Biodistribution ex vivo of $^{213}$Bi-KHEDP – a promising bone-seeking agent for targeted alpha therapy

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Abstract. Alpha-emitters are increasingly used for targeted alpha therapy because of their emission of high linear energy transfer (LET) particles with a relative short path length. Bismuth-213 ($^{213}$Bi, $T_{1/2} = 46$ min) is one of the most suitable radiation sources for medical applications. In the present work the biodistribution of $^{213}$Bi-monopotassium salt of 1-hydroxyethylidene diphosphonic acid ($^{213}$Bi-KHEDP) in intact mice was studied. It was shown that bones uptake of $^{213}$Bi-KHEDP were higher than in the most soft tissue organs throughout the study. The bone-to-soft tissue ratios for $^{213}$Bi-KHEDP were higher than the corresponding data for $^{213}$BiCl$_5$. Among the soft tissue organs, only kidneys had a high uptake of $^{213}$Bi-KHEDP and free $^{213}$Bi. In conclusion, $^{213}$Bi-KHEDP had a strong and selective bone affinity, indicating that this complex could be useful to deliver alpha-particle radiation to primary bone cancer and skeletal metastases.

1. Introduction

Targeted alpha therapy (TAT) has shown great promise in the treatment of both micrometastatic and large solid tumors in preclinical and clinical studies [1]. This term means radiopharmaceutical technology that uses α-emitting radionuclides to destroy tumor tissue. TAT offers advantages over current β-emitting conjugates radionuclide therapy of many tumors, including bone metastases. α-Particles are able to kill cancer cells due to their linear energy transfer (LET) corresponds to 60-230 keV/μm, which is more lethal to cells than LET of β-particles [2]. The primary cause for higher cell toxicity is thought to be originated from the increased frequency of DNA double strand breaks, which are difficult to repair. Besides, the cytotoxicity of α-particles is independent of both dose rate and oxygenation status of the irradiated cells [3]. Also α-particles have very short path length (< 0.1 mm), corresponding to less than 10 cell diameters, which minimizes damage from the surrounding normal tissue particularly to areas of marrow containing hematopoietic precursors [3].

The radioactive decay of bismuth-213 ($^{213}$Bi, $T_{1/2} = 46$ min) results in the emission of high-LET α-particles by $^{213}$Bi self and by its daughter $^{213}$Po around 100 keV/μm. $^{213}$Bi is obtained from a $^{225}$Ac generator with acceptable activity for about 10 days [4]. Due to the relative short half-life $^{213}$Bi can deliver a high radiation dose rate to cancer cells within a relatively short period of time and with minimal damage to surrounding normal tissue. Phosphonates are ideal bone-targeting agents and suitable vehicles for α-emitters due to their high hydroxyapatite affinity. Thus, a complex of $^{213}$Bi with phosphonate could be used as a potential agent for TAT of bone tissue disorders. The aim of this study
was to evaluate *ex vivo* biodistribution of monopotassium salt of 1-hydroxyethylidene diphosphonic acid labeled with $^{213}$Bi ($^{213}$Bi-KHEDP) in intact mice.

2. Materials and methods
Normal mice weighing 18-23 g were used in all studies. Animals were injected intravenously with 0.37 MBq of $^{213}$Bi-KHEDP or $^{213}$BiCl$_5$ ($n = 12$ each tracer) in a volume of 0.1 ml through the tail vein. Animals were sacrificed at 5 min, 1, and 3 h after injection. Four mice were used for each time points. The samples of tissues and organs were collected, weighed and counted in automatic gamma counter “Wizard” (PerkinElmer/Wallac). The uptake was expressed as a percentage of the injected dose per gram of tissue (%ID/g). All the biodistribution studies were carried out in strict compliance with the national laws related to the conduct of animal experiments.

Biodistribution data were expressed as mean value ± standard error of the mean (M ± m). Student’s t test was used to analyze data throughout all studies between groups at different time points, and $p<0.05$ was considered statistically significant. In addition, femur/blood and femur/muscle ratios were calculated.

3. Results and discussion
It was shown that bones uptake of $^{213}$Bi-KHEDP were higher than in the most soft tissue organs throughout the study. Besides, the level of $^{213}$Bi-KHEDP in bone tissue was higher as compared with $^{213}$BiCl$_5$, as shown in figures 1–4. The maximum femur uptake of $^{213}$Bi-KHEDP compared to $^{213}$BiCl$_5$ was 18.6±3.23 %ID/g versus 1.38±0.25 %ID/g at 5 min post-injection (p.i.), respectively. The amount of $^{213}$Bi-KHEDP in skull was 4.29±1.05 %ID/g at 5 min p.i., but climbed to 16.2±3.76 %ID/g at 1 h p.i., and declined to 5.28±1.21 %ID/g at 3 h p.i. The ribs uptake varied from 2.43±0.36 %ID/g to 6.15±1.76 %ID/g, spine uptake – from 1.64±0.21 %ID/g to 3.27±0.42 %ID/g. The level of activity of free $^{213}$Bi didn’t exceed 5.15±1.12 %ID/g in skull at 1 h p.i. (figures 1–4). The femur/blood and femur/muscle ratios for $^{213}$Bi-KHEDP were higher than the corresponding data for $^{213}$BiCl$_5$ (table 1).

![Figure 1. Specific amounts of radioactivity in femur of normal mice at different time after intravenous injection of $^{213}$Bi-KHEDP and $^{213}$BiCl$_5$ (in %ID/g).](image1)

![Figure 2. Specific amounts of radioactivity in ribs of normal mice at different time after intravenous injection of $^{213}$Bi-KHEDP and $^{213}$BiCl$_5$ (in %ID/g).](image2)

There are few data referring to bone-seeking complex of phosphonates and $\alpha$-emitting radionuclides. For example, bone uptake of $^{225}$Ac-EDTMP in mice was approximately 10–11 %ID/g after 15 h [5]. Similar results were obtained by Hassfjell et al. [6]: the amounts of $^{212}$Pb-EDTMP and $^{212}$Bi-EDTMP in bone tissue were 10 %ID/g and 8 %ID/g after 24 h, respectively. $^{227}$Th-EDTMP also
exhibited good and stable bone accumulation in mice: femur uptake was 8.7 %ID/g at 24 h p.i. and remained stable for 14 days [7].

![Graph showing radioactivity in skull and spine](image)

**Figure 3.** Specific amounts of radioactivity in skull of normal mice at different time after intravenous injection of $^{213}$Bi-KHEDP and $^{213}$BiCl$_5$ (in %ID/g).

**Figure 4.** Specific amounts of radioactivity in spine of normal mice at different time after intravenous injection of $^{213}$Bi-KHEDP and $^{213}$BiCl$_5$ (in %ID/g).

Higher bone accumulation revealed DOTMP chelates of α-emitters. Thus, $^{225}$Ac-DOTMP demonstrated high bone uptake (up to 20 %ID/g at 4 h p.i.) and high bone-to-soft tissue ratios in mice [8]. The amount of $^{212}$Bi-DOTMP in bone tissue of Balb/c mice was as high as 22 %ID/g after 2 h. Bone-to-soft tissue ratios were higher in young mice than in old mice, indicating enhanced uptake in areas with high bone turnover [9]. The percentage of $^{227}$Th-DOTMP in femur reached 20 %ID/g at 4 h after intravenous administration [8]. Thereby, our results are in a good agreement with literature data.

**Table 1.** Femur/blood and femur/muscle ratios in normal mice after intravenous injection of $^{213}$Bi-KHEDP and $^{213}$BiCl$_5$.

| Time after injection | Femur/blood | Femur/muscle |
|----------------------|-------------|--------------|
|                      | 5 min       | 1 h          | 3 h          | 5 min       | 1 h          | 3 h          |
| **Femur/blood**      | 4.47±0.49*  | 8.82±0.66    | 13.21±0.70   | 34.44±3.05  | 49.63±3.54   | 17.76±2.07   |
|                      | 0.11±0.02** | 0.08±0.01    | 0.09±0.01    | 1.33±0.56   | 2.88±1.70    | 1.48±0.37    |
|                      | p < 0.001   | p < 0.001    | p < 0.001    | p < 0.001   | p < 0.001    | p < 0.001    |

* – $^{213}$Bi-KHEDP
** – $^{213}$BiCl$_5$

Among the soft tissue organs, only kidneys had a high uptake of $^{213}$Bi-KHEDP and free $^{213}$Bi. Thus, the amount of $^{213}$Bi-KHEDP was 46.8±4.12 %ID/g at 5 min p.i., and then increased to
77.2±2.94 %ID/g at 3 h p.i. At contrast, kidney uptake of $^{213}$BiCl$_3$ reached 141.6±39.3 %ID/g at 1 h p.i., because kidneys are specific biological site of $^{213}$Bi deposition [10]. High kidney uptake of $^{213}$Bi-KHEDP is due to the rapid renal excretion of phosphonic acids and their derivatives [11, 12].

The amount of $^{213}$Bi-KHEDP in blood was 4.16±0.39 %ID/g at 5 min p.i., but then declined rapidly to 1.52±0.36 %ID/g at 1 h and 0.39±0.08 %ID/g at 3 h. The activity of $^{213}$BiCl$_3$ in blood was 3–10 times higher than that of $^{213}$Bi-KHEDP and varied from 3.98±0.48 to 14.8±2.90 %ID/g.

The radioactivity of $^{213}$Bi-KHEDP in liver was 4.17–5.33 %ID/g, whereas the uptake of $^{213}$BiCl$_3$ reached 15.3±3.40 %ID/g at 1 h p.i. In other organs the amounts of $^{213}$Bi-KHEDP were: 0.57–1.67 %ID/g in thyroid gland, 0.99–3.64 %ID/g in lungs, 1.02–2.61 %ID/g in spleen, 0.31–2.19 %ID/g in heart, and 0.73–1.44 %ID/g in skin. The uptake of $^{213}$Bi-KHEDP in stomach and small intestine varied from 0.62±0.14 to 1.69±0.30 %ID/g and from 0.53±0.16 to 3.72±1.11 %ID/g, respectively. Low level of activity revealed in brain: 0.10–0.29 %ID/g.

4. Summary

In conclusion, $^{213}$Bi-KHEDP had a strong and selective bone affinity, indicating that this complex could be useful to deliver $\alpha$-particle radiation to primary bone cancer and skeletal metastases.

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