{99m}$Tc-PAA) has five phosphonic groups.

2. Materials and methods

For obtaining labeled radiopharmaceuticals monopotassium salt of 1-hydroxyethyldene-1,1-diphosphonic acid (official name – Xydifon, Semashko FSU «Moschimpharmpreparat», Moscow, Russia) was used. OENTMF was obtained from JSC «Karpov institute of Physical Chemistry» (Obninsk, Russia), EDTMP and PPA were manufactured by Sigma-Aldrich (England) and Fluka (Germany), respectively. $^{99m}$Tc was received as Na$^{99m}$TeO$_4$ solution by elution from $^{99m}$Mo$^{99m}$Tc-generator in isotonic saline. $^{99m}$Mo/$^{99m}$Tc-generator was purchased from JSC «Leypunskiy institute of Physics and Power Engineering» (Obninsk, Russia).

Labeling of phosphonic acids was carried out according to the methods, which were described in the articles [12, 13]. Labeling was performed at room temperature by the addition of 2.0 ml of Na$^{99m}$TeO$_4$ solution with volume activity 3.7 MBq/ml (0.1 mCi/ml). Labeling yield and radiochemical impurities were determined using paper chromatography on Whatman paper-1 manufactured by Sigma (England). Acetone and a mixture of C$_2$H$_5$OH, 25%NH$_2$OH and H$_2$O at a ratio of 2:5:5, respectively, were used acetone as mobile phase. Quantitative analysis of each radiolabeled compound and radiochemical impurities ($^{99m}$TeO$_4$ and $^{99m}$TeO$_2$) was performed by activity measurement of strips of chromatographic paper.

Pharmacokinetic studies were carried out in 64 white outbred male rats, weighing 180 ± 40 g. All animals were divided into four groups (16 rats in each group). Pharmacokinetics of $^{99m}$Tc-HEDP, $^{99m}$Tc-OENTMF, $^{99m}$Tc-EDTMP and $^{99m}$Tc-PAA was studied in the animals of the first, the second, the third and the fourth groups, respectively.

A dose of 0.37 MBq (0.01 mCi) in a volume of 0.1 ml of each radiolabeled compound was injected intravenously via a tail vein. The amount of injected compound was 2.27-3.57 mg/kg of
animal weight. Then at various times after injection (5 min, 1, 3 and 24 h) the animals were sacrificed, and samples of various tissues were collected, placed in plastic tubes, weighed on electronic scales and counted in an automated gamma-counter with a standard of the injected dose. Then the amounts of radioactivity in each injected dose were calculated as percentage injected dose per gram of tissue (%ID/g) and as percentage injected dose per tissue (%ID/tissue). The results of radiometry were processed by estimation of standard error of mean value (M ± m). In addition, bone/blood and bone/muscle ratios were calculated.

3. Results

The chemical structure of various phosphonates derivatives almost didn’t affect the nature of complex compounds with $^{99m}$Tc. The efficacy of labeling was greater than 95%. Radiochemical impurities ($^{99m}$TcO$_4^-$ and $^{99m}$TeO$_4^-$) didn’t exceed 5%.

The results of pharmacokinetics properties of $^{99m}$Tc-HEDP, $^{99m}$Tc-OENTMP, $^{99m}$Tc-EDTMP and $^{99m}$Tc-PPA in organs and tissues of intact rats are presented in the table 1. They indicate that after intravenous injection all labeled compounds accumulated mainly in the bone tissue.

Table 1. Pharmacokinetics of $^{99m}$Tc-HEDP, $^{99m}$Tc-OENTMP, $^{99m}$Tc-EDTMP and $^{99m}$Tc-PPA in intact rats after intravenous administration (in % of injected dose per gram).

| № | Organ/tissue     | Compound          | Time after administration |
|---|------------------|-------------------|---------------------------|
|   |                  |                   | 5 min   | 1 h    | 3 h    | 24 h   |
| 1 | Blood            | $^{99m}$Tc-HEDP   | 0,78±0,17 | 0,19±0,03 | 0,048±0,004 | 0,043±0,009 |
|   |                  | $^{99m}$Tc-OENTMP | 0,56±0,09 | 0,24±0,04 | 0,11±0,01 | 0,050±0,004 |
|   |                  | $^{99m}$Tc-EDTMP  | 0,54±0,03 | 0,10±0,02 | 0,019±0,002 | 0,033±0,009 |
|   |                  | $^{99m}$Tc-PPA    | 0,71±0,13 | 0,10±0,01 | 0,020±0,001 | 0,010±0,002 |
| 2 | Thyroid gland    | $^{99m}$Tc-HEDP   | 0,32±0,07 | 0,10±0,03 | 0,12±0,02 | 0,23±0,05 |
|   |                  | $^{99m}$Tc-OENTMP | 0,31±0,05 | 0,22±0,05 | 0,30±0,10 | 1,48±0,77 |
|   |                  | $^{99m}$Tc-EDTMP  | 0,81±0,14 | 0,09±0,02 | 0,10±0,02 | 0,48±0,09 |
|   |                  | $^{99m}$Tc-PPA    | 0,70±0,11 | 0,17±0,04 | 0,11±0,03 | 0,23±0,05 |
| 3 | Lungs            | $^{99m}$Tc-HEDP   | 0,56±0,15 | 0,17±0,02 | 0,053±0,004 | 0,049±0,018 |
|   |                  | $^{99m}$Tc-OENTMP | 0,40±0,05 | 0,21±0,02 | 0,073±0,011 | 0,033±0,004 |
|   |                  | $^{99m}$Tc-EDTMP  | 0,32±0,02 | 0,06±0,01 | 0,023±0,001 | 0,018±0,004 |
|   |                  | $^{99m}$Tc-PPA    | 0,52±0,03 | 0,08±0,01 | 0,072±0,051 | 0,006±0,006 |
| 4 | Liver            | $^{99m}$Tc-HEDP   | 0,013±0,003 | 0,003±0,001 | 0,000±0,001 | 0,003±0,001 |
|   |                  | $^{99m}$Tc-OENTMP | 0,013±0,002 | 0,008±0,002 | 0,003±0,001 | 0,003±0,001 |
|   |                  | $^{99m}$Tc-EDTMP  | 0,13±0,02 | 0,044±0,001 | 0,025±0,003 | 0,014±0,004 |
|   |                  | $^{99m}$Tc-PPA    | 0,17±0,02 | 0,060±0,001 | 0,020±0,001 | 0,011±0,003 |
| 5 | Kidney           | $^{99m}$Tc-HEDP   | 1,59±0,34 | 0,74±0,10 | 0,60±0,04 | 0,59±0,20 |
|   |                  | $^{99m}$Tc-OENTMP | 1,59±0,14 | 1,46±0,17 | 1,49±0,12 | 0,93±0,07 |
|   |                  | $^{99m}$Tc-EDTMP  | 1,78±0,23 | 1,25±0,05 | 1,35±0,09 | 0,96±0,18 |
|   |                  | $^{99m}$Tc-PPA    | 2,83±0,19 | 0,63±0,03 | 0,50±0,03 | 0,27±0,01 |
| 6 | Spleen           | $^{99m}$Tc-HEDP   | 0,15±0,04 | 0,055±0,001 | 0,034±0,002 | 0,033±0,004 |
|   |                  | $^{99m}$Tc-OENTMP | 0,11±0,01 | 0,074±0,005 | 0,045±0,006 | 0,035±0,004 |
|   |                  | $^{99m}$Tc-EDTMP  | 0,11±0,01 | 0,025±0,002 | 0,015±0,001 | 0,010±0,003 |
|   |                  | $^{99m}$Tc-PPA    | 0,10±0,02 | 0,020±0,001 | 0,020±0,003 | 0,007±0,002 |
| 7 | Stomach          | $^{99m}$Tc-HEDP   | 0,30±0,06 | 0,11±0,01 | 0,15±0,02 | 0,21±0,08 |
|   |                  | $^{99m}$Tc-OENTMP | 0,23±0,03 | 0,15±0,02 | 0,09±0,02 | 0,040±0,007 |
|   |                  | $^{99m}$Tc-EDTMP  | 0,25±0,02 | 0,042±0,006 | 0,022±0,003 | 0,019±0,003 |
|   |                  | $^{99m}$Tc-PPA    | 0,26±0,06 | 0,060±0,001 | 0,070±0,010 | 0,010±0,001 |
Table 2. Ratios of $^{99m}$Tc-HEDP, $^{99m}$Tc-OENTMP, $^{99m}$Tc-EDTMP and $^{99m}$Tc-PPA in bones compared to those in blood and muscle in intact rats after intravenous administration

| №  | Ratio               | Time after administration |
|----|---------------------|---------------------------|
|    |                     | 5 мин         | 1 ч           | 3 ч           | 24 ч          |
| 1  | Femur/blood $^{99m}$Tc-HEDP | 0,87±0,19 | 10,0±0,79     | 47,2±6,62     | 57,5±6,82     |
|    | $^{99m}$Tc-OENTMP    | 0,54±0,08  | 7,91±1,81     | 20,8±3,36     | 43,1±6,50     |
|    | $^{99m}$Tc-EDTMP     | 1,84±0,20  | 22,2±5,83     | 70,0±12,3     | 29,6±5,83     |
|    | $^{99m}$Tc-PPA       | 1,30±0,15  | 22,2±5,20     | 86,9±22,0     | 174,2±56,3    |
| 2  | Femur/muscle $^{99m}$Tc-HEDP | 3,76±0,94 | 38,9±3,26     | 139,9±20,7    | 175,0±27,3    |
|    | $^{99m}$Tc-OENTMP    | 2,27±0,40  | 32,0±2,85     | 151,8±24,2    | 373,8±46,8    |
|    | $^{99m}$Tc-EDTMP     | 7,70±0,79  | 119,0±33,1    | 247,5±48,2    | 53,4±10,4     |
|    | $^{99m}$Tc-PPA       | 4,29±0,69  | 156,3±21,1    | 353,0±68,5    | 297,9±48,9    |
| 3  | Skull/blood $^{99m}$Tc-HEDP | 0,78±0,15 | 6,35±0,84     | 32,9±3,33     | 35,6±4,06     |
|    | $^{99m}$Tc-OENTMP    | 0,65±0,05  | 6,44±1,55     | 16,2±2,88     | 29,4±4,51     |
|    | $^{99m}$Tc-EDTMP     | 1,64±0,08  | 19,6±4,11     | 63,5±7,20     | 28,6±5,03     |
|    | $^{99m}$Tc-PPA       | 0,95±0,23  | 12,0±3,78     | 54,9±3,79     | 111,3±35,8    |
| 4  | Skull/muscle $^{99m}$Tc-HEDP | 3,36±0,66 | 23,9±1,43     | 97,9±10,9     | 110,5±20,5    |
|    | $^{99m}$Tc-OENTMP    | 2,89±0,64  | 26,2±2,51     | 114,3±14,9    | 254,9±31,9    |
|    | $^{99m}$Tc-EDTMP     | 7,00±0,82  | 103,5±17,5    | 222,5±25,5    | 54,7±13,5     |
|    | $^{99m}$Tc-PPA       | 3,36±0,90  | 83,1±15,7     | 253,9±72,4    | 188,4±25,7    |
Particularly high activity in the kidneys was observed for $^{99m}$Tc-PPA, so studied compounds accumulated and kept here in significant quantities during 24 h (table 1).

Some differences in the pharmacokinetic properties of $^{99m}$Tc-PPA, $^{99m}$Tc-OENTMP, $^{99m}$Tc-EDTMP and $^{99m}$Tc-HEDP were less than 1. Later, however, these values became significantly higher than 1 and reached at 3-24 hours. The highest values were noticed for $^{99m}$Tc-PPA and $^{99m}$Tc-OENTMP. For example, the ratio femur/blood for $^{99m}$Tc-PPA reached $174.2 \pm 56.3$ and femur/muscle for $^{99m}$Tc-OENTMP was $373.8 \pm 46.8$ (table 2). All these findings suggest the possibility of these radiopharmaceuticals for nuclear imaging of bone tissue.

It should be noted that all compounds were rapidly excreted from blood. During the early of the investigation there weren’t significant differences between the radioactivity of labeled compounds. Further the content of compounds declined; meanwhile, in blood the activity of $^{99m}$Tc-EDTMP and $^{99m}$Tc-PPA was lower than $^{99m}$Tc-HEDP and $^{99m}$Tc-OENTMP.

### 4. Discussion

Some differences in the pharmacokinetic properties of $^{99m}$Tc-HEDP, $^{99m}$Tc-OENTMP, $^{99m}$Tc-EDTMP and $^{99m}$Tc-PPA can be explained by various binding of these complexes to plasma proteins [11].

The disappearance of many labeled phosphonates from blood is occurred through kidneys [13 – 17], so studied compounds accumulated and kept here in significant quantities during 24 h (table 1). Particularly high activity in the kidneys was observed for $^{99m}$Tc-PPA: up to $2.83 \pm 0.19$ %g at 5 min after intravenous injection. This caused a high radiation exposure to kidneys, but led to drop of radioactivity level in non-osseous tissues.

The stability of radiopharmaceuticals labeled with $^{99m}$Tc can be assessed at their distribution in thyroid gland, since free $^{99m}$Tc has a high affinity to in thyroid gland. The concentration of complexes in the thyroid gland was low. The peak levels of $^{99m}$Tc-HEDP, $^{99m}$Tc-EDTMP and $^{99m}$Tc-PPA were noted immediately after intravenous injection. On the contrary, the amount of $^{99m}$Tc-OENTMP reached $1.48 \pm 0.77$ %g at 24 h. After 1 h there were no significant differences between the activity levels of compounds (table 1). Therefore, all radiopharmaceuticals were characterized by high stability in vivo.

The content of all complexes in non-osseous organs and tissues was low. $^{99m}$Tc-EDTMP and $^{99m}$Tc-PPA eliminated faster from all organs except the liver. Concentration of $^{99m}$Tc-EDTMP and $^{99m}$Tc-PPA in the liver was more than 10 times higher in comparison with $^{99m}$Tc-HEDP and $^{99m}$Tc-OENTMP (table 1).

### 5. Conclusion

The results of pharmacokinetics of $^{99m}$Tc-HEDP, $^{99m}$Tc-OENTMP, $^{99m}$Tc-EDTMP and $^{99m}$Tc-PPA in intact rats showed that all compounds exhibited high bone uptake and retention of radioactivity in the skeleton after their intravenous administration. The highest amounts of radioactivity were found in

|  | Rib/blood |  |  |  |  |
|---|---|---|---|---|---|
| 5 | $^{99m}$Tc-HEDP | $^{99m}$Tc-OENTMP | $^{99m}$Tc-EDTMP | $^{99m}$Tc-PPA |  |
|  | 0.57±0.04 | 1.87±0.94 | 21.9±3.77 | 16.2±2.54 |  |
|  | 0.34±0.04 | 2.38±0.49 | 5.71±1.24 | 12.0±2.68 |  |
|  | 2.01±0.18 | 18.07±6.21 | 65.1±7.40 | 25.8±5.13 |  |
|  | 0.95±0.17 | 13.3±3.60 | 75.9±19.5 | 100.6±29.3 |  |
| 6 | Rib/muscle |  |  |  |  |
|  | 2.30±0.05 | 6.17±2.73 | 65.1±11.3 | 49.5±8.98 |  |
|  | 1.55±0.47 | 9.92±1.09 | 40.4±7.68 | 100.4±17.2 |  |
|  | 8.50±0.90 | 89.1±17.8 | 228.6±28.2 | 46.7±10.0 |  |
|  | 3.20±0.65 | 97.5±20.5 | 368.2±139.1 | 180.4±40.3 |  |
| 7 | Spine/blood |  |  |  |  |
|  | 0.92±0.14 | 7.36±0.57 | 38.5±3.23 | 41.5±5.13 |  |
|  | 0.71±0.03 | 7.11±1.43 | 17.4±2.68 | 32.8±4.13 |  |
|  | 2.02±0.11 | 18.5±4.41 | 64.8±8.42 | 26.0±5.23 |  |
|  | 1.22±0.10 | 14.6±2.56 | 49.7±10.6 | 113.1±23.5 |  |
| 8 | Spine/muscle |  |  |  |  |
|  | 3.91±0.47 | 28.4±2.41 | 114.0±10.4 | 129.2±24.5 |  |
|  | 3.29±0.90 | 29.2±0.82 | 124.1±14.7 | 290.1±38.1 |  |
|  | 8.70±1.09 | 95.2±13.5 | 226.0±27.6 | 46.9±8.45 |  |
|  | 4.14±0.72 | 106.4±16.7 | 216.5±56.8 | 214.8±51.6 |  |
femur. In other bones (skull, ribs and spine) concentration of complexes was slightly lower than in femur. The retention of activity in bone tissue was high, whereas all complexes rapidly eliminated from soft organs and tissues. This was proved by the ratios of doses accumulated in bones compared to those found in other organs and tissues. The greatest values were observed after 3-24 h of injection. In addition, the studied complexes had high stability in vivo. Intensive removal of labeled compounds through the urinary system didn’t lead to the significant accumulation of radioactivity in other organs and tissues.

The main result of the carried out research was the fact that the number of phosphonic groups in phosphonic acid derivate structures did not significantly affect the pharmacokinetic properties of radiopharmaceuticals, their uptake and retention in bone tissue, as well as the excretion from soft organs and tissues. These results suggested that $^{99m}$Tc-HEDP, $^{99m}$Tc-OENTMP, $^{99m}$Tc-EDTMP and $^{99m}$Tc-PPA had the potential to become suitable bone-imaging agents.

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