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Original

Evaluation of [^{201}Tl](III) Vancomycin in normal rats

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Abstract

BACKGROUND: Tl-201 has potential in the preparation of radiolabelled compounds similar to its homologues, like In-111 and radiogallium. In this paper, recently prepared [^{201}Tl](III) vancomycin complex ([^{201}Tl](III)VAN) has been evaluated for its biological properties.

MATERIAL AND METHODS: [^{201}Tl](III)VAN was prepared according to the optimized conditions followed by biodistribution studies in normal rats for up to 52 h. The *Staphylococcus aurous* specific binding was checked *in vitro*. The complex was finally injected to normal rats. Tracer SPECT images were obtained in normal animals and compared to those of ^{67}Ga-citrate.

RESULTS: Freshly-prepared [^{201}Tl](III)VAN batches (radiochemical yield > 99%, radiochemical purity > 98%, specific activity ≈ 1.2 Ci/mmol) showed a similar biodistribution to that of unlabeled vancomycin. The microorganism binding ratios were 3 and 9 for tracer $^{201}Tl^{3+}$ and tracer ^{201}Tl(III)DTPA, respectively, suggesting the preservation of the tracer bioactivity. As a non-specific cell penetrating tracer, [^{201}Tl](III)DTPA was used.

Key words: Tl-201, vancomycin, SPECT, biodistribution, *Staphylococcus aureus*, binding study

Introduction

A wide variety of radiopharmaceuticals has been proposed for the scintigraphic detection of inflammatory and infectious disease. [^{67}Ga]Citrate, being the most primitive radiotracer for this purpose, has a high sensitivity for both acute and chronic infections and non-infectious inflammation [1]. Other radiopharmaceuticals are immunoglobulins [2], liposomes and labelled leukocytes [3], the avidin–biotin system [4] and finally radiolabelled antibiotics such as ciprofloxacin [5]. Ciprofloxacin labelled with ^{99m}Tc has already shown a high sensitivity and specificity for infection imaging, opening a new era in the imaging of infection using radiolabelled antibiotics.

As an interesting antibiotic for developing an infection-detecting agent, vancomycin has the advantage of having a significant effect on *Staphylococcus aureus*, a life-threatening microorganism in immuno-compromised patients. On the other hand, after IV administration in humans, about 75% of an administered dose of vancomycin is excreted in urine by glomerular filtration in the first 24 hours, showing no apparent metabolism of the drug [6].

The development of [^{201}Tl](III) radiopharmaceuticals could give many advantages, such as interesting physical properties, simple chemistry, high complexation constants for most Tl(III) complexes (for instance Tl(III)-DTPA at 25 C, log K = 46) [7], the possibility of kit formulation production due to high specific activity and, finally, [^{201}Tl] is available in many parts of the developed and developing world. We have recently reported the preparation and evaluation of some [^{201}Tl](III)tracers such as, [^{201}Tl](III) bleomycin [8], [^{201}Tl](III)DTPA [9], [^{201}Tl](III) human polyclonal antibody [10] and also Tl(III)-vancomycin (Tl(III)VAN) as a possible infection-imaging tracer [11].

Here we report some preclinical studies on the tracer, including biodistribution studies (ID/g%), *in vitro Staphylococcus aureus* specific binding determination as well as preliminary SPECT imaging in normal rats.

Material and methods

Production of ^{201}Tl(I) was performed in an Agriculture, Medicine and Industrial Research School (AMIRS) 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 the 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

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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 5mm strip after cutting it into pieces in a CRC Capintech Radiometer (NJ, USA). Radionuclidic purity was checked with the same detector. All calculations and RTLC counting were based on the 167 keV peak. O_3 was produced from medicinal oxygen (Air Liquide, Belgium) using a conventional O_3 generator at a flow rate of 1 litre per minute. The oxidation of $^{201}Tl^{3+}$ was checked by cellulose acetate paper electrophoresis (Gellman) in 0.05N EDTA at 200V for 10 min.

Production of [^{201}Tl](III)vancomycin

The labelled compound was prepared according to the reported method [11] using routinely-produced Tl-201 in our centre for national use. Briefly, thallous chloride solution (0.5 ml, 148 MBq) was treated with hydrogen peroxide-added (20%, 0.5 ml)-6M HCl (1 ml) mixture while ozone gas bubbled through the solution for 30 min. The conversion of Tl^+ to Tl^{3+} cation was checked by RTLC. [^{201}Tl]$TlCl_3$ (37 MBq) was evaporated and an isotonic mixture of VAN (0.25 mg) in Milli-Q® water (50μl) was then added to the residue activity and the vial was shaken for 30 seconds. The vial was left, heated or agitated at 50°C for about an hour. 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.

Preparation of [^{201}Tl]-(III)diethylenetriaminepenta acetate ([^{201}Tl](III)DTPA)

The labelled compound was prepared according to the reported method [9]. Briefly, [^{201}Tl]$TlCl_3$ (37 MBq) was dissolved in the organic medium obtained above (0.5 ml) and was immediately transferred to a 2 ml-borosilicate vial. The mixture was evaporated by slight warming (40°C) under a nitrogen flow. A portion of diethylenetriaminepentaacetic acid dianhydride (0.1 mg, 280 nmol), dissolved in normal saline (0.5 ml), was added to the thallium residue and vortexed for 1 min and incubated at 25°C for 60 min. The radiochemical purity of the final complex was checked by RTLC.

S. aureus *binding of [^{201}Tl]VAN complex*

A sample of *Staphylococcus aureus* was added to a sterile culture media and the mixture was incubated at 37°C for 15h until an O.D. of 0.5–0.7 was obtained. At this stage, the microbial count was performed (2.6×10^7mo). The bioaffinity experiments were rapidly performed on the microbial mass. One hundred microlitres of the culture was centrifuged at 2000 rpm for 15 min at 25°C. The cell pellet was carefully reconstituted in 1 ml of PBS (0.1 M, pH. 7.2) followed by the addition of 100 μCi (50μL) of the tracers ([^{201}Tl]VAN, [^{201}Tl]$IIICl_3$ and [^{201}Tl](III)DTPA) prepared above, in separate tubes. The mixtures were kept at 37°C while shaken slowly and 1 ml samples were taken from each mixture at specific time intervals. Each sample was then centrifuged in Centricon tubes and cell pellets were washed with PBS 7.5 two times. The total supernatant and pellet activities were carefully counted using an HPGe detector based on 167 keV peak for 50 seconds applying a consistent geometry. The cell uptake ratio was determined by pellet/total or pellet/supernatant.

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](III)VAN among tissues was determined for healthy NMRI male rats immediately after imaging. The total amount of radioactivity injected into each rat was measured by counting the 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 and the specific activity of different organs was calculated as a percentage of injected dose per gram of the tissue (ID/g%, based on the area under the curve of 167 keV peak using an HPGe detector).

SPECT imaging of [^{201}Tl](III)VAN in normal rats

Images were taken up to 24 hours after administration of the radiopharmaceutical by a dual-head SPECT system using a LEAP collimator. The rat-to-detector distance was 12 cm. The useful field of view (UFOV) was 540 mm × 400 mm. The spatial resolution in the coincidence mode was 10 mm FWHM at the CFOV. Sixty-four projections were acquired for 30 seconds per view with a 64 × 64 matrix.

Results and Discussion

Quality control of the tracer

In order to monitor the radiochemical yield and final radiochemical purity, RTLC experiments were performed using various mobile phases. Table 1 shows the results of the above experiments.

Biodistribution studies in normal rats

One hour post-injection, the radioactivity was enhanced in the kidneys and remained high even after 52 hours post-injection. It has been already shown that during the first 24 hours, about 75% of the administered dose of vancomycin is excreted in urine by glomerular filtration. The high accumulation of the tracer in the kidneys can be explained by this fact [12]. The activity found in the brain is low nearly all the time because it has been shown that vancomycin does not readily diffuse across normal meninges into the spinal fluid [12]. The polypeptide structure of this compound does not allow penetration through normal meninges due to the size

Table 1. RTLC results for the detection of various radiochemical species in tracer preparation; System A: acetone, System B: 10% ammonium acetate:MeOH (1:1); System C: RPTLC, MeOH

Chemical species	R_fs in various mobile phases		
	System A	System B	System C
$^{201}Tl^+$	0.6	0.8	0
$^{201}Tl^{3+}$	0.0	0.0	0
[^{201}Tl](III)DTPA	0.0	0.8	0.35
[201Tl](III)VAN	0.0	0.5	0.0

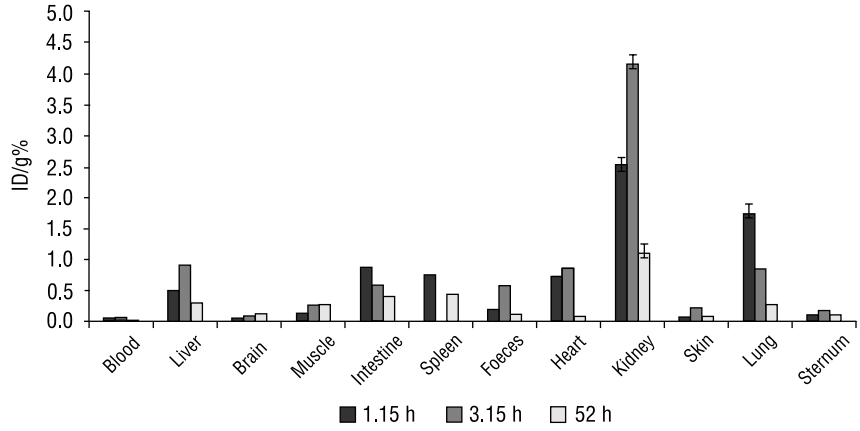

Figure 1. Biodistribution of radiotracer in normal rats at various time intervals (ID/g %, based on area under curve of the 167 keV peak in gamma spectroscopy) (decay corrected, n = 3).

and hydrophilicity. The tracer is rapidly removed from blood in the first few minutes. The GI tract, including the intestine, has a medium uptake, possibly due to liver excretion of the tracer being around 10%. The heart uptake (containing atrial appendage and pericardial tissue) is a little high during the first few hours; this has already been reported after IV administration of vancomycin [12]. However, detectable concentrations are present in pleural, peritoneal dialysis fluid, this can be observed in the chest area of the animals 2 hours post injection (Figure 1).

Streptococcus aureus *binding*

The tracer was tested for the retention of its biological activity after radiolabelling. The freshly cultivated *Streptococcus aureus* samples were prepared, and the affinity of the tracer to the microbial cell membrane was checked at 37°C. As an unspecific Tl(III)compound, [201Tl](III)DTPA was chosen for bioaffinity comparison. Tl(III) affinity cation for microbial affinity was also determined. The data (n = 5) showed that [201Tl]VAN retains its biological properties after radiolabelling (Figure 2).

SPECT imaging

The images of the tracer in the normal rats were acquired and are shown in Figure 3. Most of the infection-seeking agents such as 67Ga-citrate and radiolabelled immunoglubins possess natural accumulation in the liver, which is a disadvantage due to the high liver dose uptake as well as the inability to detect liver infections. However, radiolabelled antibiotics, such as vancomycin, with low liver uptake can be suitable substitutes for liver infection detection. Figure 4 shows the SPECT images for normal rats 24 h post injection of the tracer and 67Ga-citrate. Meanwhile, a natural brain and bone uptake is usually observed in 67Ga images which can superimpose the infection uptakes in these areas; however, for and [201Tl]VAN above uptakes are not present.

Conclusions

Optimization studies on the production of the $Tl3^{+}$ cation using oxidizing agents and HCl concentrations were performed using commercially available [201Tl]thallous chloride using a mixture

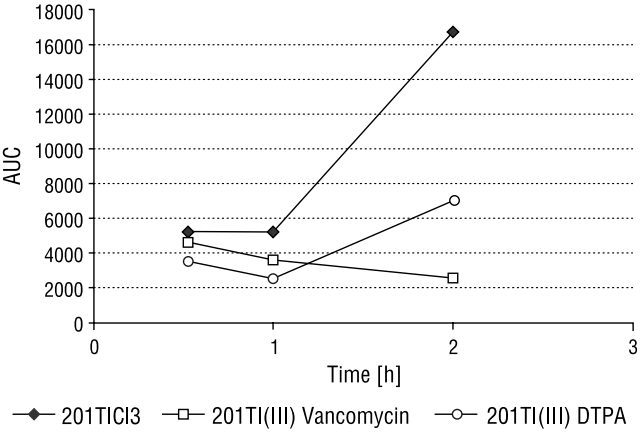

Figure 2. Binding affinity of [201Tl](III)VAN, [201Tl](III)DTPA and $^{201}TlCl_3$ with *Streptococcus aureus* at 37°C up to two hours.

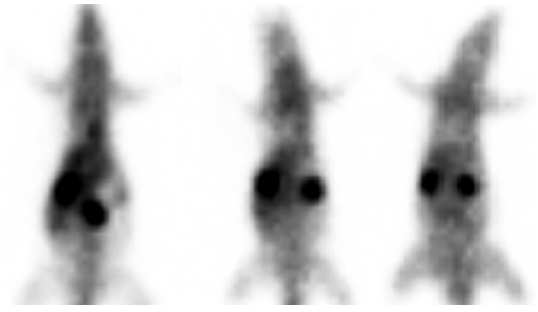

Figure 3. SPECT images of normal rats 1 h, 2 h and 4 h post injection of [201Tl]VAN (20 μCi) *via* their tail veins.

of H_2O_2, HCl and O_3. Total labelling and formulation of [201Tl](III)VAN took about 35 min with a yield of > 99%. A suitable specific activity product was formed *via* insertion of [201Tl](III) thallium cations. The final sample was checked for radiochemical and chemical purity using RTLC. The radiolabelled complex was stable in aqueous solutions for at least 5.5 days after labelling. No detectable amounts of free [201Tl](III) thallium (< 1%) were detected by TLC. The stabil-

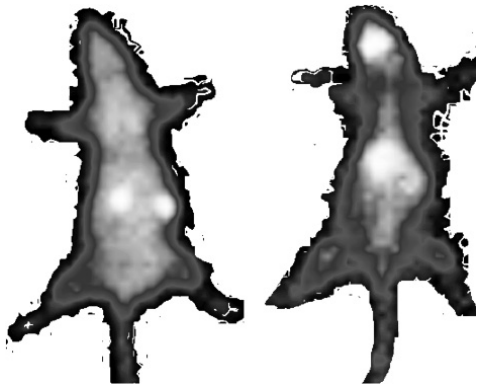

Figure 4. Biodistribution of ^{67}Ga-citrate (right) and [^{201}Tl]VAN (left) 24 h post injection.

ity of the complex was checked in the presence of freshly prepared human serum for up to 21 hours, showing the integrity of the complex. The biodistribution of the complex was studied in normal rats showing the general pattern of the vancomycin core, with excretion through the kidney in the first couple of hours. Comparison of tracer biodistribution using SPECT with ^{67}Ga-citrate revealed that the tracer can be used for the detection of liver, GI tract and cerebral infections, while Ga-67 has natural uptake among these organs. [^{201}Tl](III) vancomycin can be a SPECT radiotracer with rather a long half life, meeting radiopharmaceutical standards for use in remote nuclear medicine centres.

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