Longitudinal MicroSPECT/CT Imaging and Pharmacokinetics of Synthetic Luteinizing Hormone-releasing Hormone (LHRH) Vaccine in Rats

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Abstract. *Background: Luteinizing hormone-releasing hormone (LHRH)-derived decapeptide-based vaccines have been used in studies of immunocastration and immunotherapy of prostate cancer, but no image data are available on the kinetics of vaccines post injection (p.i.). Therefore, an ^{131}I radiolabeled LHRH-derived immunogen was developed to visualize and evaluate the retention of LHRH-derived vaccines in rats. Materials and Methods: The LHRH immunogens, which contained equal moles of ^{131}I-p607E, p667 and p500, were formulated with an equal volume of an adjuvant, Montanide ISA50. MicroSPECT/CT imaging was performed to visualize the retention of the radiolabeled immunogen up to 30 days after intramuscular inoculation of 25 μg immunogens. The pharmacokinetics, distribution and excretion were also evaluated. Results: The radiochemical purity of ^{131}I-p607E was 97.85±2.12%. The longitudinal microSPECT/CT imaging revealed that most ^{131}I-p607E was retained at the injected muscle site until 30 days p.i.. Biodistribution showed that 34.56±4.27% of radioactivity remained at the injected muscle site at 28 days p.i.. The cumulative radioactivity excreted via urine was 30.02±3.82% up to day 28 p.i.. The elimination half-life ($t_{1/2}$), T_{max}, and C_{max} were 158.67 h, 24 h, and 0.026 percentage of injected dose per gram (%ID/g), respectively. Conclusion: The LHRH immunogen, ^{131}I-p607E, was mainly retained at the intramuscular injection site during the whole study period. The microSPECT/CT imaging modality can be used to monitor the location and distribution of the LHRH immunogen, ^{131}I-p607E, in a rat model.*

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The decapeptide luteinizing hormone-releasing hormone (LHRH), *p*Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, induces the maturation of testosterone-secreting interstitial cells of the testis. In the prostate, testosterone is converted to dihydrotestosterone, the form that interacts with androgen receptors on prostatic epithelial cells to control their proliferation and apoptosis. The growth of hormone-dependent tumors arising in the prostate gland can be controlled by removal of the growth-promoting hormone(s) or by blocking the LHRH pathway with immunotherapy. Castration by surgery has been a conventional approach to stop the supply of androgens. However, castration is an invasive surgical procedure and it also removes the source of natural anabolic androgens. Active immunization against LHRH has shown the potential to shut down the reproductive system of mammals for various practical and clinical reasons (1).

An effective and practical synthetic UBITh® LHRH peptide-based immunotherapeutic vaccine for the treatment of androgen-responsive prostate cancer has been designed and tested (2). The immunological strategy of the UBITh® immunogens is to induce an "anti-self" immunity to LHRH by altering the target molecule on a synthetic peptide immunogen (Table I). Immunization with LHRH immunogens produces the desired hormone neutralization effect or "immunological castration" by eliciting anti-LHRH antibodies to the LHRH. The immunological and biological efficacy of synthetic LHRH peptide immunogens have been proven in rodents, dogs and baboons, and as an immunotherapy for androgen-responsive prostate cancer (2).

Molecular imaging has widely been applied in preclinical studies for non-invasive monitoring of the specific processes of biology and disease *in vivo* at cellular or genetic levels

Table I. *Characteristics of UBITh® LHRH immunogens.*

Code name	UBITh® LHRH immunogen
p607E[a]	Inv-GG-UBITh®4-GG-LHRH
p667	Inv-GG-UBITh®5-GG-LHRH
p500	UBITh®6-LHRH

[a]Peptide p607E: a single residue substitution of glutamic acid (E) for aspartic acid (D) in UBITh®4 peptide segment for improved peptide stability (2).

Table II. *Stability of ^{131}I-p607E peptide in normal saline (25˚C) or incubated with rat plasma (37˚C) (mean±SD, n=4 at each time-point).*

Incubation time (h)	Normal saline (%)	Plasma (%)
0	99.22±0.68	96.34±0.69
24	93.26±1.63	93.70±0.58
48	94.47±3.23	92.07±0.42
72	94.30±0.20	89.40±1.43

without sacrificing the living subject (3). Molecular imaging techniques that are currently used in clinical diagnosis can be divided into two categories – anatomical and functional modalities (4). X-ray computed tomography (CT) is utilized in examining the anatomy of tissue and organs in animals, while single photon emission computerized tomography (SPECT) provides functional or metabolic information in animals. Recently, microSPECT/CT imaging has been used for non-invasive imaging of a wide range of animal models, providing serial, longitudinal and tomographic information in the same animals thus allowing researchers to follow the biological process over time and to monitor the effects of interventions on disease progression (5).

Iodine-131 is commonly used for long term SPECT imaging due to its physical properties (mainly gamma emission at 363 keV, half-life 8.02 day). Proteins labeled with ^{131}I have been applied in clinical practices to assess the site(s) of tumors, pre-surgical staging, immunotherapy and monitoring for recurrence or response to various treatments (6). Radioiodinated LHRH agonists were originally used as tracers in competition binding assays to determine expression levels and affinity of LHRH receptors (7). Conventional techniques, such as serological assays and lymphocyte proliferation analysis, are used for the characterization of LHRH immunogens *in vivo* (2, 8). These methods, based on the evaluation of humoral and cellular immune responses to immunogens, however, can only determine the efficacy of immunogenecity. Little information about the pharmacokinetics of the immunogens can be obtained during the immunization. Although the efficacy of the immunogens has been studied, the direct visualization and pharmacokinetics of an ^{131}I-radiolabeled LHRH immunogen by SPECT/CT imaging modality in rodents has not yet been reported.

To investigate the absorption and the fate of ^{131}I-labeled LHRH immunogens, *in vivo* imaging of an LHRH immunogen, ^{131}I-p607E, was carried out by microSPECT/CT in rats and compared with the results from a conventional biodistribution method. The pharmacokinetics and the excretion profile of ^{131}I-p607E were also evaluated.

Materials and Methods

Animals and housing. Eight-week-old male Sprague-Dawley (SD) rats were obtained from the National Animal Center of Taiwan (Taipei). The rats were housed at a temperature of 20-23˚C and offered standard diet (Lab diet, PMI Feeds, St. Louis, MO, USA) with tap water *ad libitum* in the animal house of the Institute of Nuclear Energy Research (INER), Taoyuan (Taiwan, ROC). All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the INER.

Peptide synthesis. A diverse array of chimeric LHRH peptide immunogens, each carrying both the target LHRH decapeptide site and a distinct helper T cell (Th) epitope, was produced by United Biomedical Inc., (Huko, Taiwan, Asia, ROC) (2). The Th epitopes (designated as UBITh®) are covalently linked to the anti-LHRH antibody-eliciting epitope (Target B cell epitope) by continuous solid-phase synthesis. The UBITh® domains are promiscuous Th epitopes that stimulate helper T-cells of a broad range of histocompatibility backgrounds. The UBITh® epitopes used here are 15 residues in length that correspond to Th sequences found in three vaccine immunogens (Table I). The immunogens include i) p607E: hepatitis B virus surface antigen ($HBsAg_{19-33}$); the peptide sequence of p607E contained a single residue substitution of glutamic acid (E) for aspartic acid (D) in UBITh®4 peptide segment for improved peptide stability (2, 9); ii) p667: measles virus fusion protein ($MVF_{288-302}$) (10); and iii) p500: tetanus toxin ($TT_{830-844}$) (11). A further modification of the UBITh® LHRH peptides, p607E and p667, is the addition to the amino-terminus of a domain from *Yersinia* invasin protein ($Inv_{718-732}$) that has demonstrated a significant adjuvant activity. The individual functional domains of the UBITh® immunogens are separated by a glycine-glycine (GG) spacer. The UBITh® LHRH immunogens are formulated as mixtures (p607E+p667+p500) in order to maximize the immune response in individual animals (2).

Radiolabeling of p607E. The p607E peptide was radiolabeled with Na[^{131}I] using the Iodogen (1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril) method (12, 13). Briefly, borasilicate test tubes pre-coated with 50 μg of Iodogen (Pierce, Rockford, IL, USA) were prewetted with 1 ml of Tris Iodination Buffer (25 mM Tris-HCl, pH 7.5 and 0.4 M NaCl) and decanted. Then, 4-40 MBq of Na[^{131}I] in 0.1 M NaOH (PerkinElmer, Boston, MA, USA) in 100 μl of Tris Iodination Buffer was added to each tube. The reaction was allowed to proceed for 6 min at room temperature (RT) with swirling of the tube every 30 seconds. A

Table III. *Radioactivity in organs after intramuscular injection of LHRH immunogens into rats. Values were presented as percent injected dose per gram (%ID/g, mean±SD, n=5 at each time-point).*

	3 h (%ID/g)	7 day (%ID/g)	16 day (%ID/g)	21 day (%ID/g)	28 day (%ID/g)
Blood	0.00762±0.00021	0.00331±0.00032	0.00184±0.00012	0.00159±0.00009	0.00104±0.00014
Brain	0.00049±0.00002	0.00031±0.00003	0.00020±0.00001	0.00018±0.00001	0.00018±0.00002
Muscle	0.00179±0.00019	0.00110±0.00011	0.00073±0.00013	0.00083±0.00013	0.00045±0.00020
Bone	0.00363±0.00026	0.00488±0.00281	0.00149±0.00019	0.00143±0.00019	0.00167±0.00046
Testes	0.00283±0.00023	0.00146±0.00004	0.00078±0.00003	0.00065±0.00004	0.00050±0.00002
Spleen	0.00310±0.00008	0.00275±0.00031	0.00232±0.00015	0.00340±0.00058	0.00250±0.00032
Stomach	0.02306±0.00950	0.03096±0.00958	0.00380±0.00075	0.00388±0.00050	0.00174±0.00027
Small intestine	0.00370±0.00031	0.00195±0.00016	0.00096±0.00009	0.00058±0.00005	0.00045±0.00005
Large intestine	0.00325±0.00022	0.00167±0.00013	0.00092±0.00016	0.00063±0.00008	0.00059±0.00006
Kidney	0.00677±0.00097	0.01839±0.00217	0.01948±0.00229	0.01840±0.00149	0.01445±0.00086
Lung	0.00485±0.00021	0.00281±0.00041	0.00174±0.00010	0.00152±0.00017	0.00114±0.00009
Heart	0.00285±0.00013	0.00185±0.00037	0.00084±0.00005	0.00078±0.00003	0.00056±0.00004
Liver	0.00320±0.00009	0.00192±0.00018	0.00131±0.00013	0.00146±0.00024	0.00091±0.00009
Bladder	0.04044±0.03434	0.04639±0.01025	0.00492±0.00124	0.00560±0.00079	0.00560±0.00080
Thyroid	0.41086±0.20875	0.27848±0.16608	0.04070±0.02256	0.06742±0.03002	0.00555±0.00476
Plasma	0.00953±0.00014	0.00235±0.00028	0.00117±0.00053	0.00218±0.00028	0.00102±0.00031

Table IV. *Accumulation of radioactivity in the injected site after intramuscular injection of LHRH immunogens. Samples were collected for 28 days.*

Time *p.i.*	3 h	7 day	16 day	21 day	28 day
TO (%ID[a])	1.25±0.07	2.07±0.13	2.71±0.19	1.87±0.06	1.70±0.18
IMS (%ID)	97.20±2.71	92.80±4.86	42.72±3.17	39.65±2.54	34.56±4.27

TO: total organs, IMS: injected muscle site, *p.i.*: post injection. [a]%ID: radioactivity in organs/injected dose x100. Data were presented as mean±SEM (n=5 at each time-point).

total of, 100 μg of p607E in 50 μl of Tris Iodination Buffer was added to react for 6-9 minutes at RT with mixing by gently flicking the tube every 30 seconds. The sample was transferred to another tube to stop the reaction.

Quality control of radiolabeled ^{131}I-p607E. The labeling efficiency of p607E with ^{131}I was determined using radio-thin-layer chromatography (Radio-TLC) and radio-high-performance liquid chromatography (Radio-HPLC). Radio-TLC was performed by spotting samples on instant thin-layer chromatography silica gel strips (ITLC-SG, Gelman, Ann Arbor, MI, USA), which were then developed in methanol and H_2O (85/15, v/v) as the mobile phase. The developed strips were scanned by a Bioscan Imaging Scanner (Packard, USA). Radio-HPLC was performed on an HPLC system consisting of a Waters 2690 separation module (Waters Assoc. Milford, MA, USA) coupled to a Waters 2996 photodiode array UV detector and a radiomatic 625TR detector (PerkinElmer). Quality control was carried out using a reversed-phase HPLC column (0.46x25 cm, VYDAC®; Grace, Deerfield, IL, USA). Two buffers were used as the mobile phase: buffer A consisted of water for chromatography and 0.1% trifluoroacetic acid (TFA) (v/v); buffer B consisted of acetonitrile for chromatography with 0.1% TFA (v/v). The analysis of ^{131}I-p607E was run by a linear gradient from 0% to 60% of buffer B by 35 min. The radiolabeled ^{131}I-p607E was tested for its *in vitro* stability at 0, 24, 48 and 72 h after preparation at RT (14). *In vitro* plasma stability experiments were also performed by combining 500 μL ^{131}I-p607E with 500 μL of rat plasma and incubating the mixture at 37˚C. At each time-point, 5 μL of the sample was analyzed by radio-TLC as described.

Vaccine formulations. The LHRH immunogen mixtures contained equal moles of the three peptides (^{131}I-p607E, p667, p500). The immunogens were slowly mixed with an equal volume of water-in-oil adjuvant, Montanide ISA50 (50:50 (v/v)) (Seppic Inc., Fairfield, NJ, USA), for 1 h at RT. For quality control, the vaccine could be used when a drop of vaccine put into H_2O did not diffuse.

Biodistribution and excretion. Twenty-five male rats (five rats at each time-point) were used and each was immunized with the LHRH immunogens (25 μg total peptide per 250 μl dose per rat) in the right thigh by the intramuscular route at Day 0. The rats were sacrificed by CO_2 asphyxiation at 3 h, 7, 16, 21 and 28 days post injection (*p.i.*). The organs of interest were removed and weighed, and radioactivity was measured with a gamma counter (1470 WIZARD Gamma Counter, Wallac, Finland). The radioactivity of the carcass was measured by a CRC-15R dose calibrator (Capintec, Ramsay, NJ, USA) and the decay correction of all radioactivity data was calculated from the injection day (day 0). The percentage of injected dose per gram (%ID/g) and the percentage of injected dose (%ID) were calculated by comparison with standards representing

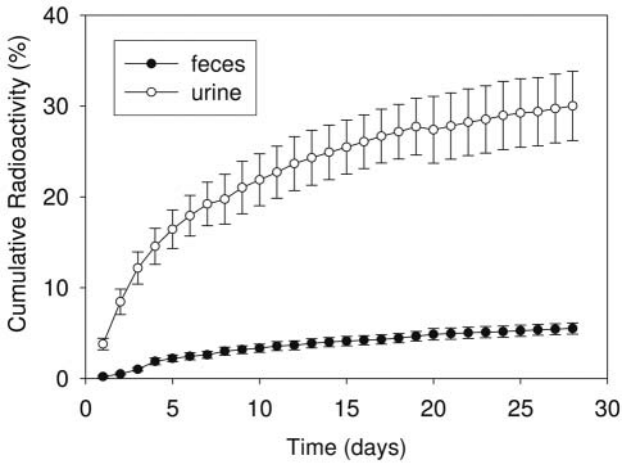

Figure 1. *Excreted radioactivity from urine and feces after intramuscular administration of 25 μg LHRH immunogens into rats. Data were presented as mean±standard error of mean (n=5).*

the injected dose per animal. When the total tissue weight (muscle, blood, bone and skin) could not be measured, it was calculated by the statistical tissue percentages of total body weights reported by Caster *et al.* (15). The weight percentages of muscle, blood, bone and skin of the total body weight used in the calculations were 45.5%, 4.95%, 6% and 18%, respectively. The percentage of total organ (TO) uptake of ^{131}I-p607E immunogens was estimated by the summation of uptake in each organ collected. The percentage of injected dose in the injected muscle site (IMS) was estimated by the total %ID (including the carcass) minus the percentage of TO. Data were expressed as mean±standard error of mean (n=5). To evaluate the excretion of ^{131}I-p607E, five conscious male SD rats (317.3±31.3 g each) were used. The rats were placed in metabolic cages individually *p.i.* of LHRH immunogens for 28 days. Food and water were adequately provided. Urine and feces were collected continuously in each individual metabolic cage, weighed at various times from 12 h to 28 days, and the radioactivity counted on a gamma counter. The %ID excreted was thus determined.

Pharmacokinetics of ^{131}I-p607E. Blood samples (300-500 μl) were collected by heart puncture under 2% isofluorine anesthesia at 1 and 4 h and 1, 2, 4, 5, 6, 7, 9, 12, 16, 21 and 28 days *p.i.* of the LHRH immunogens The samples were centrifuged at 3000 rpm to acquire plasma. The radioactivity concentration of ^{131}I-p607E in plasma was normalized to the %ID/g of tissue. The data fitted a non-compartment model and the pharmacokinetic parameters were derived by the computer software WinNonlin 4.0 (Pharsight Corporation, Mountain View, CA, USA). The $t_{1/2}$ is the elimination half-life, C_{max} is the maximum concentration reached, AUC is the area under the concentration of ^{131}I-p607E *versus* time curve.

MicroSPECT/CT imaging of ^{131}I-p607E. Three male rats were immunized in the right thigh by the intramuscular route at Day 0 with LHRH immunogens (25 μg total peptide per 250 μl dose per rat). The rats were anesthetized with 2% isoflurine and assessed with MicroSPECT/CT imaging at 3 h, 7, 14, 21 and 30 days *p.i.* with LHRH immunogens. The animal imaging system was

Table V. *Pharmacokinetic parameters of radioactivity in rats after intramuscular injection of LHRH immunogens (25 μg/rat) (n=5 for the study).*

Parameter	Unit	Value
$t_{1/2}$	h	158.67
T_{max}	h	24
C_{max}	%ID/g	0.026
$AUC_{0\rightarrow 28\ d}$	h*%ID/g	4.5

AUC: Area under the plasma concentration *versus* time curves.

X-SPECT/CT, the first commercially available, dual modality with SPECT and X-ray CT for imaging animals in medical research (Gamma Medica, Northridge, CA, USA). The fusion of X-SPECT/CT imaging has been established in our laboratory (16). The microCT element comprised a micro focus X-ray tube and a flat-panel X-ray detector. The X-ray source was a fixed anode tube with 250 mm spot size. The flat-panel detector was a 120 mm gadolinium oxysulfide/complementary metal oxide silicon (GOS/CMOS) detector with a 50 μm pitch size. The animals were positioned horizontally on a stationary bed and the X-ray tube was rotated around the subject. The distance from the X-ray tube to the detector was 298.0 mm, while the distance to the center of rotation was 225.0 mm. The operating current and voltage of the X-ray tube were set to 0.5 mA and 50 kVp, respectively. Each scan had 256 projections with a 0.5 s exposure per projection. Tomograms were reconstructed by applying the Feldkamp algorithm on a volume of 512x512x512 voxels with isotropic 0.15 mm voxel size. The compact gamma camera used in X-SPECT was based on pixilated NaI(TI) scintillators in combination with a position-sensitive photomultiplier tube readout. The scintillator array had a 58x58 matrix of 2x2x6 mm^3 crystals. Two gamma cameras were mounted at 180˚C opposite to the axis of rotation. Various interchangeable pinhole and parallel-hole collimators, with hole-sizes ranging from 0.5 to 2.0 mm, were used to optimize the resolution, the sensitivity and the field of view for particular applications. For the X-SEPCT/CT system, the object in the fusion study did not move, and the registration was performed using built-in spatial transformation. After registration, both the SPECT and the CT tomograms had 256x256x256 voxels in an isotropic 0.3 mm voxel size.

Results

Radiolabeling of ^{131}I-p607E. The labeling efficiencies of ^{131}I-p607E were 97.85±2.12% by radio-TLC. The retention time of free ^{131}I and ^{131}I-p607E was 3.4 and 29.5 min, respectively, by radio-HPLC analysis. Because the labeling efficiencies were over 95%, no further purification was performed for the animal studies. In normal saline, the ^{131}I-p607E stability ranged from 99.22±0.68% at 0 h to 94.30±0.20% at 72 h at RT (Table II). After incubation with rat plasma at 37˚C, the ^{131}I-p607E stability was 96.34±0.69% and 89.40±1.43% at 0 and 72 h, respectively.

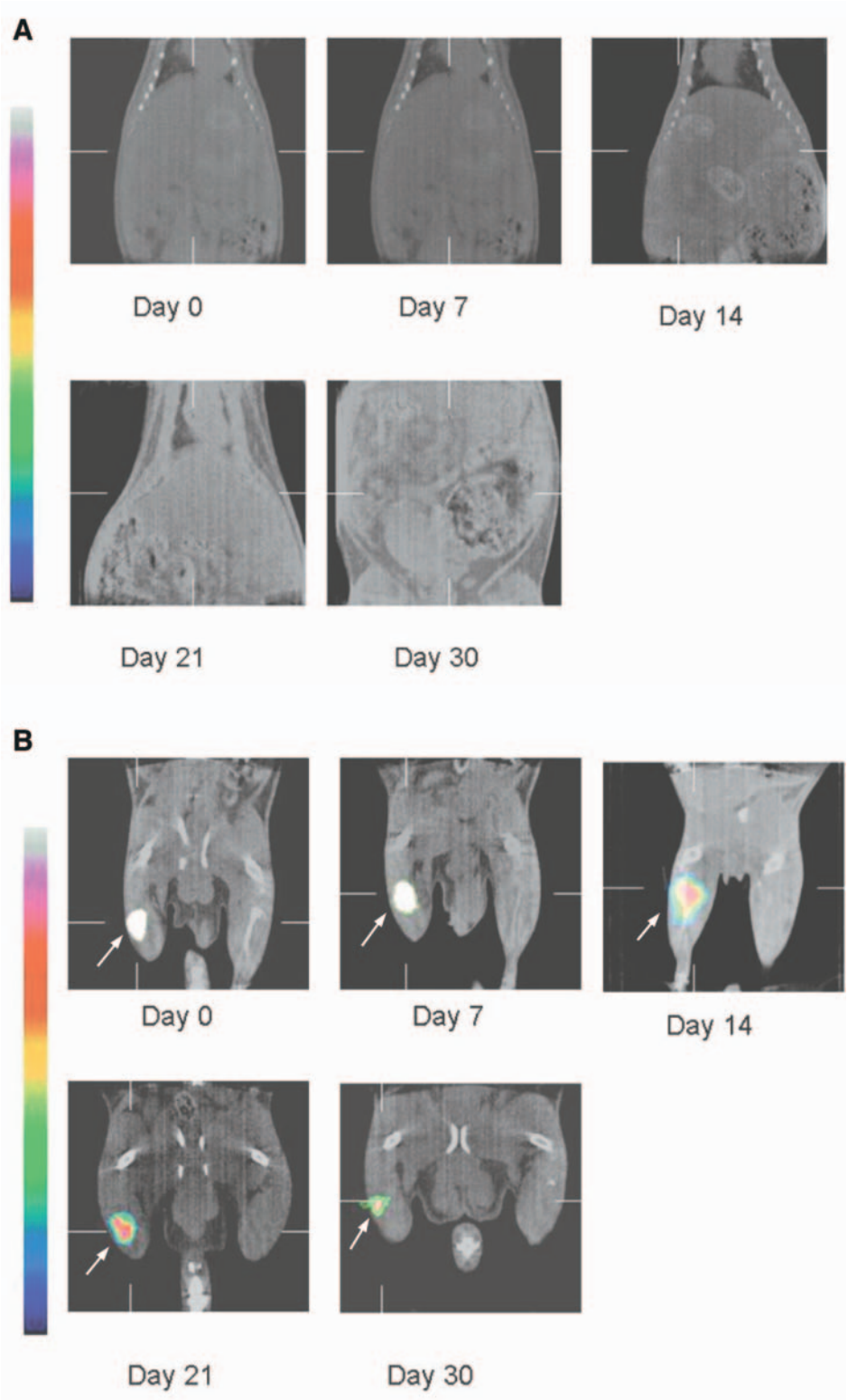

Figure 2. *The microSPECT/CT fused coronal images after administration of LHRH immunogens into a rat. These fused abdominal (A) and leg (B) region imaging scans were performed at 3 h, 7, 14, 21 and 30 days after intramuscular administration of 25 μg LHRH immunogens in the rat. Most of the radioactivity indicated by arrows remained in the initial injected area up to 30 days and remained away from the heart, liver and kidneys. The highest amount of radioactivity present in the image is indicated by white, the lowest amount of radioactivity present is indicated by black. Similar results were obtained in three different rats.*

Biodistribution and excretion. Biodistribution data of the ^{131}I-p607E (%ID/g) in selected organs at 3 h, 7, 14, 21 and 28 days *p.i.* are shown in Table III. Due to the formulation of the ^{131}I-p607E immunogens with water-in-oil adjuvant, most of the immunogens were retained at the injected site. The thyroid uptake was 0.41086±0.20875%ID/g and 0.27848±0.16608%ID/g at 3 h and 7 days *p.i.*, respectively, then declined to 0.00555±0.00476%ID/g at 28 day *p.i.*. The stomach uptake was 0.02306±0.00950%ID/g and 0.03096±0.00958% ID/g at 3 h and 7 days *p.i.*, respectively, then declined to 0.00174±0.0027%ID/g at 28 days *p.i.*. The spleen is the major organ for generation of immune responses. The accumulation of ^{131}I-p607E immunogens in the spleen reached the maximum of 0.0034±0.00058%ID/g at Day 21 *p.i.*.

To investigate the retention of ^{131}I-p607E immunogens during the generation of immune responses, the percentage of injected dose (%ID) remaining at the injected muscle site was analyzed (Table IV). The total organ uptake of ^{131}I-p607E immunogens remained low (1.25-2.71%ID) up to 28 days *p.i.*. The radioactivity in the injected muscle site was 97.20±2.71%ID at 3 h *p.i.* and gradually declined to 34.56±4.27%ID at Day 28 *p.i.*. More than 90% of the ^{131}I-p607E immunogens in the whole body stayed at the injected muscle site at each time-point. The excretion data of ^{131}I-p607E from the SD rats are presented in Figure 1. The cumulative radioactivity excreted *via* urine was 30.02±3.82% up to Day 28 *p.i.*. The radioactivity was primarily cleared through the urine, while some excretion *via* feces was also observed (5.51±0.60% up to day 28 *p.i.*).

Pharmacokinetics of ^{131}I-p607E. The radioactivity data (%ID/g) at various time-points fitted a non-compartment model for pharmacokinetics (Table V). The radioactivity was 0.005%ID/g at 3 h, reached a maximum at 24 h (C_{max}=0.026%ID/g), then slowly declined to 0.002%ID/g at day 28 *p.i.*. The elimination half-life ($t_{1/2}$) was 158.67 h *p.i.*. The $AUC_{0\rightarrow 28\ d}$ was 4.5 h*%ID/g.

Longitudinal monitoring of the location of ^{131}I-p607E by microSPECT/CT imaging. The microSPECT/CT imaging was performed at 3 h, 7, 14 and 30 days *p.i.* of ^{131}I-p607E immunogens into the right thigh of the SD rats. The SPECT/CT fused coronal images of the abdominal (Figure 2A) and leg (Figure 2B) regions of the rats are presented. The SPECT/CT fused images of the abdominal regions displayed the heart, chest, ribs, liver and kidney, where no radioactivity of ^{131}I-p607E was visualized. The radioactivity of the ^{131}I-p607E was mostly retained at the injection site on the different days, as shown by the microSPECT/CT images, which correlated well with the biodistribution results.

Discussion

In companion animals, LHRH immunotherapy has been reported for immunocontraception (17). In the swine industry, the LHRH vaccine can be used for removal of androstenones or boar taint, known to cause off-flavor of the pork from boars, as well as for growth promotion (2, 18). The radioactivity of ^{131}I-p607E in the present study was mostly retained at the injection site throughout the study period, as shown by conventional biodistribution and microSPECT/CT images, which correlated well with the effects of LHRH immunogens in previous animal studies (2).

These LHRH agonists contain a Tyr residue in position 5 for radioiodination. The LHRH receptor-binding affinities for iodine-radiolabeled LHRHs are the same as those for the corresponding noniodinated peptides (19, 20). Some ^{125}I labeled LHRH peptide agonists have also been studied in respect of the functions of LHRH receptors (21, 22). Recently, a growth hormone-releasing hormone (GHRH) antagonist, JV-1-42, has been radiolabeled with ^{131}I to evaluate its ability to cross the blood-brain barrier (23). The qualitative microSPECT/CT information in the present study demonstrated that the distribution of ^{131}I-p607E immunogens *in vivo* agreed with the findings of biodistribution. Our preliminary semi-quantitative analysis of ^{131}I-p607E immunogens by microSPECT/CT imaging also presented the same distribution trend with the results of biodistribution (data not shown).

The pharmacokinetics of an LHRH antagonist was studied by carbon-14 labeled cetrorelix (24). The T_{max} and $t_{1/2}$ were 2 h and 35.6 h after a single subcutaneous injection of ^{14}C-cetrorelix, respectively. In our study, the T_{max} and $t_{1/2}$ were 24 h and 158.67 h, respectively, after a single intramuscular injection of ^{131}I-p607E immunogens formulated with adjuvant. These results may suggest that the ^{131}I-p607E immunogen formulated with adjuvant was retained longer after immunization at the right thigh than ^{14}C-cetrorelix. Some important parameters to be considered in designing pharmacokinetic studies include the choice of radioisotopes, the position of the radiolabel in the molecule, the specific activity, radiochemical purity, analysis protocol and the imaging system. Iodine-131 has the advantages of a well-known chemistry, longer half-life (8 days), easy availability and low cost for medical research and radiotherapy (25). These advantages also make it useful for monitoring the location and distribution of vaccine during the immune response. In this regard, the data obtained from conventional biodistribution are consistent with the microSPECT/CT imaging results. This molecular imaging approach for visualizing the distribution of immunogens provides a potentially useful technique for investigating the action mechanisms of immunological processes.

In conclusion, the radioactivity of ^{131}I-p607E was mainly retained at the injection site during the study period as represented by biodistribution, which correlates well with the microSPECT/CT images. These results have demonstrated an application of non-invasive microSPECT/ CT molecular imaging in immunological studies which may be helpful for investigating the staging of vaccine treatment.

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