The addition of DTPA to [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate prior to administration reduces rat skeleton uptake of radioactivity

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Abstract. Peptide receptor-targeted radionuclide therapy is nowadays also being performed with DOTA-conjugated peptides, such as [DOTA^{0},Tyr^{3}]octreotate, labelled with radionuclides like ^{177}Lu. The incorporation of ^{177}Lu is typically ≥99.5%; however, since a total patient dose can be as high as 800 mCi, the amount of free $^{177}Lu^{3+}$ (= non-DOTA-incorporated) can be substantial. Free $^{177}Lu^{3+}$ accumulates in bone with unwanted irradiation of bone marrow as a consequence. ^{177}Lu-DTPA is reported to be stable in serum in vitro, and in vivo it has rapid renal excretion. Transforming free Lu^{3+} to Lu-DTPA might reroute this fraction from accumulation in bone to renal clearance. We therefore investigated: (a) the biodistribution in rats of $^{177}LuCl_3$, [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate and ^{177}Lu-DTPA; (b) the possibilities of complexing the free $^{177}Lu^{3+}$ in [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate to ^{177}Lu-DTPA prior to intravenous injection; and (c) the effects of free $^{177}Lu^{3+}$ in [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, in the presence and absence of DTPA, on the biodistribution in rats. $^{177}LuCl_3$ had high skeletal uptake (i.e. 5% ID per gram femur, with localization mainly in the epiphyseal plates) and a 24-h total body retention of 80% injected dose (ID). [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate had high and specific uptake in somatostatin receptor-positive tissues, and 24-h total body retention of 19% ID. ^{177}Lu-DTPA had rapid renal clearance, and 24-h total body retention of 4% ID. Free $^{177}Lu^{3+}$ in [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate could be complexed to ^{177}Lu-DTPA. Accumulation of ^{177}Lu in femur, blood, liver and spleen showed a dose relation to the amount of free $^{177}Lu^{3+}$, while these accumulations could be normalized by the addition of DTPA. After labelling [DOTA^{0},Tyr^{3}]octreotate with ^{177}Lu the addition of DTPA prior to intravenous administration of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate is strongly recommended.

Keywords: DOTA – DTPA – Radiolabelling – Biodistribution

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Introduction

Peptide receptor-targeted radionuclide therapy of somatostatin receptor (SSR)-positive tumours is currently also being performed using DOTA-chelated SS analogues such as [DOTA^{0},Tyr^{3}]octreotate (DOTA-tate), radiolabelled with ^{177}Lu [1, 2, 3]. Although incorporation of the radiolabel is typically ≥99.5%, there is always a free fraction. Since therapeutic doses (up to 800 mCi per patient) are involved, the absolute amount of free $^{177}Lu^{3+}$ can be significant, and since free $^{177}Lu^{3+}$ acts as a Ca^{2+} mimic, it accumulates in bone [4]. Muller et al. reported in a study with mice that 60% of the injected dose (ID) of $^{177}LuCl_3$ is incorporated in the skeleton at 48 h p.i. [5, 6]. Li et al. reported the stability of ^{177}Lu-DTPA in vitro in serum [4]. In order to investigate the in vivo characteristics of the three ^{177}Lu-labelled compounds separately – i.e. $^{177}LuCl_3$, ^{177}Lu-DTPA and ^{177}Lu-DOTA-tate –, biodistribution studies were performed in rats.

Based on these results (reported here) with the three forms of ^{177}Lu, we hypothesized that after labelling DOTA-tate with ^{177}Lu, the addition of excess DTPA might be able to complex the fraction of free $^{177}Lu^{3+}$ by transformation to ^{177}Lu-DTPA, with rerouting from accumulation in bone to renal clearance. Therefore, we investigated the biodistribution of ^{177}Lu-DOTA-tate with 0% and free $^{177}Lu^{3+}$ (up to 10%), and in the presence and absence of DTPA.

Materials and methods

^{177}Lu-labelled compounds. $^{177}LuCl_3$ (IDB Holland, Baarle Nassau, The Netherlands) was provided in 0.05 *N* HCl. The labelling (20 min at 80°C), high-performance liquid chromatography and instant thin-layer chromatography were performed as described previously [2]. The amount of injected of DOTA-tate was 0.5 µg and the amount of DTPA (added to $^{177}LuCl_3$ or ^{177}Lu-DOTA-tate prior to the i.v. administration) was 4 µg.

Animals and tissue distribution. Male Wistar rats (10–14 weeks old, 225–250 g, Harlan-CPB, Austerlitz, The Netherlands, $n \geq 3$ rats per group) were kept under standard laboratory conditions (12 h light/12 h dark) and were given standard laboratory diet (Hope Farms, Woerden, The Netherlands) and water ad libitum. The experimental protocol adhered to the rules laid down by the Dutch Animal Experimentation Act and was approved by the Committee on Animal Research of the Erasmus Medical Centre, Rotterdam. Radioactive material, 10 MBq $^{177}LuCl_3$, ^{177}Lu-DTPA or ^{177}Lu-DOTA-tate, was injected intravenously in the penis vein. The rats were housed in metabolic cages for urine and faeces collection. Blood, spleen, pancreas, adrenals, pituitary, kidney, liver, lungs, thymus, sternum, muscle and femurs were isolated 24 h p.i. and concentration of radioactivity measured in the well-type gamma counter LKB-282 compugamma, as described previously [7]. From one femur the inner part was rinsed with saline in order to collect the bone marrow. Bone and bone marrow were analysed for radioactive concentration.

Ex vivo autoradiography. Localization of radioactivity in tissues after injection of $^{177}LuCl_3$, ^{177}Lu-DTPA or ^{177}Lu-DOTA-tate in rats was investigated by ex vivo autoradiography, as described previously [3]. The sections and spliced femur were exposed to phosphor imaging screens (Packard Instruments Co., Meriden, USA) for 1 day in X-ray cassettes and analysed using a Cyclone phosphor imager and a computer-assisted OptiQuant 03.00 image processing system (Packard Instruments Co, Groningen, The Netherlands).

Data acquisition. The injections of radioactivity were monitored with a gamma camera (Siemens, ROTA-II Erlangen). During the first 25 min and at 4 and at 24 h, radioactivity accumulation was measured using regions of interest, as described previously [7]. Statistical analysis was performed using Student's *t* test. Statistical significance was defined at $P<0.05$.

Results

The dynamic studies in rats injected with $^{177}LuCl_3$, ^{177}Lu-DOTA-tate and ^{177}Lu-DTPA showed rapid distribution of radioactivity (data not shown). The ^{177}Lu-DTPA-injected rats revealed instant uptake of radioactivity in kidney and rapid appearance of radioactivity in the urinary bladder. Twenty-five minutes following the injection of ^{177}Lu-DTPA, 50% ID was accumulated in the urinary bladder (calculated using regions of interest), while at this time point the rats injected with $^{177}LuCl_3$ or ^{177}Lu-DOTA-tate had accumulated less than 5% ID there (data not shown). The images from static gamma camera studies at 4 and 24 h of rats injected with $^{177}LuCl_3$

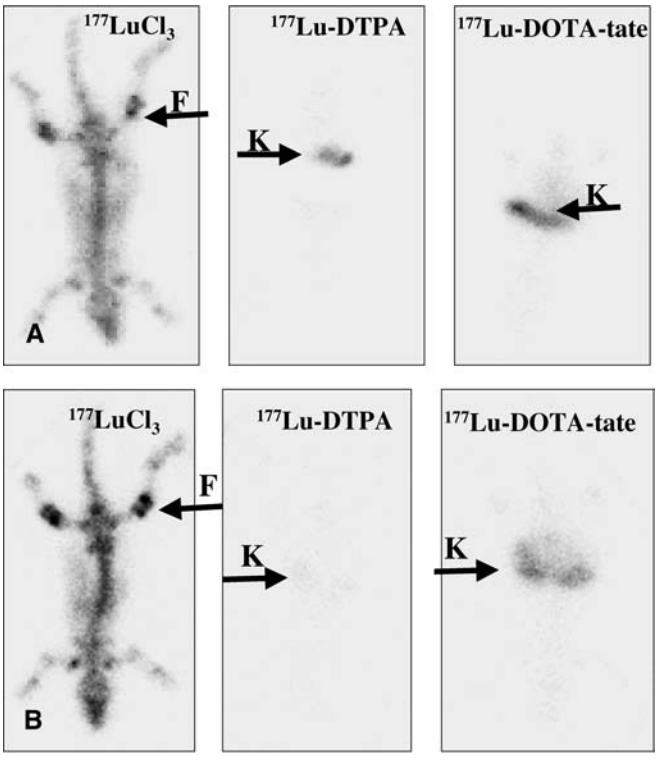

Fig. 1A, B. The results of a static gamma camera study at 4 h p.i. (**A**) and 24 h p.i. (**B**) in rats injected with $^{177}LuCl_3$, ^{177}Lu-DTPA or ^{177}Lu-DOTA-tate. The *arrows* indicate: *K*, kidney; *F*, head of the femur

(Fig. 1) showed mainly uptake in skeleton, with total body retention of 83% and 80% ID, respectively. At these time points, the ^{177}Lu-DTPA-injected rats had total body retention of 20% and 4% ID, respectively. The rats injected with ^{177}Lu-DOTA-tate showed clear kidney retention at both time points, with total body retention of 28% and 19% ID, respectively. The % excreted radioactivity, as measured from rats in metabolic cages, corresponded to these data (i.e. retained and excreted radioactivity totalled 100%), confirming the total body data obtained using gamma camera measurements.

Ex vivo autoradiography of kidney showed uptake of radioactivity in the proximal renal tubuli (located in the outer part of the renal cortex) of rats injected with $^{177}LuCl_3$, ^{177}Lu-DOTA-tate or ^{177}Lu-DTPA (data not shown). The $^{177}LuCl_3$-injected rats also showed accumulation of radioactivity on the border of the medulla and cortex, and since this is where ions are reabsorbed, the accumulation of radioactivity is most probably due to the uptake of free ^{177}Lu (data not shown). The distribution of radioactivity in liver and spleen was homogeneous (data not shown).

Photographs of femur and the corresponding ex vivo autoradiography are presented in Fig. 2. Figure 2A shows the femur of a rat injected with ^{177}Lu-DOTA-tate (0% free ^{177}Lu). In this rat, 0.28±0.02% ID/g was found in the total femur, of which 95% was SSR mediated (as

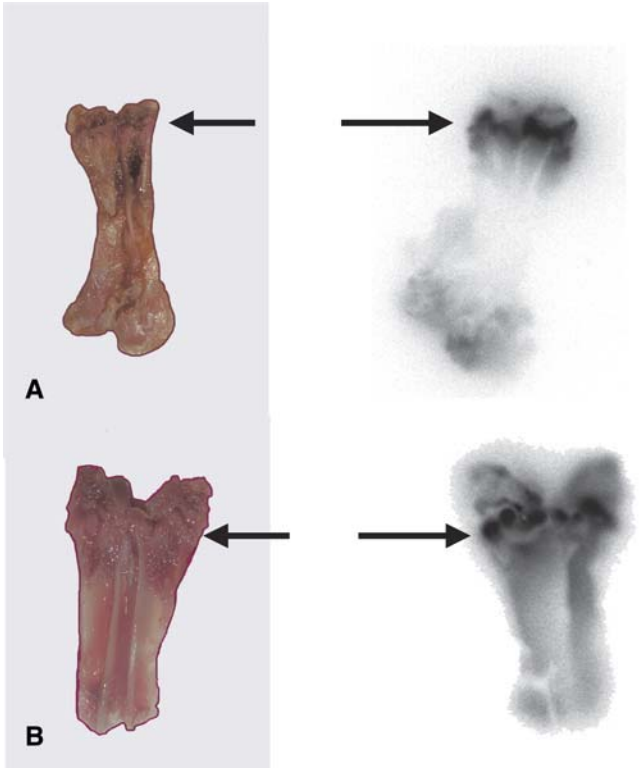

Fig. 2A, B. Ex vivo autoradiography of femurs at 24 h p.i. of rats injected with ^{177}Lu-DOTA-tate (**A**) or $^{177}LuCl_3$ (**B**). The uptake in **A** is 0.28% ID per gram femur. This value could be lowered to 0.02% ID per gram femur by co-injection with an SSR-blocking amount of octreotide. Femoral uptake in the rat injected with $^{177}LuCl_3$ (**B**) was 5.1% ID per gram. This value could be lowered to 0.02% ID by the addition of DTPA (data not shown). The *arrow* indicates the epiphyseal plate

found when rats were co-injected with 0.1 mg octreotide, data not shown), and localized in the epiphyseal plate. Of the total amount of radioactivity in femur, 0.7±0.1% was localized in bone marrow: 140 times lower than the amount localized in bone. Figure 2B shows the femur of a rat injected with $^{177}LuCl_3$. In this rat, 5.1±0.3% ID/g was found in the total femur, of which 0.015±0.002% was recovered in the bone marrow: 6,000 times lower than the amount localized in bone. The addition of DTPA resulted in a >95% reduction in total femoral uptake and strongly reduced uptake in the epiphyseal plate (data not shown). In ^{177}Lu-DOTA-tate-injected rats with 10% free ^{177}Lu in DOTA-tate, the femoral uptake was 1.2±0.1% ID/g, and this dropped to 0.02±0.01% ID/g in the presence of DTPA. In ^{177}Lu-DTPA-injected rats the femoral uptake was 0.017±0.002% ID/g, of which 8±2% was in the bone marrow: 12 times lower than the amount localized in bone.

The concentration of radioactivity in blood, liver and spleen in rats injected with 10% free ^{177}Lu in DOTA-tate in the absence of DTPA was significantly higher (expressed as % ID/g) than that in rats injected with 0% free ^{177}Lu (data not shown). No significant differences were found in other organs between rats injected with 0% free ^{177}Lu in the absence or presence of DTPA and the rats injected with 10% free ^{177}Lu in the presence of DTPA.

Discussion

Peptide receptor-targeted radionuclide therapy of SSR-positive tumours with ^{177}Lu-DOTA-tate is currently being performed [1, 2, 3]. Although the amount of free ^{177}Lu is ≤0.5%, there are reports on its incorporation in the skeleton [5, 6, 8]. Therefore, preclinical experiments were performed to obtain detailed morphological information. Autoradiographic studies of femurs of rats injected with $^{177}LuCl_3$ or ^{177}Lu-DOTA-tate showed localization in the epiphyseal plates (Fig. 2). (In humans these plates disappear after early adulthood.) In rats injected with ^{177}Lu-DOTA-tate, the uptake in the epiphyseal plates could be strongly reduced by the co-injection of DTPA (if free $^{177}LuCl_3$ was present) or octreotide. The uptake in bone of $^{177}LuCl_3$ and ^{177}Lu-DOTA-tate can be explained by the following facts: (a) Lu^{3+} mimics Ca^{2+} and participates in hydroxyapatite formation [4], and (b) the epiphyseal plates are reported to be SSR positive [9]. The addition of DTPA reduced uptake in epiphyseal plates, most probably due to the rapid renal clearance of the formed ^{177}Lu-DTPA, as has also been reported for ^{177}Lu-DTPA in mice [4]. Radioactivity was recovered in the bone and not in the bone marrow: the ratio of bone vs bone marrow radioactivity was 6,000:1 and 140:1 for $^{177}LuCl_3$ and ^{177}Lu-DOTA-tate, respectively. A retrospective study of nine young patients (1.5–14 years old) with non-growth-related diseases who were undergoing OctreoScan scintigraphy was performed, and the epiphyseal plates were not visualized. The concentration of radioactivity in blood, liver and spleen increases when 10% free ^{177}Lu is present in ^{177}Lu-DOTA-tate, and this increase can simply be abolished by the addition of DTPA. The explanation for this is most likely the formation of ^{177}Lu-DTPA in vitro, which will reroute the free $^{177}Lu^{3+}$ to rapid renal clearance in vivo. Therefore, it may be concluded that ^{177}Lu-DOTA-tate with 10% free $^{177}Lu^{3+}$ in the presence of DTPA is comparable with 0% free $^{177}Lu^{3+}$.

In conclusion, after labelling DOTA-tate with ^{177}Lu, the addition of DTPA prior to intravenous administration of ^{177}Lu-DOTA-tate is strongly recommended.

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