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^{99m}Tc-glucarate for detection of isoproterenol-induced myocardial infarction in rats

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Abstract

Infarct-avid radiopharmaceuticals are necessary for rapid and timely diagnosis of acute myocardial infarction. The animal model used to produce infarction implies artery ligation but chemical induction can be easily obtained with isoproterenol. A new infarct-avid radiopharmaceutical based on glucaric acid was prepared in the hospital radiopharmacy of the INCMNSZ. ^{99m}Tc-glucarate was easy to prepare, stable for 96 h and was used to study its biodistribution in rats with isoproterenol-induced acute myocardial infarction. Histological studies demonstrated that the rats developed an infarct 18 h after isoproterenol administration. The rat biodistribution studies showed a rapid blood clearance via the kidneys. Thirty minutes after ^{99m}Tc-glucarate administration the standardised heart uptake value S_hUV was 4.7 in infarcted rat heart which is six times more than in normal rats. ROIs drawn over the gamma camera images showed a ratio of 4.4. The high image quality suggests that high contrast images can be obtained in humans and the 96 h stability makes it an ideal agent to detect, in patients, early cardiac infarction.

Keywords: ^{99m}Tc-glucaric acid; Isoproterenol; Rat myocardial infarction; ^{99m}Tc-glucarate

1. Introduction

Worldwide, acute myocardial infarction is a very common cause of mortality and a rapid and timely diagnosis is an important problem which may be solved with specific radiopharmaceuticals. Several animal models have been used to study

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myocardial infarction. In the rat model the animals are anesthetised and the left anterior descending coronary artery is ligated producing several degrees of myocardial infarction. Because of the fast rat heart rate the ligation procedure results in occlusion of the LAD in only 2/3 of the animals while producing ischemia in the rest (Othani et al., 1992). The same cumbersome methodology has been used with rabbits (Narula et al., 1997) and dogs (Orlandi et al., 1991). Since 1959 it is known that isoproterenol (dihydroxyphenylethanol–isopropylamine), a synthetic β-adrenergic agonist, is a myocardial infarction inducer in rats (Chagoya et al., 1997). Glucaric acid, a six carbon, glucose derived dicarboxilic acid, is a non-toxic natural compound which is normally present in tissues and body fluids. Labelled with ^{99m}Tc it is similar to other ^{99m}Tc-based infarct-avid agents. It concentrates in cerebral and myocardial infarcts (Khaw et al., 1997) and there is low uptake in tissues subjected to brief episodes of ischemia (Bianco et al., 1995; Yaoita et al., 1991). In one reported formulation, ^{99m}Tc-glucarate was prepared with a 97 glucaric acid/$SnCl_2$ molar ratio and it required a 60 min incubation period before administration (Othani et al., 1992). Considering our expertise in technetium radiopharmaceuticals preparation and that chemical induced infarction is easier to achieve than with artery ligation, we speculated that an easily prepared ^{99m}Tc-glucarate in an animal model could detect acute myocardial infarction. Therefore, the objective of this investigation was to formulate and characterise a new ^{99m}Tc labelled glucaric acid and use it to detect chemically produced myocardial infarction in rats, as the basis for future clinical studies.

2. Materials and methods

2.1. Labelling

Technetium-99m (^{99m}Tc) was obtained from a *GETEC* ^{99}Mo/^{99m}Tc generator (ININ) and the activity was determined with a *Capintec RC-15* dose calibrator. Reagent grade chemicals from *Sigma Chem. Co.* were used. D-glucaric acid ($C_6O_8H_{10}$) was used as purchased as were all the other reagents. Molar ratios of the reducing agent ($SnCl_2{\cdot}2H_2O$) with a stabiliser or antioxidant (gentisic acid) were studied in order to use 5 mg of glucaric acid, dissolved in 1 ml 0.9% NaCl solution. The experimental design is shown in Table 1.

The procedure was to add 0.0125 mg of gentisic acid to the glucarate solution, adding stannous chloride, sodium pertechnetate, recently eluted from the generator, and adjusting the pH. The radioactive mixture was incubated at 18–25 °C for 10 min and millipore filtered (0.22 μm). Radiochemical purity was performed with HPLC and ITLC. Since ^{99m}Tc-perthecnetate and -glucarate are both highly hydrophilic molecules, a HPLC molecular exclusion system was preferred to separate them instead of a reverse phase or adsorption system. For molecular exclusion HPLC a ProteinPak 125, 3.9×300 mm column, with UV photodiode array detector, was eluted with phosphate buffer 0.1 M pH 7.4 at a flow rate 1.0 ml/min. The radiochromatographic profile was determined by collecting samples (Waters fraction collector) of 0.5 ml each for counting in an external NaI(Tl) detector (NML Laboratories). For ascending mini-chromatography with ITLC silica gel glass fiber strips were used (Gelman-Sciences, Ann Arbor, Mich, USA) the mobile phase was saline plus glucaric acid 0.006 N. For Whatman paper ascending chromatography acetone was used to detect free ^{99m}Tc-pertechnetate.

2.2. Animal studies

Male Wistar, INCMNSZ rats, weighing 250–300 g, maintained on standard PMI 5001 feed, were used in accord with the rules and regulations of the Institute, for infarct production and for normal rat biodistribution studies. To determine

Table 1
Experimental design for ^{99m}Tc-glucarate formulation

Class	Levels	Values
$SnCl_2$ (mg/ml)	3	0.05, 0.125, 0.5
pH	3	3, 4.5, 6

Dependent variable: radiochemical purity.

the best time for heart infarction DL-isoproterenol hydrochloride was dissolved in 0.9% NaCl solution to give a 20 mg/ml concentration. Sixty-seven milligram per kilogram body weight were subcutaneously injected and 28 rats (four rats for each time) were CO_2 sacrificed after 2, 4, 6, 8, 18, 24 and 48 h. A surgical midline incision was made and the heart was taken out and placed in a test tube containing 10% neutral buffered formaldehyde solution for histological studies.

2.3. Biodistribution

Normal Wistar rats (three per time point) were injected in the tail vein 1.85 MBq (50 μCi) in 0.1 ml of the glucarate-technetium radiopharmaceutical and CO_2 sacrificed after 0.083, 0.25, 0.5, 1, 2 and 24 h. A surgical midline incision was made and blood was drawn into a disposable syringe and approximately 1 ml was placed into a test tube. Then the organs of interest were extracted. Each organ (heart, lung, liver, spleen, stomach, intestines, left kidney, muscle and bone) were saline rinsed, paper blotted, placed into pre weighed test tubes and the activity was determined with a well type scintillation detector (Canberra). The mean cpm were used to obtain accumulation in percent injected activity per gram of tissue % IA/g. Distribution studies, 30 min after the radiopharmaceutical was injected into four normal and four infarcted rats, were undertaken as above mentioned. Standardised heart uptake value S_hUV was calculated as follows (Wahl, 1994):

$$\mathrm{SUV} = \frac{\text{Decay corrected activity (MBq) in the heart/mass of heart(g)}}{\text{Total injected activity/rat weight (g)}}.$$

2.4. Histological studies

The isoproterenol injected rat hearts were paraffin embedded, 5 μm thick sections were cut and hematoxylin–eosin stained. The slides were examined with a Zeiss light microscope.

2.5. Infarct imaging

After the time for the isoproterenol induced infarct was determined four rats were injected ^{99m}Tc-glucarate in the lateral tail vein and imaged 30 min later after being sedated with a combination ketamine–xylazine (50, 5 mg/kg) subcutaneously injected. A Siemens SPECT 2 scintillation gamma camera with a parallel hole, all purpose collimator, was used to localise radioactive concentration. A region of interest (ROI) was drawn on the heart image and cpm/pixel/ROI were determined. Normal ^{99m}Tc-glucarate injected rats were used for comparison.

3. Results

3.1. Radiopharmaceutical formulation

The optimal formulation contained 5 mg glucaric acid; 0.0125 mg gentisic acid, 0.125 mg stannous chloride and $^{99m}TcO_4^-$ in a final 1.5–3 ml saline solution volume and pH 6. The molar glucarate/stannous chloride ratio was 37. The radiochemical purity was greater than 95% determined by both ITLC-SG and HPLC. The radiochemical impurities ($< 5\%$) observed were ^{99m}Tc-pertechnetate and hydrolysed-reduced ^{99m}Tc. The hydrophilic ^{99m}Tc-glucarate and $^{99m}TcO_4^-$ migrated with the solvent front on ITLC-SG (Rf 1.0) while hydrolysed-reduced technetium remained at the origin (Rf 0.0). Retention time in the HPLC column for ^{99m}Tc-glucarate is 10 and 7 min for ^{99m}Tc-pertechnetate. The radiopharmaceutical was prepared with 68.5–1665 MBq (1.85–45 mCi) with labelling efficiency $>$ 95% up to 96 h.

3.2. Animal studies

In normal rats the activity is concentrated in blood, heart and is eliminated by the kidneys (12.3% IA/g tissue in 24 h). Lungs, liver, spleen, intestine, leg muscle and femur bone show low ^{99m}Tc-glucarate concentration. Blood and heart concentration reach a peak 30 min after labelled glucarate administration and 24 h blood concen-

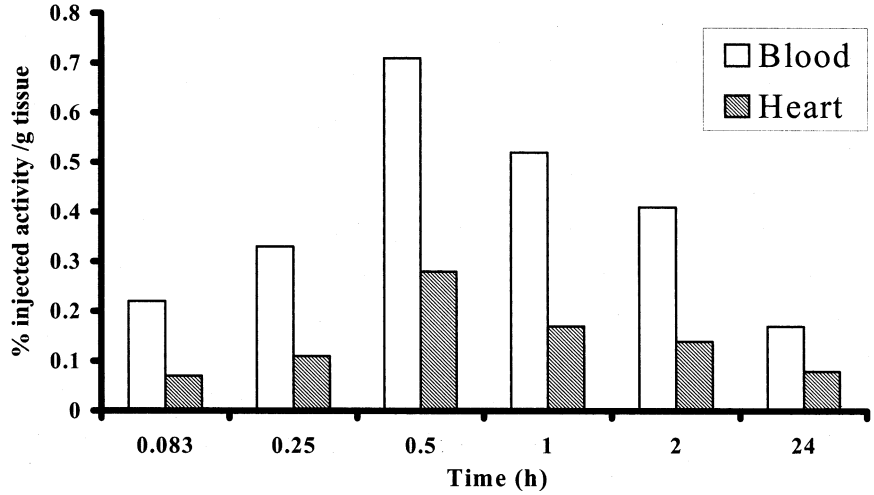

Fig. 1. Time–concentration of ^{99m}Tc-glucarate in blood and heart of control Wistar rats (0.083–24 h).

tration was $0.12 \pm 0.06\%$ IA/g in control rats. Thirty minuteS heart concentration in infarcted heart ($n = 4$) showed a $S_hUV = 4.7$ which corresponds to six times more than the normal rat value (Figs. 1 and 2).

3.3. Histological studies

Microscopic findings in the hearts of the rats which had been injected isoproterenol 18 h previously included myocardial necrosis foci intercalated among normal muscle fibers. In the necrosis foci there were lymphocytic exudate, atrophy of the remaining normal muscle and also elongation, undulation of the fibers and formation of contractile band lesions characteristic of the pre-infarction stage. The hearts belonging to the rats injected with isoproterenol 24 h previously, showed coagulative necrosis, polymorphonuclear cell exudates, edema and fragmentation of myocardial fibers all characteristic of acute myocardial infarction. The presence of macrophages, fibroblasts and collagen fibres was considered as a post-infarction stage.

3.4. Infarct imaging

Scans were taken 30 min after ^{99m}Tc-glucarate was injected into the infarcted rats and showed significant concentration in the heart compared with the heart of control rats. The concentration was 12.7 vs 2.9% IA/g ($P < 0.05$), respectively, calculated by cpm/pixel/ROI. The ratio being 4.4 times higher in infarcted rats. Besides the heart, the kidneys and bladder images were observed.

4. Discussion

Infarct avid imaging ^{99m}Tc labelled pyrophosphates have been used but the uptake mechanism is related to the amount of calcium present in the mitochondria of dead cells and therefore ^{99m}Tc-PYP does not provide data in the critical early hours following onset of chest pain and thus imaging has to be delayed for 2–3 days. In 1992, ^{99m}Tc-glucarate was described as a radiopharmaceutical for imaging acute myocardial infarction but the preparation required 1 h incubation period which is too much time for such a study (Othani et al., 1992). Glucaric acid is a small molecule (FW 210.1) and the radiopharmaceutical ^{99m}Tc-glucarate might be a five co-ordinated dimeric technetium negatively charged complex $[^{99m}TcV(glucarate)_2]^-$ still small enough to diffuse rapidly and come in contact with tissue that has low blood flow. The exact mechanism of intracellular accumulation of ^{99m}Tc-glucarate is not

known. Sub-cellular distribution studies show that 75% is incorporated into the nuclear fraction and the remainder is equally distributed in cytosol and mitochondrial fractions. Of the nuclear fraction 83% is associated with histones, pole-like nucleoproteins into which DNA chains are rolled up, and 17% is associated with DNA of the non-viable cells. Therefore, ^{99m}Tc-glucarate is associated with disruption of the cell and nuclear membranes allowing free intracellular diffusion and electrochemical binding of the negatively charged glucarate complex to positively charged histones. This hypothesis would also explain the lack of ^{99m}Tc-glucarate uptake in dead cells because histones are washed out rapidly on full cell death (Mariani et al., 1999). The high activity of ^{99m}Tc-glucarate for the nucleohistones together with the fast blood clearance should permit a high target to background ratio that makes possible early visualisation of the infarcted myocardium (Khaw, 1999). In human breast tumour xenografted in immune deficient mice intracellular distribution of ^{99m}Tc-glucarate 5 h after administration was $50.91 \pm 6.55\%$ in the nuclear fraction; $34.34 \pm 2.88\%$ in the cytoplasmic fraction and $14.75 \pm 7.66\%$ in the nuclear mitochondrial fraction (Petrov et al., 1997).

In humans it is difficult to diagnose acute myocardial infarction since it can be associated with atypical symptoms or even absence of chest pain. Early diagnosis is necessary in order to initiate therapy to salvage myocardium at risk and to avoid the risk of unnecessary thrombolytic therapy. Previous studies in humans suggest that ^{99m}Tc-glucarate localisation can only occur if the administration of the radiopharmaceutical is done within 1–10 h of onset of necrosis ^{99m}Tc-glucarate imagining can be postponed 2–18 h after administration of the radiopharmaceutical or until the patient has been stabilised or controlled (Strauss, 1996; Gerson and McGoron, 1997). Because our radiopharmaceutical is stable for 96 h the resident physician can have it at hand and inject it into the patient as soon as the acute myocardial infarction is suspected. Later on the patient can be taken to the nuclear medicine department for a heart scan.

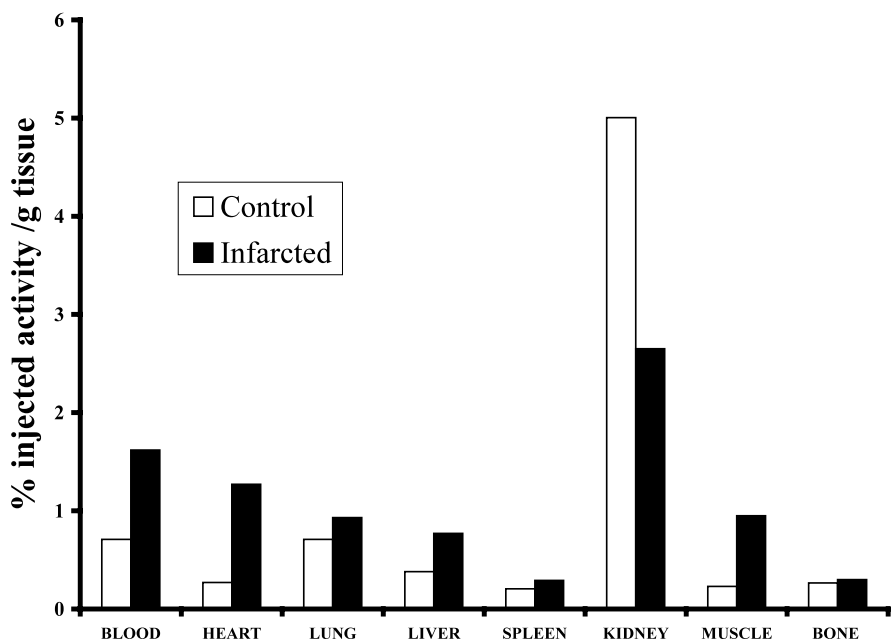

Fig. 2. Uptake of ^{99m}Tc-glucarate (% injected actitity/g tissue), in several organs, at 30 min P.I. in control and infarcted Wistar rats.

5. Conclusion

The proposed glucaric acid ^{99m}Tc-labelling technique is easy and can be rapidly performed in any hospital radiopharmacy. The radiopharmaceutical is a stable, high radiochemical purity agent useful for the detection of acute myocardial infarction in rats. The present study indicated an in vitro infarcted-/normal-heart ratio 4.7 and in vivo, using ROIs on the scan heart images, the ratio was 4.4. Rapid blood clearance and high S_hUV of the infarcted rat myocardium provided images with excellent resolution which suggest that high contrast images of normal and infracted myocardium can be obtained in humans.

Our animal model of chemically produced myocardial infarction was designed to detect early myocardial changes with ^{99m}Tc-glucarate. Studies in patients with acute myocardial infarction symptoms might prove the usefulness of ^{99m}Tc-glucarate, prepared at the radiopharmacy of the INCMNSZ, to detect early cardiac problems. It might also be worthwhile to investigate its use as a diagnostic agent in breast cancer.

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