Development of Tc99m-Saccharic Acid for Heart Imaging

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Abstract

Cardiovascular disease especially the coronary heart disease (CHD) is the leading cause of death worldwide. Coronary heart disease is a common term for the buildup of plaque in the heart coronary arteries that could block the blood supply to the myocardial and this could lead to heart attack. An estimated 17 million people died from cardiovascular disease in 2008 representing 30% of all global death.In United Kingdom, coronary heart disease killed as much as 82,000 people each year. Hence, early detection of the coronary heart disease is very important in reducing the mortality among the world population. One of the most sensitive detection method is by radio imaging using Technetium-99m radiopharmaceuticals.

Several different radio imaging agents such as 99mTc-radiopharmaceutical were developed as radiagnostic agent in determining the CHD especially in identifying the blockage of the coronary artery of the heart muscle. Despite the success of Tc99m-sestamibi and ^{99m}Tc-tetrofosmin as an effective agents for myocardial perfusion study, the search for other ^{99m}Tc heart imaging agents has never been interrupted.

This report described the formulation of the ^{99m}Tc-saccharate radiopharmaceutical kit , radiolabelling of the kit, radiochemical purity evaluation of the ^{99m}Tc labeled saccharic acid, and animal study involving radio imaging using gamma camera. The animal are then sacrificed and the biological distribution of the ^{99m}Tc-saccharic acid in-vivo was determined. Comparative study was also conducted using commercially available Tc99m-tetrafosmin, a CHD radiopharmaceutical kit.

The ^{99m}Tc-saccharic acid was successfully formulated and gave a very high labeling efficiency of >92% with ^{99m}Tc and gave good uptake in the necrosis skeletal muscle.

Keywords : Coronary heart disease, Myocardial infarct imaging ^{99m} Tc-saccharate

INTRODUCTION

Cardiovascular disease especially the coronary heart disease (CHD) is the leading cause of death worldwide. The World Health Organization (WHO) reported that out of 17 million death due to cardiovascular diseases globally in 2008, 7.2 million were due to coronary heart disease, and another 5.7 million were due to stroke. . In United Kingdom, coronary heart disease killed as much as 82,000 people

each year. In the USA, approximately 1.5 million cases of myocardial infarction occur annually. In Malaysia, heart disease is the number killer since 1980 (R. Jeyamalar).

Coronary heart disease is a common term for the buildup of plaque in the heart coronary arteries that could block the blood supply to the myocardial and this could lead to acute myocardial infarction (AMI) or heart attack. Hence, early detection of the coronary heart disease is very important in reducing the mortality among the world population.

The diagnosis of AMI is normally based on typical chest pain, the ECG changes, and the pattern of serum cardiac enzyme releases in the majority of the patients. Within hours of AMI, necrosis, edema and inflammation are reported to be localized in the infarcted area, which are followed by a long-term period of fibroblast proliferation and collagen deposition which lead to scar formation. An imaging agent that localized quickly and specifically in areas of this 'scarred' or dead heart muscle could provide critical diagnostic information. Infarct-avid radiopharmaceuticals are useful for rapid and timely diagnosis of acute myocardial infarction. One of the most sensitive non-invasive early detection method is by radio imaging using radiopharmaceuticals.

A number of radiopharmaceuticals have been reported for early detection of myocardial infarction. Among them Thallium-201 is the procedure of choice for the diagnosis and evaluation of coronary artery disease to determine the location and extent of ischemia and infarction. Since thallium-201 is costly and not easily available at all the nuclear medicine centers, a number of ^{99m}Tc based radiopharmaceutical have been developed.

Since 2002 several different radio imaging agents such as ^{99m}Tc radiopharmaceutical were developed as radio diagnostic agent in determining the CHD especially in identifying ischemic and infarcted heart muscle due to the blockage of the coronary artery of the heart muscle. Despite the success of Tc99m-sestamibi, ^{99m}Tc-teboroxime and Tc99m-tetrofosmin as reasonably effective agents for myocardial perfusion study, the search for other more inexpensive and effective ^{99m}Tc heart imaging agents has never been interrupted.However,all these radiopharmaceutical kits are not available indigenously and need to be imported at exorbitant cost. This coupled with increased cost of diagnostic cardiological investigation and therapeutic management prompted us to develop ^{99m}Tc-saccharate radiopharmaceutical for early diagnosis of coronary artery diseases and AMI. ^{99m}Tc-saccharate has been shown to have good potential for early detection of AMI. Hence, we have undertaken thepresent study to develop stannous saccharate kit and have studied the kit formulation, labeling efficiency, in-vitro stability and its biological distribution using animal infarct models.

MATERIALS AND METHODS

^{99m}Tc pertechnetate

^{99m}Tc pertechnetate solution was obtained by eluting the Mo99/Tc99m generator provided bythe Medical Technology Division, Malaysian Nuclear Agency.

Formulation of stannous saccharate kit

Several formulation of the saccharate kits were prepare in-house. Two set of the stannous saccharate kits were prepared. For set A, each kit contained fixed amount of potassium saccharate (15 mg) and stannous chloride dihydrate (450 ug) but with variable pH. The pH of the preparation varied from 2 to 7. The study was to determine the effect of pH of the preparation on the radiochemical purity of the 99mTc-saccharate.

For set B, each kit contained 15 mg of potassium saccharate but with different amount of stannous chloride dihydrate (0.2 to 0.8 ug). The pH of the preparation was set at 4.5. The purpose was to study the effect of stannous chloride dihydrate content on the radiochemical purity of the ^{99m}Tc-saccharate.

Radiolabelling

^{99m}Tc-saccharate was prepared by reconstituting the stannous saccharate kit with ^{99m}Tc-pertechnetate obtained from the Medical Technology Division. Two mCi of ^{99m}Tc pertechnetate in 2 ml volume was added to the kit. The contents were mixed thoroughly and incubated at room temperature from 5 to30 minutes.

Radiochemical Purity Determination

The radiochemical purity of ^{99m}Tc-saccharate was assessed by ascending instant thin layer chromatography-silica gel (ITLC-SG) strips using solvent system, namely acetone (100%) and saline (0.9% NaCl) as mobile phases. The radioactive impurities were identified as reduced hydrolyzed Tc99m and free pertechnetate (99mTcO4-).

Biological distribution

In vivo biological distribution of ^{99m}Tc-saccharate was studied in Sprague-Dawley female rats each with body weight between 200 to 220 gram. Thermal infarct or necrosis of skeletal muscle was produced in the rats by touching the hot tip of a car cigarette lighter to the exposedthigh muscle for a few seconds as per Adler's method. The whole procedure was carried out under general anesthesia. Thirty µl of ^{99m}Tc-sacharate (100 uCi) was intravenously administered into the tail vein of each rat after about 1 hour post-infarct induction. Radio imaging of the animals were carried out at different time interval using gamma camera at 15, 120 and 180 minutes. The animals were then sacrificed at different time intervals after giving an overdose of anesthetic ether. Different organs were quickly removed, washed with normal saline, dried in the paper folds and placed in pre-weighed counting tubes. The tube containing the organs were each weighed and the radioactivity of the organswere counted using a well-type gamma counter (Perkin Elmer, USA) and expressed as percent injected dose/organ and percent injected dose/gm.

Comparative study

Comparative study between ^{99m}Tc-saccharate and commercially available ^{99m}Tc-tetrofosmin was not done due to the delay in receiving the commercial kit.

RESULTS

Radiolabelling

The labelling yield so far achieved was approximately 94% when 0.7 mg of stannous chloride dihydrate was used for complexing 15 mg potassium saccharic acid with 150 uCi ^{99m}Tc-pertechnetate at pH 4.5 (Tables 1 and 2). The labelling of saccharate at pH4.5 with ^{99m}Tc-pertechnetate seemed to be dependent on the amount stannous chloride dihydrate content of the kit. No significant degradation of the reconstituted ^{99m}Tc-saccharate was noted for up to 5 hours at room temperature.

Table 1. The effect of $SnCl_2.2H_2O$ concentration on radiochemical purity of ^{99m}Tc-saccharate.Potassium saccharate content and pH of the preparations were kept constant at 15 mg and 4.5, respectively. Only the amount of $SnCl_2.2H_2O$ was varied.

$SnCl_2.2H_2O$ (ug)	% Free $^{99m}TcO_4^-$	% Reduced Hydrolyzed Tc99m	% ^{99m}Tc-saccharate
200	23.9	41.9	34.2
400	0.2	13.5	86.3
500	5.8	12.24	93.9
600	0.68	3.2	96.2
700	2.48	2.12	89.9
800	2.88	21.7	77.8

Table 2. Effect of pH on the formulation of ^{99m}Tc-saccharate. While keeping other reaction conditions constant, only the pH of the reaction was varied from 2 to 7.

pH	% Labelling Efficiency at different incubation period (min)			
	5	10	15	30
2	32.5	40.2	49.3	52.1
3	28.5	30	35.5	33.7
4	81.2	95.4	94.3	95.6
5	78.9	92.6	95.8	96.1
6	45.4	47.8	53.3	56.2
7	90.2	94.7	95.2	95.7

Biological distribution

Biological distribution of ^{99m}Tc-saccharate in liver, skeletal muscle, heart, blood, kidneys and necrotic thigh muscle at 3 hours post-injection is shown in Table 4. Among all the organs studied, the skeletal muscle exhibited the highest uptake of the radiopharmaceutical (5.2% of the radioactive injected dose) at 30 min which decreased to 2.47% at 3 hours. Although liver and kidney exhibited relatively less accumulation of radioactivity per whole organ, it is significantly high relative to skeletal muscle when

calculated on weight basis. ^{99m}Tc-saccharate showed high necrosis or infarct to muscle and necrosis to blood ratios after injection into animal model. The high radioactivity in the kidney, bladder and urine indicated that the radiopharmaceutical was excreted from the body through the kidney.

Table3. Tissue distribution in normal female rats at 0.5, 1.5 and 3 hour post-injection of 99mTc-saccharate. Data expressed as percent of injected dose ± sd per whole organ of 3 animals

Organ/Tissue	Percent of Injected dose/whole organ		
	0.5 hour post-injection	1.5 hours post-injection	3 hours post-injection
Blood	8.1	2.82	1.14
Liver	2.33	1.07	0.04
Heart	0.35	0.28	0.07
Kidneys	5.55	7.12	2.42
Guts	2.27	3.7	3.44
Skeletal muscle	6.17	8.21	2.15
Necrotic skeletal muscle	1.15	3.92	4.33

The radiopharmaceutical was examined for its ability to concentrate in infarcted muscle. When 99mTc-saccharate was administered to a rat having 1 hour old thermal infarct in thigh muscle, a significant amount of radioactivity was found concentrated in the infarct as is evident from Table 4 and Figure 1.

Table 4. . Tissue distribution in normal rat at 0.5, 1.5 and 3 hour post-injection of 99mTc-saccharate. Data expressed as percent of injected dose/gm weight ± sd of 3 animals.

Organ/Tissue	Percent of Injected dose/gm organ		
	0.5 hour post-injection	1.5 hours post-injection	3 hours post-injection
Blood	0.54	0.07	0.04
Liver	0.19	0.08	0.05
Heart	0.44	0.35	0.28
Kidneys	2.64	3.39	1.22
Skeletal muscle	0.11	0.15	0.25
Necrotic skeletal muscle	1.64	6.6	8.45

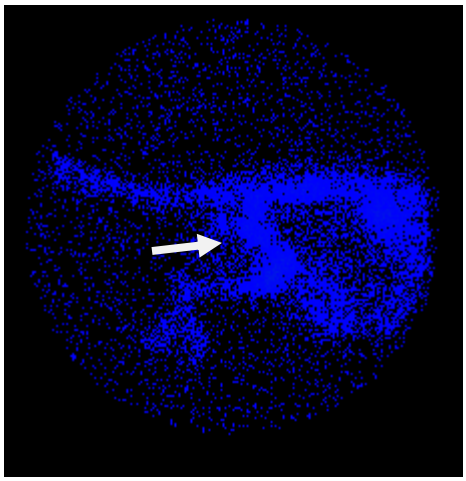

Figure 1. Scintigraphy in rat after 1.5 hour i.v. administration of 99mTc-saccharate. Right lateral view image of experimental rat model of thermal infarct in right thigh muscle (Arrow)

DISCUSSION

Saccharate or saccharic acid is also known as glucaric acid, is a carboxylic acid analogue of glucose. From the result obtained the most appropriate formulation of stannous sacharate kit comprised of potassium saccharate (15 mg), stannous chloride dihydrate (0.6 mg) and pH 4.5 (Table 1 and 2).The formulation gave very high labelling efficiency (92 to 95%) when the saccharate kit were reconstituted with ^{99m} Tc pertechnetate. The ^{99m}Tc-saccharate prepared was also found to be stable post-reconstitution for not less than 5 hours at room temperature. The addition of antioxidant such as ascorbic acid or gentisic acid to the formulation as suggested by Ballinger *et al*., may enhance the stability of the reconstituted ^{99m}Tc-saccharate at room temperature.

The high uptake of the radiotracer in the kidney, bladder, and liver indicated that the radiotracer was excreted from the body mainly through both the hepatobiliary and renal routes. The radiotracer rapid clearance from various organs including blood is of considerable interest since this improves the target to non-target ratios and results in low background apart from a low radiation burden to the organism(Table 3). The uptake of the radiotracer per gram in the infracted skeletal muscle at 1.5 hr post-injection was found to be more than 6 time the uptake in the viable skeletal muscle and about 8 times at 3 hour post injection (Table 4). The high and rapid uptake but slow clearance of the radiotracer in the infarct skeletal muscle indicated that the radiotracer has great potential as infarct imaging agent. All this findings demonstrates the suitability of the ^{99m}Tc-saccharate radiotracer for the early detection of myocardial infarction as was previously reported (Narula J *et al*,. and Orlandi C).

^{99m}Tc-Saccharate uptake by the necrosis or infarcted skeletal tissue may be due to a possible increase in the utilization of the sugar transport system in the hypoxic state. Saccharate is structurally similar to fructose and enters the cells through D-fructose sugar transport system (Yaoita H *et.al*). ^{99m}Tc-saccharate has been proved capable of locating necrotic lesions in the experimental models of infarction.

^{99m}Tc-saccharate formulated and prepared in this study was found to accumulate in the region of skeletal muscle necrosis. This correlate well with those results reported earlier (Khaw BA *et al.*, Babbar AK *et al*.,). Further in-vivo study using infarcted myocardial tissue rather than skeletal muscle necrosis need to be carried out in order to clarify the true potential of this radiotracer. This may be done by inducing infarct to the animal heart using correct dose of isoproterenol.

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