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ORIGINAL PAPER

Preparation and biodistribution of [^{20l}Tl](III)vancomycin complex in normal rats

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Abstract Thallium-201 ($T_{1/2}$ = 3.04 days) in Tl^+ form was converted to Tl^{3+} cation in presence of O_3 in 6 M HCl controlled by RTLC/gel electrophoresis methods. The final evaporated activity was reacted with vancomycin (VAN) in water to yield [^{201}Tl](III)VAN. The best results were obtained at room temperature in water after 30 min with a radiochemical yield > 99%, after mixing the reactants followed by SPE purification using Si Sep-Pak. The studies showed that thallic ion is mostly incorporated into vancomycin with a radiochemical purity of more than 98 ± 1% by RTLC. A specific activity of about 4.14×10^{10} Bq/mmol was obtained. Radiochemical purity and stability of ^{201}Tl-VAN in the preparation and in presence of human serum was determined up to 5.5 days. Biodistribution study of ^{201}Tl(III)-vancomycin in normal rats was performed up to 52 h.

Key words thallium-201 • vancomycin • labeling • SPECT • radiopharmaceuticals • infection

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Introduction

Thallium-201 ($T_{1/2}$ = 3.04 days) has been used in clinical nuclear cardiology and oncology for the last 3 decades. The development of [^{201}Tl](III)radiopharmaceuticals could provide many advantages: a) the physical properties of ^{201}Tl are interesting; b) the chemistry of [^{201}Tl](III)-complexation molecule is simple; c) the complexation constant for most of Tl(III) complexes are among the highest (for instance Tl(III)-DTPA at 25°C, log K = 46) for all metal-chelator complexes; d) high specific activity radiopharmaceuticals can be synthesized in kit formulations; e) [^{201}Tl] is available in many parts of the developed and developing world and can offer alternatives for other metallic radioisotopes including [^{111}In]In^{3+}. Alternatively any new [^{201}Tl](III)labeled compound can prove to be useful in any routine nuclear medicine department, especially if the starting material is [^{201}Tl]TlCl, and oxidation process would be feasible using ordinary chemicals.

Despite the above advantages, Tl-201 labeled compounds are rare in the literature. In one study, a peptide-DTPA-^{201}Tl(III) conjugate was prepared for possible scintigraphy, but further biological evaluation of the conjugate was not reported [4]. In another study, DTPA conjugation was used in human polyclonal antibody [18].

A wide variety of radiopharmaceuticals have been proposed for the scintigraphic detection of inflammatory and infectious disease [1]. [^{67}Ga]Citrate, being the most primitive radiotracer for this purpose, has a high sensitivity for both acute and chronic infections and

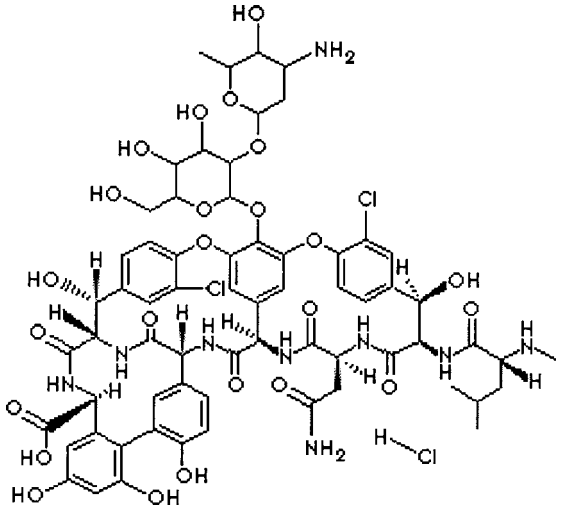

Fig. 1. Chemical structure of vancomycin hydrochloride.

noninfectious inflammation [4]. Other radiopharmaceuticals are immunoglobulins [3, 13, 14], liposomes and labeled leukocytes [3, 14, 18], antimicrobial peptides [5], avidin-biotin system [7], nanocolloids [9] and finally radiolabeled antibiotics such as ciprofloxacin [2]. Ciprofloxacin labeled with ^{99m}Tc has already shown a high sensitivity and specificity for infection imaging, opening a new era in the imaging of infection using radiolabeled antibiotics.

Vancomycin hydrochloride is an antibiotic produced by the growth of certain strains of *Streptomyces orientalis*. Vancomycin HCl has the chemical formula $C_{66}H_{75}Cl_{12}N_9O_{24}$ HCl and formula weight 1486 (Fig. 1). In the first 24 h, about 75% of the administered dose of vancomycin is excreted in urine by glomerular filtration. There is no apparent metabolism of the drug. The bactericidal action of vancomycin results primarily from inhibition of cell-wall biosynthesis. In addition, vancomycin alters bacterial-cell membrane permeability and RNA synthesis. There is no cross resistance between vancomycin and other antibiotics. Vancomycin is active against staphylococci, including *Staphylococcus aureus*.

There are studies concerning radiolabeled vancomycins in the literature for drug interaction investigations [15] and use of ^{99m}Tc-vancomycin for possible infection imaging [16]. Based on our previous experiences on the production and biological evaluation of radiolabeled glycopeptide antibiotics [11, 12] and vast clinical application of vancomycin for therapeutic purposes [6], we were interested in preparation of radiolabeled vancomycin as a possible infection imaging agent.

Because of ^{201}Tl availability as a SPECT radionuclide and its suitable physical properties, developing radiotracers by Tl(III)-complexation in the form of thallic cation will introduce a new generation of radiopharmaceutical kits.

We optimized [^{201}Tl](III)complex formation conditions with vancomycin. The stability of [^{201}Tl](III)vancomycin complex in the presence of human serum and final preparation was determined for 5.5 days. Finally the optimized tracer was administered to normal rats for biodistribution studies.

Experimental

Materials and methods

Production of ^{201}Tl(I) was performed in the Nuclear Research Center for Agriculture and Medicine (NRCAM) 30 MeV cyclotron (Cyclone-30, IBA) based on the routine production of ^{201}Tl-thallous chloride for country use. $^{203}Tl_2O_3$ with isotopic enrichment of more than 95% was supplied by Kurchatov Institute (Russia). Ammonium acetate and methanol were purchased from Aldrich (Germany). Thin-layer chromatography (TLC) was performed on polymer-backed silica gel (F 1500/LS 254, 20 × 20 cm, TLC Ready Foil, Schleicher & Schuell®, Germany). The distribution of radioactivity along the RTLC chromatograms was performed by counting 5-mm portions of the strip using an in-house made radiochromatogram scanner equipped with a Canberra™ high purity germanium (HPGe) detector (model GC1020-7500SL) or counting each 5 mm-strip after cutting it into pieces in a CRC Capintech Radiometer (NJ, USA). Radionuclide purity was checked with the same detector. All calculations and RTLC counting were based on the 167 keV peak. O_3 was produced by medicinal oxygen (Air Liquide, Belgium) using a conventional O_3 generator at a flow rate of 1 liter per minute. The oxidation of $^{201}Tl^{3+}$ was checked by cellulose acetate paper electrophoresis (Gellman) in 0.05 N EDTA at 200 V for 10 min. Pyrogen test was performed using a commercial LAL kit (sensitivity 0.125 EU/ml, Charles River Endosafe Co., USA).

Production of thallium-201 in [^{201}Tl]$TlCl_3$ form

The production of Tl-201 in our Center is routinely performed for country use. Briefly, Tl-203 electroplated on a copper support was irradiated by 28.5 MeV (1400 μAh) to produce Pb-201 via ^{203}Tl(p,3n)^{201}Pb nuclear reaction. Pb-201 is purified by the carrier added precipitation method, followed by incubating the bulk for 36 h. After Pb-201 decay to Tl-201, the product is purified by column chromatography. For the production of thallic cation various modalities were considered. Thallous chloride solution (0.5 ml, 148 MBq) was treated with hydrogen peroxide added (20%, 0.5 ml)-6 M HCl (1 ml) mixture while ozone gas bubbled through the solution for 30 min. The aqueous solution was evaporated to the dryness under a flow of N_2 at 90°C. The conversion of Tl^+ to Tl^{3+} cation was checked either by RTLC using 2 solvent systems (system A: 10% ammonium acetate : MeOH: 1:1, system B: acetone) or gel electrophoresis using Gellman papers.

Labeling of vancomycin with [^{201}Tl]$TlCl_3$

[^{201}Tl]$TlCl_3$ (37 MBq) dissolved in acidic medium obtained above (0.5 ml) was transferred to a 2 ml-borosilicate vial. The mixture was evaporated by slight warming (50°C) under nitrogen flow. An isotonic mixture of VAN (0.25 mg) in Milli-Q® water (50 μl) was then added to the residue activity and the vial was shaken for 30 s. The vial was left or heated or agitated

at different temperatures (25°C, 50°C, 80°C and 100°C) up to an hour for obtaining the best conditions. The vial was finally cooled to room temperature. The active solution was checked for radiochemical purity by polymer-backed silica gel layer chromatography using a 1:1 mixture of 10% ammonium acetate and methanol as mobile phase. The mixture was injected into a Si Sep-Pak column and then fractions were passed through the column to remove possible ionic impurities. The final solution was then passed through a 0.22 μm filter and pH was adjusted to 5.5–7 by the addition of 1 M sodium acetate buffer.

Radiochemical purity

Radio thin-layer chromatography was performed using a mixture of ammonium acetate and 10% methanol (1:1) as eluent. Thus, the radiochemical yields were determined by comparison of un-complexed ^{201}Tl(III) and labeled compound radio peak using the RTLC method.

[^{201}Tl]VAN complex in final product

Stability studies were based on the previous studies performed for other radiolabeled glycopeptide antibiotics [9]. A sample of [^{201}Tl](III)VAN 1.85×10^7 Bq was kept at room temperature for 5 days while checked by RTLC every 12 h. A micro-pipette sample (5 μl) was taken from the shaken mixture and the ratio of free radiothallium(III) to [^{201}Tl]VAN was checked by radio thin-layer chromatography (eluent: 10% NH_4OAc buffer and methanol 1:1).

Serum stability studies

To 36.1 MBq (976 μCi) of [^{201}Tl](III)VAN was added to 500 μL of freshly prepared human serum. The resulting mixture was incubated at 37°C, and 1.5-μL aliquots were analyzed by radio-TLC after 0, 1, 16.5, 18, 21 and 132 h of incubation to determine complex stability.

Biodistribution in normal rats

Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd ed. The distribution of [^{201}Tl]VAN among tissues was determined for healthy NMRI male rats . The total amount of radioactivity injected into each rat was measured by counting a 1-ml syringe before and after injection in a dose calibrator with fixed geometry. The animals were sacrificed by CO_2 asphyxiation at selected times after injection (1, 3 and 52 h), the tissues (blood, heart, lung, brain, intestine, urine, skin, stomach, kidneys, liver, muscle and bone) were weighed and rinsed with normal saline. The activity of each sample was determined using the HPGe detector equipped with a sample holder device as AUC of injected dose per gram of tissues (AUC: area under curve for the 167 keV peak).

Results and discussion

[^{201}Tl]Tl^{3+} production

The inversion of the Tl^+/Tl^{3+} was studied using the oxidizing agent O_3/hydrogen peroxide and was based on RTLC and paper electrophoresis experiments. The most effective and user-friendly reagent in our hand was shown to be a mixture of H_2O_2 and 6 M HCl in the presence of O_3. The residue was re-dissolved in normal saline and checked for the results. In RTLC, two solvent systems were used as eluents. System A consisted of 10% ammonium acetate : MeOH (1:1), while system B consisted of pure acetone.

In solvent system A, the more charged Tl^{3+} stayed at the origin (R_f = 0.0) using normal phase, while less charged Tl^+ exhibit a higher R_f at about 0.7. In the second system, pure acetone, the R_f = 0.1 for Tl^{3+} and R_f = 0.9 for Tl^+ were observed. In paper electrophoresis 5 μl of the final sample was transferred onto the paper and the migration of the Tl-EDTA$^-$ to the cathode was compared with that of Tl^+ migrating to the anode. The method showed a > 99% conversion of $Tl^+ \rightarrow Tl^{3+}$. Figures 2 and 3 show the RTLC of Tl(I) and Tl(III) in both systems.

Radiolabeling

The labeled vancomycin exhibits a lower R_f (0.52), while unlabeled vancomycin exhibits a higher R_f of 0.9 (Fig. 4).

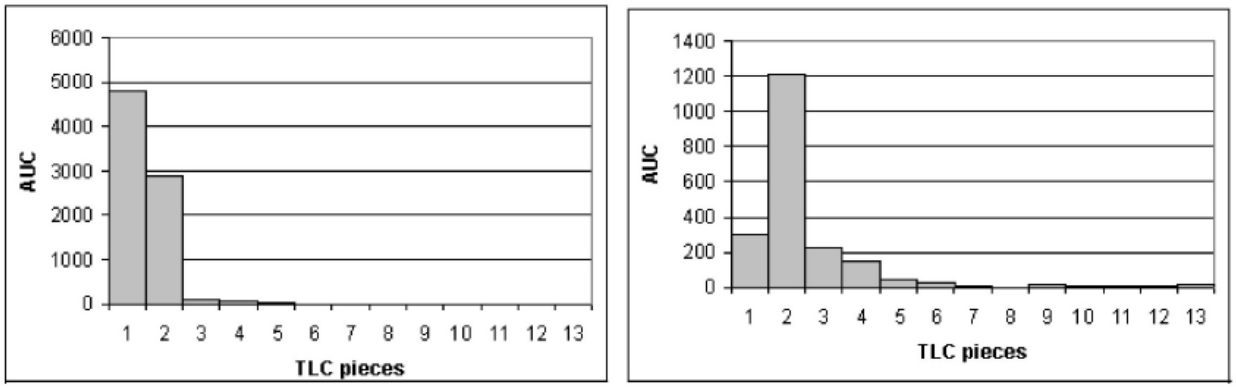

Fig. 2. Radio-chromatogram of [^{201}Tl]$TlCl_3$ in acetone (right) and in 10% ammonium acetate : MeOH (1:1) as mobile phase (left); AUC: area under curve of the 167 keV peak in the gamma spectrum.

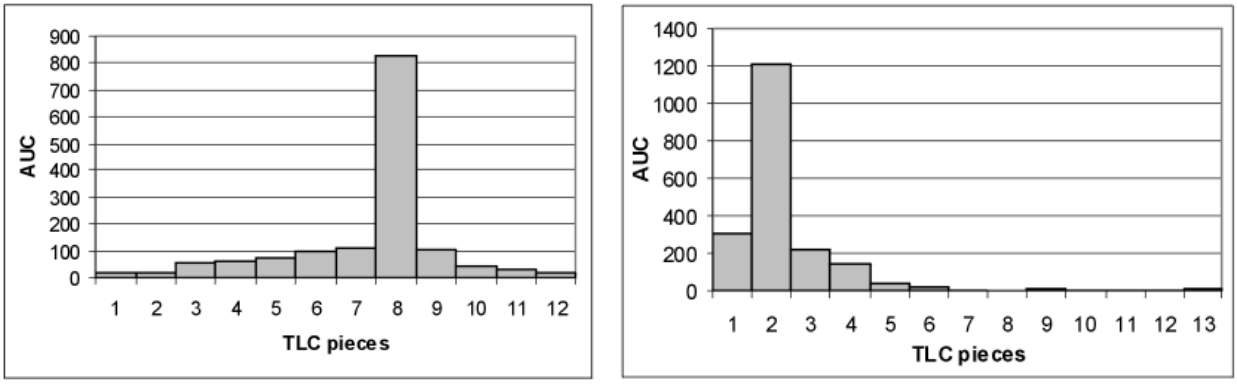

Fig. 3. Radio-chromatogram of [^{201}Tl]TlCl in acetone (right) and in 10% ammonium acetate:MeOH (1:1) (left) as mobile phase; AUC: area under curve of the 167 keV peak in the gamma spectrum.

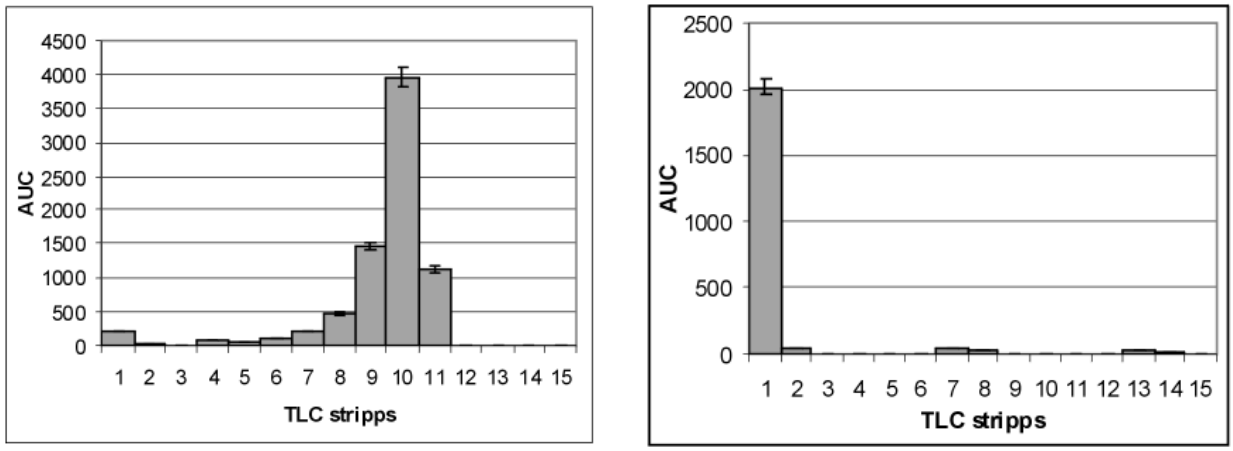

Fig. 4. Radio-chromatogram of [^{201}Tl]vancomycin in acetone (right) and 10% ammonium acetate:MeOH (1:1) (left) as mobile phase; AUC: area under curve of the 167 keV peak in the gamma spectrum.

Due to the difference in the polarity, the best way to avoid contamination with thallic and thallous impurities seemed to be solid phase extraction (SPE) of the labeling mixture.

In order to obtain the best labeling reaction conditions, the complex formation was optimized for temperature, time, and the amount of vancomycin in the physiological pH range (5.5–7). At room temperature, neutral pH yielded a successful labeling. Increasing the amount of vancomycin per radioactivity unit starting from 20 up to 80 nanomoles for 3.7×10^7 Bq of the activity, enhanced the radiochemical yield up to 84%. In order to get a radiochemical yield higher than 90%, the amount of the vancomycin had to be higher than 800 nmoles (Fig. 5).

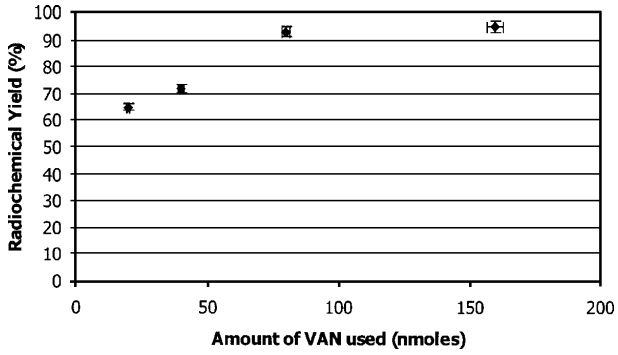

Fig. 5. Effect of vancomycin amount (nM) on [^{201}Tl]VAN radiochemical yield at an experimental temperature (room temperature) at pH 6–7 (n = 5).

Heating the reaction mixture to 90°C did not increase the yield. Higher temperatures reduced the radiochemical yield due to the decomposition of vancomycin and/or product, resulting in decomposition products with visible wavelength color (yellow). Thus, room temperature was considered the best temperature while it was more facile. At room temperature the radiolabeling was completed in 30 min, while the mixture must have been shaken at 10 min-intervals.

[^{201}Tl]VAN preparation was sterilized by 0.22 μ filtration. The chemical stability in the final product lasted for 5 days post labeling, being suitable to perform further studies. The RTLC of the final product showed no change in stability and [^{201}Tl]Tl^+/Tl^{3+}[^{201}Tl]VAN were not changed during 3 days.

Serum stability

The final product: human serum mixture was incubated at 37°C for up to 5.5 days and samples went through RTLC tests for study of the complex integrity. No change in stability was observed in the first 21 h and [^{201}Tl]Tl^{3+}[^{201}Tl]VAN were not changed (Fig. 6).

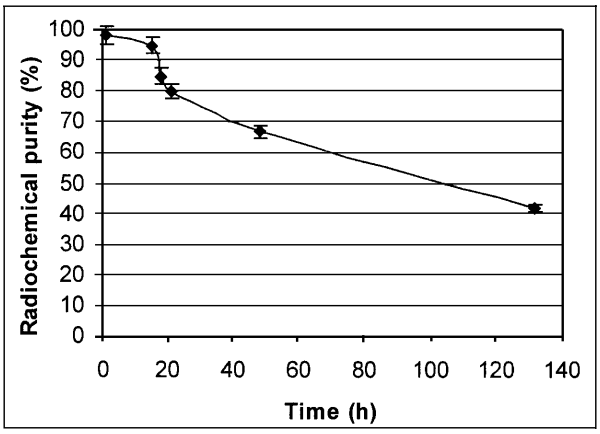

Fig. 6. Radiochemical purity of the [^{201}Tl]VAN in presence of human serum at 37°C.

Biodistribution studies in normal rats

An hour post-injection the radioactivity was enhanced in the kidneys and remained high even after 52 h post injection. It has been already shown that during the first 24 h, about 75% of the administered dose of vancomycin is excreted in urine by glomerular filtration. High accumulation of the tracer in the kidneys can be explained by this fact [17]. The activity found in brain is almost low at all times, because it has been shown that vancomycin does not readily diffuse across normal meninges into the spinal fluid [8] (Figs. 7–9).

Optimization studies on the production of Tl^{3+} cation using oxidizing agents and HCl concentrations was performed using commercially available [^{201}Tl]thallous chloride using a mixture of H_2O_2/HCl/O_3. Total labeling and formulation of [^{201}Tl]VAN took about 35 min, with a yield of > 99%. A suitable specific activity product was formed via insertion of [^{201}Tl](III)thallium cation. The final sample was checked for radiochemical and chemical purity using RTLC. The radiolabeled complex was stable in aqueous solutions for at least 5.5 days after labeling. No detectable amounts of free [^{201}Tl](III)thallium (< 1%) were detected by TLC. The stability of the complex was checked in the presence of freshly prepared human serum for up to 140 h, showing the integrity of the complex up to 21 h. The

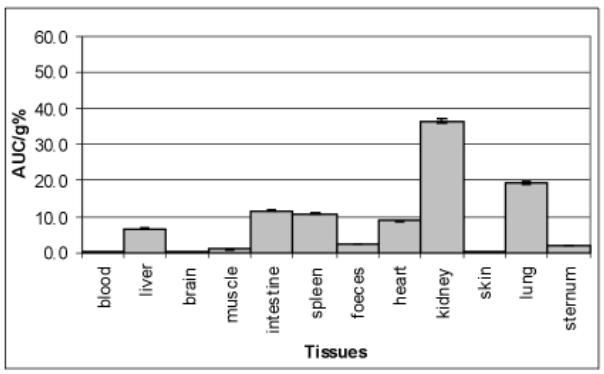

Fig. 7. Biodistribution of radiotracer in normal rats 1 h post-injection (AUC/g%); AUC: area under curve of the 167 keV peak in the gamma spectrum.

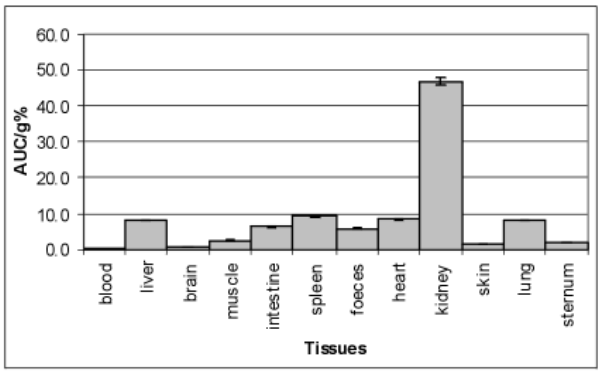

Fig. 8. Biodistribution of radiotracer in normal rats 3 h post-injection (AUC/g%); AUC: area under curve of the 167 keV peak in the gamma spectrum.

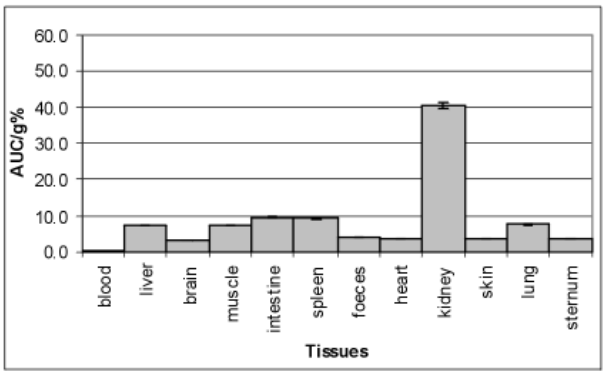

Fig. 9. Biodistribution of radiotracer in normal rats 52 h post-injection (AUC/g%); AUC: area under curve of the 167 keV peak in the gamma spectrum.

biodistribution of the complex was studied in normal rats showing the general pattern of vancomycin core, with excretion through kidney in the first couple of hours. This pattern was also observed after IV injection to the normal rats at the same time intervals. [^{201}Tl](III)vancomycin, can be SPECT radiotracer with a rather long half-life, meeting radiopharmaceutical standards for use in remote nuclear medicine centers.

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