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Preparation and evaluation of [^{201}Tl](III)-DTPA-HIgG for inflammation detection

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Background: Radiolabeled polyclonal human immunoglubins are useful in the detection of inflammations. In this work a novel approach has been presented to use thallium-201 as a complexing nuclide for the development of radioimmunoconjugates. ***Materials and Methods:*** Thallium-201 ($T_{1/2}$=3.04 d) in Tl^+ form was converted to Tl^{3+} cation in presence of O_3 in 6M HCl, controlled by RTLC/gel electrophoresis methods and used in the labeling of human polyclonal antibody (HIgG) after residulation with freshly prepared cyclic DTPA-dianhydride. The best results of the conjugation were obtained by the addition of 1 ml of a HIgG pharmaceutical solution (5 mg/ml, in phosphate buffer, pH=7) to a glass tube, which was pre-coated with DTPA-dianhydride (0.01 mg) at 25°C with continuous mild stirring for 30 min. ***Results:*** The final isotonic [^{201}Tl](III)-DTPA-HIgG complex was checked by radio-TLC using several solvent systems to ensure the formation of only one species, and it was followed by filtration through a 0.22 µm filter (specific activity= 33.7 TBq/mM, radiochemical purity>95%). Preliminary bio-distribution studies in normal and inflammation-bearing rats were performed. The target/skin and target/blood ratios were 4 and 6 after 28h, respectively, showing the selectivity of the radiopharmaceutical for the inflammatory lesions. ***Conclusion:*** The incorporation of Tl(III) cation into a immunoconjugate was performed using the known methods. The biodistribution of the immunocomplex was shown to be consistent with a stable complex for the detection of inflammations. Significant inflammation detection was observed for the final complex in rats with turpentine oil-induced inflammation. **Iran. J. Radiat. Res., 2006; 4 (3): 105-114**

Keywords: *^{201}Tl(III)-complex, biodistribution, human polyclonal antibody, inflammation.*

INTRODUCTION

There are a couple of radiotracers used in the detection of inflammation and infection in human [1], but just a few of them have been widely used in routine clinical trials. ^{111}In-labeled HIG has been extensively tested in a large number of clinical studies. It has shown excellent performance in the localization of musculoskeletal infection and inflammation [2]. In addition, good results have been reported in pulmonary infection, particularly in immunocompromised patients [3, 4], and abdominal inflammation [5].

The other radioelement of group IIIA, thallium-201 ($T_{1/2}$=3.04 d), has been used in clinical nuclear cardiology and oncology for three decades. The development of [^{201}Tl](III) radiopharmaceuticals could provide many advantages due to the physical properties, the simple chemistry of [^{201}Tl](III) complexation and the high complexation constant for Tl(III)-DTPA (at 25°C, log K=46). On the other hand, high specific activity Tl-radiopharmaceuticals can be synthesized in kit formulations and [^{201}Tl] is available in many parts of the developed and developing world and it can offer alternatives for other metallic radioisotopes including [^{111}In] In^{3+}. Despite the mentioned above advantages, Tl-201 labeled compounds are rare in the literature [6]. Recently, we have reported the evaluation of a biologically active Tl(III) compound, i.e. bleomycin [7].

In continuation of our efforts to create new Tl(III)labeled compounds we focused on the ^{201}Tl(III)-HIgG labeling.

[^{201}Tl](III)-DTPA-HIgG was prepared as a

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model of Tl-chelated immunoconjugate for biodistribution studies in normal and inflammation-induced rats based on our recent experiences with radiometal-labeled antibodies [8]. A precise labeling strategy was employed using freshly-prepared DTPA cyclic dianhydride, with various HIgG concentrations. Finally, an optimized radiolabeling method for developing a highly reactive DTPA-conjugated HIgG kit for possible inflammation studies was introduced.

MATERIALS AND METHODS

Production of ^{201}Tl was performed in Nuclear Research Center for Agriculture and Medicine (NRCAM) using a 30 MeV cyclotron (Cyclone-30, IBA) based on the routine production of thallous chloride for country use. $^{203}Tl_2O_3$ with an enrichment of more than 95% was supplied by the Kurchatov Institute (Russia). All chemicals were purchased from the 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). Normal saline used for labeling was of high purity and had been filtered through 0.22 µ Cativex filters. 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 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 countings 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 0.05N EDTA at 200V for 10 min. 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. AUC/gr% (AUC: area under the curve of 167 keV photopeak) was considered as a instructive method of injected dose percentage per gram of the organs to be used for organ dose calculations.

Conversion of thallium-201 to [^{201}Tl]$TlCl_3$ form

Thallous chloride solution (0.5 ml, 4 mCi) was treated with a mixture of *di*-isopropyl ether, hydrogen peroxide, (20%, 0.5 ml) and 6M HCl (1 ml) while ozone gas bubbled through the solution for 30 min. The organic layer was separated, and 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. The organic layer was evaporated to dryness under a flow of N_2 at 30°C for labeling use.

Preparation of fresh cyclic DTPA dianhydride for radiolabeling

Cyclic DTPA dianhydride was prepared according to the methods previously given in the literature with slight modifications[9]. Briefly, DTPA in acidic form (0.1 mol) was heated with a 4-fold molar excess of acetic anhydride (0.4 mole), dissolved in 50 ml of pyridine and heated at 65°C for 24 h. The resulting anhydride was insoluble in pyridine and was collected by filtration, purified by repeated washing with acetic anhydride, and finally with anhydrous ether. Drying in an oven at 50-60°C had removed the last traces of solvent. The melting point was 178-180°C. ^1H NMR and IR spectra were consistent with the spectra reported in the literature [9].

Conjugation of cyclic DTPA di-anhydride with the HIgG

The chelator diethylenetriamine penta-acetic acid dianhydride prepared above was conjugated to the antibody using a small modification of the well-known cyclic anhydride method [10]. Conjugation was performed at a 1: 1 molar ratio. In brief, 20 µl

of a 1 mg ml^{-1} suspension of DTPA anhydride in dry chloroform (Merck, Darmstadt, Germany) was pipetted under ultrasonication and transferred to a glass tube. The chloroform was evaporated under a gentle stream of nitrogen. Commercially available HIgG (5 mg, 0.5 ml, pH=8) was subsequently added and gently mixed at room temperature for 60 min. Conjugation mixture was then passed through a Sephadex G-50 column (2×15 cm, 2 gr in 50 ml of Milli-Q® water) separately and one-milliliter fractions were collected and checked for the presence of protein using UV absorbance at 280 nm or visible folin-phenol colorimetric assay. The fractions containing the highest concentration of the immunoconjugate was chosen and kept at 4°C and for radiolabeling.

Radiolabeling of the antibody conjugate with ^{201}Tl(III)

The antibody conjugate was labeled using an optimization protocol according to literature [11]. Typically, 37-40 MBq of evaporated ^{201}Tl(III) activity (prepared above) was added to a conical vial and the vial was purged under a flow of nitrogen. Conjugated fraction was added to the Tl^{3+} containing vial in 1 ml of phosphate buffer (0.1 M, pH=8), and mixed gently for 30 second. The resulting solution was incubated at room temperature for 30 minutes. Following incubation, the radiolabeled antibody conjugate was purified from free ^{201}Tl by gel filtration on a Sephadex G-50 column (15-20 ml bed volume), and eluted with PBS. The latter gel purification step was finally omitted by optimizing the ratio of the radioactivity having been used, and conjugated antibody. Fractions (1 ml) were collected, and the radioactivity of each fraction was measured by a recently calibrated radioisotope dose calibrator (CRC-7, Capintec Instruments, Ramsey, NJ). The protein presence in each fraction was determined, using a fast protein assay method by mixing freshly prepared Folin-Colciteau® reagent, and 10 µl of the eluted fractions. The fractions containing the proteins with the maximum radioactivity were combined and tested for purity by ITLC using a radio TLC scanner. Control labeling experiments were also performed using $^{201}TlCl_3$, and DTPA with $^{201}TlCl_3$. Both reaction mixtures were passed through separate gel filtration columns and eluted with PBS. Usually the fraction number 5-7 showed the presence of protein, which fraction 6 was used in the other experiments (n=3).

Quality control of ^{201}Tl(III)-DTPA-HIgG

a. Thin layer chromatography; System I: A 5 µl sample of the final fraction was spotted on a silica gel paper, and it was developed in a mixture of 10% ammonium acetate:methanol (3:1) as the mobile phase, in order to observe the R_fs of free $^{201}Tl^{3+}$, and ^{201}Tl-DTPA while radiolabeled protein stays at the bottom [12].

System II: Another applied system was used performed on silica-impregnated glass fibre sheets. From the final product, 5 µl was applied to the ITLC strip which was developed with 0.9% NaCl for 5 min. Radioactivity was determined by a chromatography scanner equipped with an HPGe crystal. [^{201}Tl](III)-DTPA moved to the front, and the ^{201}Tl-labelled antibody remained at the starting position [13].

System III: The next system was performed on silica-impregnated glass fiber sheets. From the final product, 5 µl was applied to the ITLC strip that was developed with a mixture of pyridine:ethanol:water (1:2:4) for 5 min. Radioactivity was determined by a chromatography scanner equipped with an HPGe crystal. ^{201}Tl-DTPA moved to the front, the ^{201}Tl-labelled antibody remained at the starting position [14]. Some other solvent systems were also used as shown in table 1.

b. Paper chromatography: Paper chromatography [Whatman No. 1 (Whatman, Maidstone, UK), methanol/water (55:45)] of the eluate showed that >94% of the activity remained at the origin corresponding to the [^{201}Tl](III)-DTPA-HIgG. The labeling yield was 45±5% (n=5), and a specific activity of 300-500 MBq per 1 mg DTPA-conjugate was obtained [15].

c. Radiolabeled Antibody integration: Gel electrophoresis was performed in order to

evaluate possible radiolysis/chemolysis of HIgG in the course of reaction and purification. The samples of rainbow protein ladder standard, pure unlabeled HIgG, and ^{201}Tl-DTPA-conjugate were loaded over a 16% bis-acrylamide/acrylamide gel and it was followed by running at the constant voltage of 285V for 40 min. The current was then disconnected, and the gels were stained in bromphenol blue staining mixture for 3 hours, followed by de-staining by MeOH, and acetic acid mixture. In case of dilute protein samples, silver staining of the gel was employed [16].

Maximum capacity of the radiolabeling

Ten microlitres of DTPA-conjugated samples of HIgG (1-3 mg) were incubated with 1, 2, 4 and 8 MBq of Tl(III)$^{3+}$ at room temperature for 1 hour. The radiochemical purity of the final sample was then checked by RTLC using 10% ammonium acetate: methanol (1:1) as the eluent, as well as other solvent systems. The radiochemical purity over 95% was accepted as a radiopharmaceutical product, and the specific activity of the final radioimmunoconjugate was calculated using the latter sample.

Stability testing of the radiolabeled compound

Stability of [^{201}Tl](III)-DTPA-HIgG in PBS was determined by storing the final solution at 4°C for 14 days and performing frequent ITLC analysis to determine radiochemical purity. Frequent ITLC analysis was performed. Furthermore, the stability of the conjugated DTPA-HIgG, stored at -20°C for more than 1 month was investigated. ITLC analysis of the conjugated product was performed to monitor for degradation products or other impurities. After subsequent ^{201}Tl-labelling of the stored conjugated product, both labeling efficiency and radiochemical purity were determined.

Stability testing of the radiolabeled compound in presence of serum

For studies of radiolabel stability in serum, radiolabel stability was assessed by gel filtration on a Sepharose column (1×30 cm). The column was equilibrated with PBS and eluted at a flow rate of 0.5 mL/min at room temperature; 0.5 mL fractions were collected.

Biodistribution of [^{201}Tl](III)-DTPA-HIgG in normal rats

To determine its biodistribution, [^{201}Tl](III)-DTPA-HIgG was administered to normal rats. A volume (25-50 µl) of final [^{201}Tl](III)-DTPA-HIgG solution, containing 1.5 MBq radioactivity, was injected intravenously to rats through their tail vein. The animals were sacrificed at the exact time intervals (2, 4, 24 and 28 h). The specific activity of different organs was calculated as the percentage of the injected dose per gram in tissues, using HPGe detector equipped with a sample holder device.

Induction of inflammation in normal rats

Inflammation-bearing rats: turpentine oil (40 µl) was injected subcutaneously (SC) to the dorsal area of 5 groups of rats weighing 150-175 grams. After 7 days sample animals were ready for biodistribution studies with subcutaneously of inflammatory tissues (0.7±0.05 g).

Biodistribution of ^{111}In-DTPA-HIgG to inflammatory-lesion bearing rats

The distribution of [^{201}Tl]-DTPA-HIgG among tissues was determined for untreated rats, and for the rats with inflammatory lesions. A volume of final [^{201}Tl]-DTPA-HIgG solution (0.1 ml) containing an activity of 1.5 MBq (≤15 µg in 100 µL) was injected to the animals *via* their dorsal tail vein. The total amount of radioactivity injected into each rat was measured by counting the 1-ml syringe before and after the injection in a dose calibrator with fixed geometry. The animals were sacrificed by ether anesthesia at selected times after the injection (24, 48, 72, 144 h), the tissues (blood, heart, lung, spleen, intestine, foeces, skin, bladder, kidneys, liver, muscle and bone) were weighed and rinsed with normal saline, and their specific activities were determined with a HPGe detector equipped with a sample holder

device as percentage of injected dose per gram of tissues.

RESULTS

Since the complexes of DTPA with many metals with a valence of III are stable, the idea was to prepare Tl^{3+} cation in high chemical purity followed by complexation. It has been recently shown that Tl^{3+} ion is a highly toxic cation, but when complexation happens the toxicity decreases drastically [17]. The toxicity is possibly due to glutathione metabolism impairs [18], or major alterations in the rheology of the bi-layer, which could be partially responsible for the neurotoxic effects of this metal [19]. But, all the above features occur when Tl is present at nano molar amounts, and the specific activity of ^{201}Tl is far below toxicity values.

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

The conversion of the Tl^{+} to Tl^{3+} using oxidizing agent, O_3, was performed in presence of hydrogen peroxide. The residue was re-dissolved in normal saline, and the results were checked. In RTLC two solvent systems were used as eluents. System A consisted 10% ammonium acetate: MeOH (1:1), while system B contained pure acetone.

In the mentioned system, more polar Tl^{3+} stayed at the origin (R_f=0.0), while less polar Tl^{+} were showing a high R_f (about 0.7). In the second system, i.e. pure acetone, 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 on the paper, and the migration of the Tl-EDTA- to the cathode was compared with that of Tl^{+} migrating to anode. The method showed a conversion of >99% for Tl^{+}/Tl^{3+}.

Preparation and structure confirmation of DTPA cyclic di-anhydride

In order to prepare the bi-functional ligand, DTPA cyclic *di*-anhydride, which was not cost effective, we tried the general procedure for its preparation. The reaction was performed in pyridine containing DTPA acid form acetic anhydride. The filtered mass was washed with cold acetic anhydride to remove the residues of the reactant. The solid was dried in oven for a couple of hours and finally it was re-crystallized to get a high purity product, suitable for spectroscopic and radiolabeling steps (figure 1). Washing/drying steps were very important, because more repetition of these steps afforded high-purity product with rather long shelf-life. Such samples can be stored at room temperature under a blanket of N_2 for up to one year.

Conjugation of HIgG with DTPA cyclic di-anhydride and radiolabeling of HIgG with ^{201}Tl

Tl(I) is stable in aqueous media, but Tl(III) is reactive and can be hydrolysed in alkaline or neutral solutions and reduced by common reducing agents. It can, however, be stabilised by complexation. Thus, if the complexation of the DTPA-moiety with Tl(III) was fast enough, no detectable Tl(I) cation would be observed in the final sample. In many studies, using DTPA as a chelating reagent, hydrolysis and reduction of Tl(III) in the aqueous media were prevented, and the stabilized species in aqueous solution was used for the Tl detection [20]. Tl(III) forms a very stable complex, $[Tl(DTPA)]^{2-}$, with DTPA with stability constant of logK = 46 [21]. A Tl(I)-DTPA complex can never be formed.

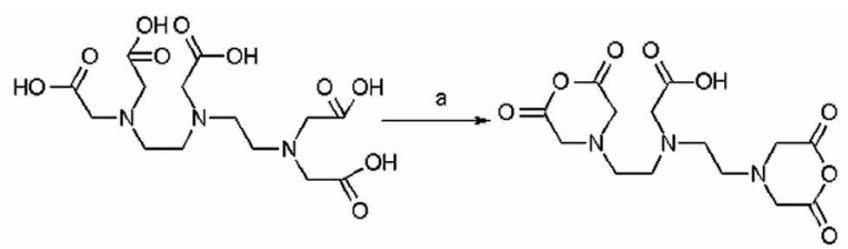

Figure 1. Schematic diagram of the synthesis of DTPA cyclic di-anhydride.

The labeling yield of [^{201}Tl](III)-DTPA-HIgG has been studied in a wide range of antibody/DTPA ratios in order to optimize the process, and to improve [^{201}Tl](III)-DTPA-HIgG performance in vitro. The overall radiolabeling efficiency has been over 77% and the specific activity has been kept in the range of 37TBq/mM.

The conjugated [^{201}Tl](III)-DTPA-HIgG fractions, containing the maximum protein content, were mixed with ^{201}Tl-$TlCl_3$ solution, vortexed and kept at room temperature. Small fractions were taken from the mixture, and tested by RTLC to find the best time scale for labeling. After an hour, free ^{201}Tl/conjugated ^{201}Tl ratio in the labeled sample remained unchanged. The mixture was then passed through another Sephadex G-50 gel filtration column in order to remove trace amounts of unbound ^{201}Tl cation.

The eluted fractions were checked by Folin-Colciteau® reagent, and for presence of radioactivity in order to determine the [^{201}Tl](III)-DTPA-HIgG containing fractions. Fraction number 6 was chosen as the suitable final product with appropriate specific activity for animal tests.

Instant thin layer chromatography, using various mobile and stationary phases, was performed in order to ensure the existence of the desired radiolabeled antibody only. Five different solvent systems and two stationary phases were applied. In all RTLC tests radiolabeled antibody stayed in the origin, while other species migrated to other R_fs depending on the mobile phase used. The R_fs of the possible occurring chemical species in chromatography of the reaction steps are summarized in the table 1.

Table 1. Chromatography results for the quality control of Tl-DTPA-HIgG (n=5).

Chemical species	Mobile phase	Stationary phase	R_f
^{210}Tl-DTPA-HIgG	10% Ammonium acetate:methanol (1:1)	Silicagel	0.3
$^{201}Tl^{3+}$	//	//	0.2
^{201}Tl-DTPA	//	//	0.7
^{201}Tl-DTPA-HIgG	0.9% sodium chloride solution	//	0.2
$^{201}Tl^{3+}$	//	//	0.0
^{201}Tl-DTPA	//	//	0.5
^{201}Tl-DTPA-HIgG	pyridine:ethanol:water (1:2:4)	//	0.0
$^{201}Tl^{3+}$	//	//	0.1
^{201}Tl-DTPA	//	//	0.7
^{201}Tl-DTPA-HIgG	Water:methanol (45:55)	Paper chromatography Whatman No. 1.	0.2
$^{201}Tl^{3+}$	//	//	0.1
^{201}Tl-DTPA	//	//	0.8
^{201}Tl-DTPA-HIgG	1 mM DTPA	//	0.4
$^{201}Tl^{3+}$	//	//	0.9
^{201}Tl-DTPA	//	//	0.6
^{201}Tl-DTPA-HIgG	//	Silicagel	0.0
$^{201}Tl^{3+}$	//	//	0.0
^{201}Tl-DTPA	//	//	0.8

Maximum capacity of the radiolabeling

In order to obtain the highest possible specific activity the equal amounts of the conjugated protein solution were labeled using various amounts of Tl^{3+} activity. The minimum acceptable radiochemical purity was considered to be 95%. Figure 2 shows the radiochemical purity of the radiolabeling reaction with increasing radioactivity. The best specific activity was calculated, using the sample with a radiochemical purity of >95%. Thus, the best acceptable radiochemical purity (95%) was obtained using 12 MBq of ^{201}Tl(III) and 0.05 mg of IgG.

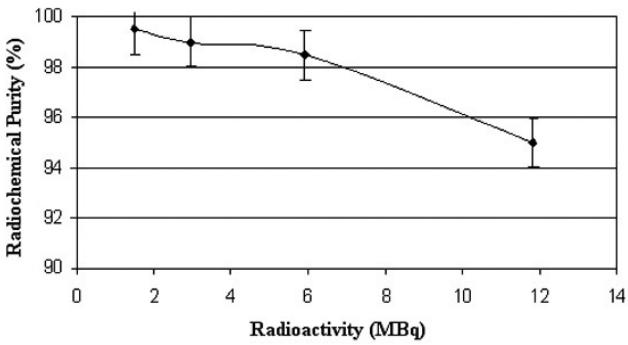

Figure 2. Radiochemical purity of [^{201}Tl](III)-DTPA-HIgG as a function of radioactivity for radiolabeling of 0.05 mg of IgG (n=5).

Stability of Radiolabeled Protein in Vitro

The stability of the radiolabeled protein in vitro was determined after challenge with phosphate-buffered saline and serum. ITLC analysis showed that the proteins retained the radiolabel over a period of several hours, indicating that the Tl-protein chelate was of high affinity. These results were confirmed by gel filtration chromatography. After incubation of [^{201}Tl](III)-DTPA-HIgG with PBS for 24 h, almost all the eluted radioactivity was in the same position as [^{201}Tl](III)-DTPA-HIgG; there was no evidence for large-scale release of free Tl. Similarly, gel filtration chromatography of [^{201}Tl](III)-DTPA-HIgG after a 24 h incubation with human serum showed that the radioactivity had still been eluted in the same position.

Thus, there was no evidence for either degradation or transchelation of ^{201}Tl to other serum proteins over a time period, consistent with the normal blood clearance time of HIgG.

Biodistribution studies in normal rats

The distribution of [^{201}Tl](III)-DTPA-HIgG among tissues were determined for untreated rats and for rats with inflammatory lesions (figure3). A volume (0.05 ml) of final [^{201}Tl](III)-DTPA-HIgG solution containing 4.4-5.2 MBq radioactivity (≤6 µg IgG in 100 µL) was injected into the dorsal tail vein. The total amount of radioactivity injected into each mouse was measured by counting the 1-ml syringe before and after injection in a curiemeter with a fixed geometry. The animals were sacrificed by ether asyxphycation at selected times after injection (2, 4, 24 and 28 h), the tissues

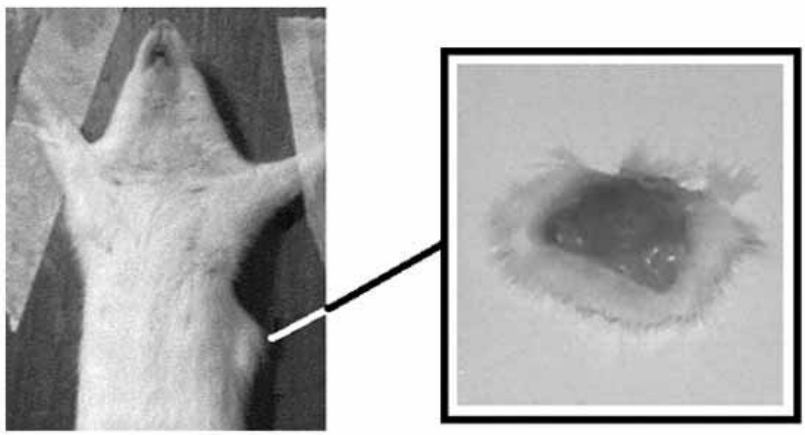

Figure 3. The size and shape of inflammatory lesions in the animal and after dissection.

(blood, heart, spleen, kidneys, liver, intestine, stomach, lung, skin) and foeces were weighed, and their specific activities were determined with a HPGe detector as a percent of the area under the curve of 167 keV peak per gram of tissues. (figures 4-7).

In the first couple of hours, the blood radioactivity was detectable, but after 2h almost no detectable counts were observed in the samples, showing the tissue uptake of the labeled antibody, and low/or no free metal release in the circulation. Liver uptake was significant (15%) in the first few hours, while it decreased after 4h and remained almost constant up to 28 hours. Stool radioactive content was increased after 28 h which could have been to GI disposal of the activity into intestine.

Biodistribution studies in inflammation-bearing mice

As it can be concluded from figure 7, after 28 hours the maximum activity has been present in kidney which was possibly due to excretion of ^{201}Tl after complex dissociation in liver and a rather nice accumulation of radioimmunoconjugate in the inflamed tissue. Lower accumulations have been observed in the gastrointestinal system due to entro-hepatic excretion.

From this data, it is understandable that 24-30 hour range is a rather suitable time for

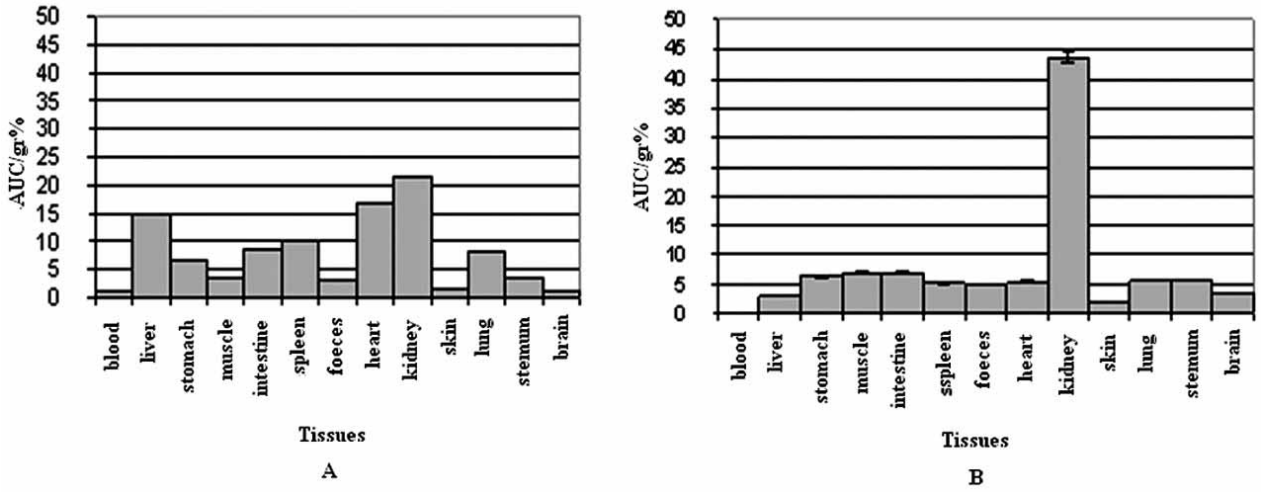

Figure 4. Bio-distribution of [^{201}Tl](III)-DTPA-HIgG in normal rats 2 (A) and 4 h (B) post-injection, (AUC: area under the curve of 167 keV peak) (n=5).

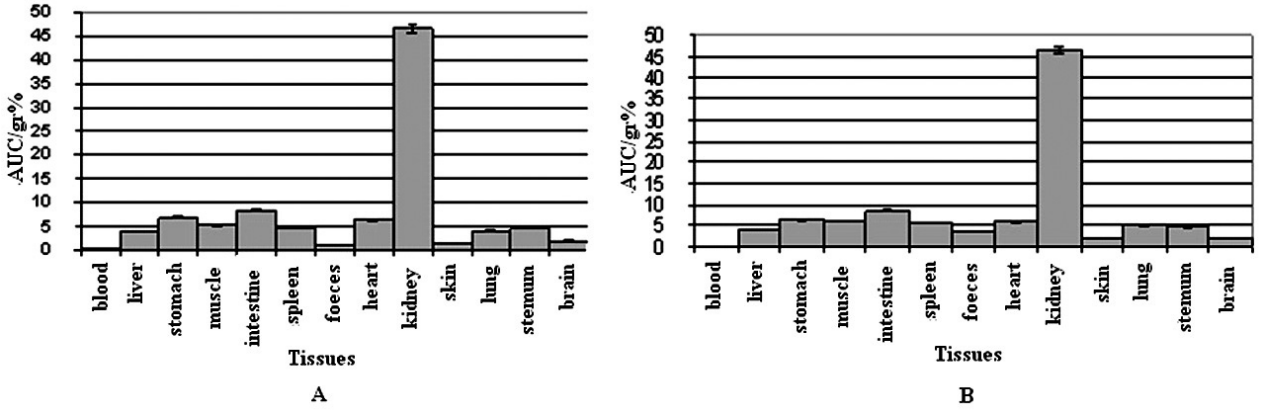

Figure 5. Bio-distribution of [^{201}Tl](III)-DTPA-HIgG in normal rats 20 (A) and 24 h (B) post-injection, (AUC: area under the curve of 167 keV peak) (n=5).

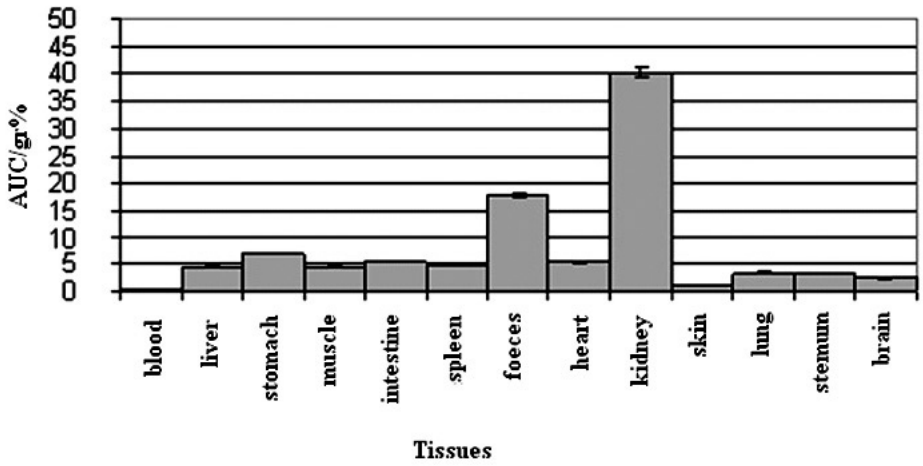

Figure 6. Bio-distribution of [201Tl](III)-DTPA-HIgG in normal rats 28h post-injection, (AUC: area under the curve of 167 keV peak) (n=5).

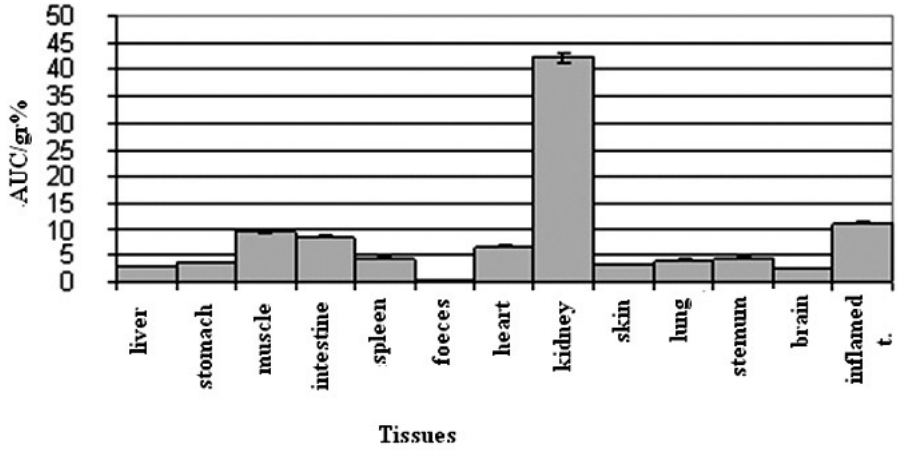

Figure 7. Bio-distribution of [201Tl](III)-DTPA-HIgG in inflammated rats 28h post-injection 7 days after pretreatment with turpentine-oil injection, (AUC: area under the curve of 167 keV peak)

future SPECT studies *in vivo* due to better target accumulation. At this time range, possible abdominal inflammations can not be diagnosed due to significant gastrointestinal activity. The use of inflammation inducers in the limbs, for example, can overcome the interaction of the real inflammation data with the GI artifacts.

DISCUSSION

[201Tl](III)-DTPA-HIgG had a significant accumulation in the turpentine-induced rats after 28 h. A significant inflammation lesion uptake was observed 28 hours after administration of the tracer. However, the 201Tl abundance (9%) in the inflamed tissue, was much lower than the other reported radiolabeled immunoglubins, such as 111In (90%) [4] and 99mTc (89%) [22]. The higher kidney uptake of the tracer was a result of the instability of the conjugate in serum and/or release of non-specifically binded Tl(III) from HIgG. According to these results, 201Tl can not be a competitor for other radiolabeled immunoglubins. Due to the extensive excretion of radioactivity from urine a complementary RTLC study on the urine sample can be helpful to figure out the nature of the excreted compound (201Tl(III) or 201Tl(III)-DTPA). A future 201Tl(III)-DTPA

biodistribution study can be instructive to compare the data.

In conclusion, total labeling and formulation of [^{201}Tl](III)-DTPA-HIgG took about 60 minutes, with a yield of 99%. A suitable specific activity product was formed *via* insertion of ^{201}Tl cation. No unlabelled and/or labeled conjugates were observed upon RTLC analysis of the final preparations. A significant inflammation lesion uptake was observed 28 hours after administration of the tracer. The use of thallium-201 labeled compounds in nuclear medicine can be an interesting option in the future.

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