Multimodality Imaging and Preclinical Evaluation of 177Lu-AMBA for Human Prostate Tumours in a Murine Model

I-HSIANG LIU1, CHIH-HSIEN CHANG1,2, CHUNG-LI HO1, SHU-PEI CHIU1, WAN-CHI LEE1, TSUI-JUNG CHANG1, LIANG-CHENG CHEN1, YU-HSIEN WU1, CHENG-HUI CHUANG1, YING-KAI FU1 and TE-WEI LEE1

1Institute of Nuclear Energy Research, Taoyuan, Taiwan, R.O.C.; 2Biomedical Imaging and Radiological Sciences, National Yang-Ming University, Taipei, Taiwan, R.O.C.

Abstract. AMBA (DO3A-CH2CO-G-(4-aminobenzoyl)-QWAVGHLM-NH2) is a bombesin (BN)-like peptide having high affinity with gastrin-releasing peptide receptors (GRPr). 177Lu-AMBA is currently undergoing clinical trial as a systemic radiotherapy for hormone refractory prostate cancer (HRPC) patients. This study evaluated the biodistribution, pharmacokinetics, bioluminescent imaging (BLI) and microSPECT/CT imaging of 177Lu-AMBA in PC-3M-luc-C6 luciferase-expressing human prostate tumour-bearing mice. Plasma stability of 177Lu-AMBA could be maintained up to 55.67±6.07% at 24 h in a protection buffer. High positive correlations of PC-3M luc-C6 tumour growth in SCID mice between caliper measurement and BLI were observed (R2=0.999). Both the biodistribution and microSPECT/CT imaging in PC-3M-luc-C6 tumour-bearing mice showed that 177Lu-AMBA in tumour uptake could be retained for 24 h. The distribution half-life (t1/2α) and the elimination half-life (t1/2β) of 177Lu-AMBA in mice were 0.52 h and 26.6 h, respectively. These results indicated that BLI could be used to monitor the growth of tumour. High uptake of 177Lu-AMBA in PC-3M-luc-C6 tumour-bearing mice by microSPECT/CT imaging can further evaluate the potential of 177Lu-AMBA therapy for PC-3M-luc-C6 tumours.

Prostate cancer is estimated to be first in the number of cancer cases and second in the number of deaths due to cancer among men in the Western world (1). The prostate-specific antigen (PSA) has been used for the diagnosis and staging of prostate cancer, and its detection sensitivity has made it effective for the evaluation of therapeutic efficacy (2). However, there are still a number of limitations that need to be addressed, such as the facts that PSA detection alone tends to underestimate the stage of prostate cancer (3) and that therapy makes prostate tumours become hormone-independent growths after treatment with androgen-deprivation therapy (4). Therefore, efforts to establish new therapeutic approaches for prostate cancer are continuing. In addition to PSA, there are other biomarkers that are overexpressed in prostate cancer such as prostate stem cell antigen (PSCA), prostate-specific membrane antigen (PSMA) (5), early prostate cancer antigen (EPCA), EPCA-2 (6) and gastrin-releasing peptide receptor (GRPr). GRPr, one kind of G-protein-coupled receptor, is a subtype of bombesin (BN)-like peptide receptors, which are normally expressed in non-neuroendocrine tissues of the pancreas and the breast and neuroendocrine tissues of the brain, the gastrointestinal (GI) tract, the lung and the prostate, but are expressed abnormally in cancers of the prostate, the colon and the lung (7-9). Because of the hormone-independent growth of prostate cancer after androgen-deprivation therapy, overexpressed GRPrs on these tumour cells are considered a promising approach for prostate cancer therapy. BN-like peptides can target GRPr and may be considered as one kind of probe for prostate cancer (10, 11). In humans, GRPr is over-expressed in androgen-independent prostate cancer (12). The PC-3 xenograft tumour is one of the suitable models for evaluating the antineoplastic activity of BN-like peptides in the treatment of androgen-independent prostate cancer (13). AMBA (DO3A-CH2CO-G-(4-aminobenzoyl)-QWAVGHLM-NH2), a well-known BN-like compound, is considered to have high affinity with GRPr and NMBr (neuromedin B receptor) (14, 15). AMBA has a DO3A structure that can chelate tripositive lanthanide isotopes, such as 68Ga, 90Y, 111In and 177Lu, thus it can formulate many kinds of radiolabelled probes for various purposes (16). 177Lu-AMBA is one radiolabelled probe that can be used for the diagnosis and therapy of prostate tumours. Its lack of radiostability is...
its only disadvantage but this can be addressed by mixing it with a protection buffer of ascorbic acid (17). In a previous study, not only did the PC-3 cell line express high GRPr, but also the LNCaP and DU145 cell line expressing low GRPr could be effectively treated by $^{177}$Lu-AMBA (18). Overall, $^{177}$Lu-AMBA is a promising drug for prostate cancer and is already in phase I clinical trials (14).

Bioluminescent imaging (BLI) is often applied for tumour model systems and can be used to monitor tumour growth (19). PC-3M-luc-C6 is a PC-3 cell expressing firefly luciferase, which has been proven to be an efficient and sensitive measure for prostate cancer in murine models (20). In this study, the growth of PC-3M-luc-C6 tumour in SCID mice was evaluated using a BLI system. Although previous studies have demonstrated the radiostability, binding properties in vitro and biodistribution in vivo (14), the pharmacokinetics and microSPECT/CT imaging of $^{177}$Lu-AMBA in PC-3M-luc-C6 xenograft SCID mice have not yet been reported. In-depth in vivo studies of $^{177}$Lu-AMBA could help to evaluate its properties. Furthermore, combining BLI and microSPECT/CT imaging could have the potential for evaluating the therapeutic efficacy of $^{177}$Lu-AMBA at early disease stages.

Materials and Methods

Chemicals. Protected Nα-Fmoc-amino acid derivatives were obtained from Novabiochem (Merck Schuchardt OHG, Germany), Fmoc-amide resin and coupling reagent were obtained from Applied Biosystems Inc. (Foster City, CA, USA) and DOTA-tris (t-Bu ester) was obtained from Macroyclics (Dallas, TX, USA). Fmoc-4-azido-4-H was obtained from Bachem (Chauptstrasse, Switzerland). Bombesin was obtained from Fluka (Buchs, Switzerland).

Synthesis of AMBA. AMBA was synthesized using solid-phase peptide synthesis (SPPS) using an Applied Biosystems Model 433A equipped with an automated peptide synthesizer, employing the Fmoc (9-fluorenylmethoxy-carbonyl) strategy. Carboxyl groups on Fmoc-protected amino acids were activated by (2-(1-Fmoc (9-fluorenylmethoxy-carbonyl) strategy. Carboxyl groups on N-terminal amino group on the growing peptide, anchored via the C-terminus to the resin, and providing for stepwise amino acid addition. Rink Amide resin (0.25 g) and Fmoc-protected amino acids (1.0 mM), with appropriate side chain protections, and DOTA-tris (t-Bu ester) were used for SPPS of the BBN conjugates. Side chain protecting groups in the synthesis were Trt for Gin and His, and Boc for Trp.

The protected peptide-resin was cleaved and deprotected with a mixture of 50% trifluoroacetic acid (TFA): 45% chloroform, 3.75% anisole, 1.25% 1, 2-ethanedithiol (EDT) for 4 h at room temperature (RT). The crude peptide was isolated by precipitating with cold diethyl ether. After centrifugation, the collected precipitate was dried (RT). The crude peptide was isolated by precipitating with cool anisole, 1.25% 1, 2-ethanedithiol (EDT) for 4 h at room temperature.

Radiolabelling and purification of $^{177}$Lu-AMBA. The labelling method for AMBA with $^{177}$Lu was previously described (14). Briefly, 3 μg of AMBA and about 37 MBq $^{177}$LuCl$_3$ (PerkinElmer, USA; 37 GBq/ml in 0.05 M HCl) were added in 0.2 M sodium acetate (pH4.8) until there was 50 μl per vial, and then heated at 95°C for 10 min. The labelling efficiency of $^{177}$Lu-AMBA was analysed by instant thin-layer chromatography (ITLC SG; Pall Corporation, New York, NY, USA) with 0.1 M Na-citrate (pH 5.0) as solvent ($^{177}$LuCl$_3$; RF=0.9–1.0, peptide-bound $^{177}$Lu: RF=0–0.1) (17). Radiocolloid was also monitored using ITLC strips developed with acetone/0.9% saline (1:1) as solvent (radiocolloid: RF=0–0.1). Radio high performance liquid chromatography (Radio-HPLC) analysis was performed using a Waters 2690 chromatography system with a 996 photodiode array detector (PDA), a Bioscan radiodetector (Washington, DC, USA) and a FC 203B fraction collector by Gilson (Middleton, WI, USA). $^{177}$Lu-AMBA was purified by an Agilent (Santa Clara, CA, USA) Zorbax bonus-RP HPLC column (4.6×250 mm, 5 μm) eluted with a gradient mixture from 80% solvent A (0.1% TFA in H$_2$O) (v/v)/20% B (0.1% TFA in acetonitrile) (v/v) to 70% A/30% B in 15 min. Flow rate was 1.5 ml/min at RT and the retention time for $^{177}$Lu-AMBA was 7.5 min.

In vitro plasma stability. After radiolabelling, $^{177}$Lu-AMBA (10 μl, 7.4 MBq) was first diluted with 40 μl protection buffer [8:2 mixture of 0.9% sodium chloride injection USP and ascorbic acid (250 mg/ml; Tai Yu Chemical & Pharmaceutical, Taiwan, ROC), with 0.025% (w/v) Na$_2$EDTA], and the same volume of 0.9% sodium chloride injection USP was used as a control. The sample was incubated in normal saline, rat and human plasma at a ratio of 1:1 (v/v) at 37°C. At several time points after administration (1, 4, 8, 24 and 48 h), 1 μl and 5μl of $^{177}$Lu-AMBA in plasma or normal saline were analyzed using ITLC-SG and radio-HPLC, respectively. The analysis method was the same as that applied for purification.

Cell culture and animal model. Bioluminescent human prostate adenocarcinoma cell line PC-3M-luc-C6 (Caliper Life Sciences, MA, USA) was maintained in Ham’s F-12K medium supplemented with 10% heat-inactivated fetal bovine serum and incubated in 5% CO$_2$ at 37°C, and subcultured by 0.05% trypsin/ethylenediaminetetraacetic acid (all from Gibco, Grand Island, NY, USA). The cell line was thawed from an aliquot vial, grown and used within 10 passages for animal inoculation. Male severely compromised immunodeficient (SCID) mice at 4 weeks old were obtained from the Laboratory Animal Center of National Taiwan University (Taipei, Taiwan, ROC) and maintained on a standard diet (Lab diet; PMI Feeds, St. Louis, MO, USA) at RT, with free access to tap water in the animal house of the Institute of Nuclear Energy Research (INER), Taoyuan, Taiwan, ROC. Each of the SCID mice was subcutaneously injected with $2\times10^6$ PC-3M-luc-C6 cells (in 100 μl of 0.9% sodium chloride injection USP) in the right hind flank. The animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the INER.

BLI. BLI was performed with a highly sensitive, cooled CCD camera mounted in a light-tight specimen box (IVIS Imaging System 100 Series; Xenogen, USA) using protocols similar to those.
Imaging Box was in the range of $1 \times 10^4$ photons or 1-2×$10^5$ Image software (Xenogen). Background bioluminescence from the and quantified as total photon counts or photons/s using the Living display images were designated around each well by grid mode Quantification of signals in the regions of interest (ROI) from 10,000 to 20 cells into appropriate cell culture media in black, clear bottom, 96-well plates. The luciferase activity was measured by adding 200 μl luciferase reagents (150 μg/ml) (Xenogen, Alameda, CA, USA) 10 min prior to imaging. Quantification of signals in the regions of interest (ROI) from culture media in black, clear bottom, 96-well plates. The luciferase activity was measured by adding 200 μl luciferase reagents (150 μg/ml) (Xenogen, Alameda, CA, USA) 10 min prior to imaging. Quantification of signals in the regions of interest (ROI) from Images were designated around the tumour sites and quantified as total photons/s. For in vivo BLI, five PC-3M-luc-C6 tumour-bearing mice were given the substance D-luciferin in DPBS intraperitoneally at 150 mg/kg. 10-15 min after luciferin injection, five mice were placed onto the stage inside the light-tight camera box with continuous exposure of 1-2% isoflurane and imaged with the IVIS system. Imaging time ranged from 30 s to 2 min, depending upon tumour bioluminescence and size. ROIs from displayed images were designated around the tumour sites and quantified as total photons/s by the Living Image software.

Biodistribution. At 3 weeks after PC-3M-luc-C6 cell inoculation, developed tumour weights ranged from 0.01 to 0.2 g. Twenty-four PC-3M-luc-C6 xenograft SCID mice (n=4 for each group) were injected with 0.37 MBq (0.1 μg) of the 177Lu-AMBA in 80 μl of 0.9% sodium chloride injection USP via the tail vein. The mice were sacrificed by CO₂ asphyxiation and tissues and organs were excised at 30 min, 1, 4, 8, 24 and 48 h post injection (p.i.). Subsequently, the tissues and organs were weighed, radioactivity was counted in a Packard Cobra II gamma-counter by Perkin-Elmer (Waltham, MA, USA) and the percentage of injected dose per gram (% ID/g) for each organ or tissue were calculated (22).

Pharmacokinetic studies. Four PC-3M-luc-C6 xenograft SCID mice were injected with 0.37 MBq (0.1 μg) of the 177Lu-AMBA in 80 μl of 0.9% sodium chloride injection USP via the tail vein. At 5 min, 1, 4, and 24 h p.i., 20 μl of blood was collected from the heart puncture, then the blood weighed and radioactivity was counted in the Cobra II gamma-counter and the percentage of injected dose per gram (% ID/g) was calculated. The data were fitted to a two-compartment model and the pharmacokinetic parameters were derived by the WinNonlin 5.0 software (Pharsight Corporation, Mountain View, CA, USA).

Micro-SPECT/CT imaging. Two male SCID mice bearing human PC-3M-luc-C6 tumours of approximately 0.1 g were i.v. injected with 14.8 MBq/0.95 μg 177Lu-AMBA after purification by radio-HPLC. The SPECT and CT images were acquired by a micro-SPECT/CT scanner system (XSPECT; Gamma Medica-Ideas Inc., Northridge, CA, USA). SPECT imaging was performed using medium-energy, parallel-hole collimators at 1, 4, 8, 24, and 48 h. The source and detector were mounted on a circular gantry allowing them to rotate 360 degrees around the subject (mouse) positioned on a stationary bed. The field of view (FOV) was 12.5 cm. The imaging acquisition was accomplished using 64 projections at 90 seconds per projection. The energy windows were set at 113 keV±10% and 209 keV±10%. SPECT imaging was followed by CT imaging (X-ray source: 50 kV, 0.4 mA; 256 projections) with the animal in exactly the same position. A three dimensional (3-D) Feldkamp cone beam algorithm was used for CT image reconstruction and a two-dimensional (2-D) filtered back projection algorithm was used for SPECT image reconstruction. All image processing software, including SPECT/CT co-registration, was provided by Gamma Medica-Ideas Inc (Northridge, CA, USA). After co-registration, both the fused SPECT and CT images had 256×256×256 voxels with an isotropic 0.3-mm voxel size.

Results

Radiolabelling and plasma stability studies of 177Lu-AMBA. The labelling efficiency of 177Lu-AMBA by ITLC analysis was 91.14±2.59%. The protection buffer may have affected the stability of 177Lu-AMBA in different conditions by radio-HPLC analysis (Table I). For radiolabelling of 177Lu-AMBA, the radiochemical purity was 70.8±7.51% by radio-HPLC analysis. The stability of 177Lu-AMBA rapidly reduced at 24 h and completely degraded at 48 h in normal saline without the protection buffer. However, 177Lu-AMBA retained 55.67±6.07% and 38.44±3.83% at 24 and 48 h in the protection buffer, respectively. These results indicated the effect of the protection buffer on the storage of 177Lu-AMBA. The stability of 177Lu-AMBA in rat and human plasma without the protection buffer was not significantly different from that with the protection buffer. The stability of 177Lu-AMBA in human plasma was slightly higher than that in rat plasma.

Table I. In vitro stability of 177Lu-AMBA by HPLC analysis at different time points after incubation in normal saline, human plasma and rat plasma at 37°C (mean±SEM, n=4 at each time point).

<table>
<thead>
<tr>
<th>Time point (h)</th>
<th>Control</th>
<th>With protection buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal saline (%)</td>
<td>Rat plasma (%)</td>
</tr>
<tr>
<td>0</td>
<td>70.8±7.51</td>
<td>74.3±7.58</td>
</tr>
<tr>
<td>1</td>
<td>63.6±11.5</td>
<td>65.1±12.0</td>
</tr>
<tr>
<td>4</td>
<td>52.9±12.2</td>
<td>58.7±19.7</td>
</tr>
<tr>
<td>24</td>
<td>14.8±13.0</td>
<td>25.0±18.1</td>
</tr>
<tr>
<td>48</td>
<td>0.00±0.00</td>
<td>24.3±21.9</td>
</tr>
</tbody>
</table>

Liu et al: Pharmacokinetics and Imaging of 177Lu-AMBA
The photon emission from suspensions of human prostate (PC-3M-luc-C6) tumour cell lines expressing luciferase is shown in Figure 1A. The minimum number of detectable cells in the suspension was approximately 156 cells per well. Bioluminescence per well correlated to the total number of PC-3M-luc-C6 cells ($r^2=0.96$; Figure 1B). To evaluate the tumour growth in vivo, the subcutaneous growth of the PC-3M-luc-C6 tumour cells was sequentially measured from day 0 to day 14 by BLI and compared to external caliper measurements of the same tumour sites. On day 0, xenografted mice showed successful tumour imaging by BLI (Figure 2A). Bioluminescent photon counts in the tumour sites correlated to the growth of PC-3M-luc-C6 cells during the study period ($r^2=0.96$; Figure 2B). In contrast, accurate measurements of tumour volume by the caliper method could be obtained in mice only after 7 days post implantation (Figure 3A). After tumours were measurable by the caliper method, BLI correlated well with caliper-measured data ($r^2=0.99$; Figure 3B).

**Biodistribution.** $^{177}$Lu-AMBA significantly accumulated in the tumour, the adrenal, the pancreas, the small intestine and large intestines (Table II). Fast blood clearance and fast excretion from the kidneys were observed. This indicated that the radioactivity was excreted rapidly in the urine. The uptake of $^{177}$Lu-AMBA still remained 10.4±3.49, 3.52±0.17.
Figure 2. Growth of PC-3M-luc-C6 tumours in SCID mice. A: Bioluminescent images of two SCID mice are displayed on day 0, 3, 7, 10 and 14. B: Tumour size was measured by calipers ($n=5$, mean±SEM).
and 1.32±0.61% ID/g in the pancreas, the kidney and the tumour at 24 h, respectively (Table II). The tumour-to-blood ratio reached a maximum within 24 h and then declined.

**Pharmacokinetic studies.** The mean radioactivity (% ID/ml) of \(^{177}\text{Lu-AMBA}\) with log scale in the plasma is plotted in Figure 4. The radioactivity declined to under detection limit after 24 h. The pharmacokinetic parameters derived by a two-compartment model (23) indicated that the distribution half-life (\(t_{1/2\alpha}\)) and elimination half-life (\(t_{1/2\beta}\)) of \(^{177}\text{Lu-AMBA}\) were 0.52±0.05 h and 26.63±11.74 h, respectively (Table III).

**MicroSPECT/CT imaging.** Micro-SPECT/CT imaging of \(^{177}\text{Lu-AMBA}\) demonstrated a significant uptake in the tumours at 4 and 24 h after intravenous injection (Figure 5). The longitudinal micro-SPECT/CT imaging showed high accumulation of \(^{177}\text{Lu-AMBA}\) in the kidney, the pancreas and the GI tract at 4, 8, 24 and 48 h after intravenous injection. The trend of the uptake shown in the imaging data was similar to the results of the biodistribution study.

**Discussion**

GRP receptors are expressed in many kinds of cancers, and bombesin shows a high affinity for the GRP receptor, which has led to the development of the most appropriate radiolabelled analogues of the BN-like peptide (24, 25). \(^{177}\text{Lu-AMBA}\) was developed (14) due to its high-affinity binding of GRP-R expressing cells (15, 17, 18, 26). Although excellent probes have been evaluated in treating prostate cancer, no study on the plasma stability of \(^{177}\text{Lu-AMBA}\) in the protection buffer up to 72 h has yet been reported. Furthermore, multi-imaging modalities to monitor the growth of PC-3M-luc-C6 tumour by a bioluminescence system and to measure the uptake of \(^{177}\text{Lu-AMBA}\) by microSPECT/CT have also not yet been reported.

For the plasma stability of \(^{177}\text{Lu-AMBA}\), the results of ITLC analysis could not distinguish the stability of \(^{177}\text{Lu-AMBA}\) in different environments (data not shown), whereas HPLC analysis was able to determine the decrease in the stability of \(^{177}\text{Lu-AMBA}\) during the study period (Table I). Taken together, these data indicated that ITLC analysis is suitable only for labeling yields but not for radiochemical purity. The stability of \(^{177}\text{Lu-AMBA}\) in normal saline decreased rapidly when no protection buffer was added and was completely degraded in 48 h, whereas 38.4±3.83% of \(^{177}\text{Lu-AMBA}\) was contained in the protection buffer after 48 h (Table I). These results were in agreement with a previous report by Chen et al. (17). Although there were no significant differences whether mixing with the protection buffer in the
Figure 5. Micro-SPECT/CT imaging of $^{177}$Lu-AMBA was performed in PC-3M-luc-C6 tumour-bearing mice. Mice were intravenously injected with 14.8 MBq/0.95 μg $^{177}$Lu-AMBA. The energy windows and the image size were set at 113 keV±10%, 209 keV±10% and 80×80 pixels, respectively.

Table II. Biodistribution of $^{177}$Lu-AMBA after i.v. injection in PC-3M-luc-C6 prostate tumour-bearing SCID mice. Values are expressed as % ID/g, mean±SEM (n=4 at each time point).

<table>
<thead>
<tr>
<th>Organ/time</th>
<th>0.5 h</th>
<th>1 h</th>
<th>4 h</th>
<th>8 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>2.01±0.46</td>
<td>0.69±0.09</td>
<td>0.04±0.00</td>
<td>0.02±0.01</td>
<td>0.01±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Brain</td>
<td>0.07±0.01</td>
<td>0.03±0.01</td>
<td>0.02±0.00</td>
<td>0.01±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.53±0.15</td>
<td>0.32±0.05</td>
<td>0.02±0.02</td>
<td>0.04±0.01</td>
<td>0.03±0.01</td>
<td>0.01±0.01</td>
</tr>
<tr>
<td>Bone</td>
<td>0.52±0.07</td>
<td>0.35±0.02</td>
<td>0.27±0.05</td>
<td>0.18±0.02</td>
<td>0.20±0.04</td>
<td>0.16±0.04</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.96±0.16</td>
<td>0.61±0.06</td>
<td>0.47±0.11</td>
<td>0.77±0.38</td>
<td>0.36±0.07</td>
<td>0.22±0.10</td>
</tr>
<tr>
<td>Pancreas</td>
<td>18.5±4.10</td>
<td>19.0±1.33</td>
<td>25.6±16.9</td>
<td>15.9±2.57</td>
<td>10.5±3.49</td>
<td>7.81±1.05</td>
</tr>
<tr>
<td>S.I.</td>
<td>1.47±0.27</td>
<td>1.55±0.42</td>
<td>1.88±1.10</td>
<td>0.67±0.10</td>
<td>0.41±0.06</td>
<td>0.37±0.13</td>
</tr>
<tr>
<td>L.I.</td>
<td>2.29±0.45</td>
<td>1.62±0.28</td>
<td>3.79±2.19</td>
<td>1.72±0.25</td>
<td>1.18±0.10</td>
<td>0.39±0.07</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.81±0.38</td>
<td>1.20±0.21</td>
<td>1.24±0.46</td>
<td>0.58±0.10</td>
<td>0.39±0.03</td>
<td>0.34±0.02</td>
</tr>
<tr>
<td>Kidney</td>
<td>8.07±0.94</td>
<td>6.05±0.94</td>
<td>4.70±0.91</td>
<td>5.22±0.51</td>
<td>3.52±0.17</td>
<td>2.17±0.09</td>
</tr>
<tr>
<td>Bladder</td>
<td>5.06±1.40</td>
<td>2.95±1.43</td>
<td>0.40±0.26</td>
<td>0.30±0.08</td>
<td>0.15±0.04</td>
<td>0.13±0.02</td>
</tr>
<tr>
<td>Testis</td>
<td>0.49±0.09</td>
<td>0.37±0.16</td>
<td>0.08±0.03</td>
<td>0.05±0.01</td>
<td>0.05±0.02</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Liver</td>
<td>0.91±0.15</td>
<td>0.58±0.13</td>
<td>0.49±0.13</td>
<td>0.24±0.03</td>
<td>0.25±0.02</td>
<td>0.21±0.02</td>
</tr>
<tr>
<td>Heart</td>
<td>0.65±0.13</td>
<td>0.25±0.04</td>
<td>0.10±0.04</td>
<td>0.07±0.02</td>
<td>0.02±0.01</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Lung</td>
<td>1.62±0.34</td>
<td>0.43±0.15</td>
<td>0.21±0.07</td>
<td>0.15±0.03</td>
<td>0.11±0.05</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td>Tumour</td>
<td>2.33±0.52</td>
<td>1.07±0.11</td>
<td>0.88±0.48</td>
<td>0.73±0.04</td>
<td>0.45±0.09</td>
<td>0.33±0.03</td>
</tr>
<tr>
<td>Faeces</td>
<td>0.77±0.12</td>
<td>1.12±0.43</td>
<td>7.22±5.52</td>
<td>0.80±0.21</td>
<td>1.29±0.34</td>
<td>1.09±0.15</td>
</tr>
<tr>
<td>Urine</td>
<td>518.8±69.29</td>
<td>644.1±45.64</td>
<td>16.0±10.23</td>
<td>1.17±0.37</td>
<td>1.36±0.42</td>
<td>1.61±0.28</td>
</tr>
</tbody>
</table>

S.I.: Small intestine; L.I.: Large intestine.
The biodistribution of $^{177}$Lu-AMBA in PC-3M-luc-C6 bearing mice was similar to that of the PC-3 tumour model (14). $^{177}$Lu-AMBA was excreted primarily by the urine and accumulated most in the pancreas, in addition to the kidneys, the GI tract and the tumour (Table II). Compared to other BN-analogues, the rapid excretion of urine makes $^{177}$Lu-AMBA suitable for radiotherapy since there is less detrimental effect to other organs from the radiation. Although GRPr is a predominant receptor subtype in the pancreas of rodents, such high uptake of $^{177}$Lu-AMBA in the tumour may not affect the physiological condition in mice (22). Roger et al. found that the concentration of GRPrs on the mouse pancreas was 27 fmol/mg (30), while Fanger et al. reported a level of 75 fmol/mg (31). The higher pancreas uptake of the radiolabelled bombesin agonist may be due to its higher metabolic stability in vivo. Waser et al. reported that, in contrast to the strongly labelled GRPR-positive mouse pancreas with $^{177}$Lu-AMBA, the human pancreas did not bind $^{177}$Lu-AMBA unless chronic pancreatitis was diagnosed (15).

In the pharmacokinetic studies (Figure 4 and Table III), the elimination half-life ($t_{1/2\beta}$), distribution half-life ($t_{1/2\alpha}$) and the area under the concentration curve (AUC) of $^{177}$Lu-AMBA demonstrated the behaviour of fast peptide distribution and elimination.

In contrast to BLI, radionuclide-based techniques have good spatial resolution though they are somewhat limited by their higher cost and production of isotopes. Various methodologies have been developed for imaging the reporter gene expression in living cells, non-invasively and repetitively in animals. Each of these methodologies has unique applications, advantages and limitations that can be complementary to other modalities (32). Micro-SPECT/CT imaging is a non-invasive imaging modality that can determine the distribution of radiotherapeutic drugs in vivo at different time points. Micro-SPECT/CT imaging correlated well with the biodistribution study. $^{177}$Lu-AMBA was clearly distributed in the pancreas, the GI tract and the tumour 4 to 48 h after administration (Figure 5). Many studies have focused on micro-SPECT/CT imaging of BN-analog and AMBA, which were labeled with $^{111}$In (21, 33-35). Recently, imaging of $^{177}$Lu-AMBA by Maddalena et al. using planar gamma imaging showed results similar to the present study (18). Overall, micro-SPECT/CT imaging can be a very accurate tool for evaluating the quantity and quality of radiotherapy. In conclusion, the BLI system could effectively evaluate the PC-3M-luc-C6 tumour model and, combined with experiments of $^{177}$Lu-AMBA in vitro and in vivo, provides a multimodality imaging method for the preclinical study of $^{177}$Lu-AMBA.

**Acknowledgements**

The Authors would like to thank Chun-Lin Chen for his help with the preparation of AMBA.
References


Received June 6, 2010
Revised August 6, 2010
Accepted August 24, 2010