BIODISTRIBUTION OF TECHNETIUM- 99m LABELED DILTIAZEM AND NIFEDIPINE IN NORMAL MICE

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SUMMARY: Diltiazem and nifedipine were labeled with ^{99m}Tc by Sn^{++} reduction method with an efficiency of greater than 99% as determined by ITLC. The biodistribution of both agents were studied in normal mice at various intervals. Scintigrams were also obtained at 1 hour post-injection of mice and at 30 min intervals up to 3 hours in rabbits. Our results indicated a very high accumulation in liver (max. 61.4±10.0 %/g for ^{99m}Tc-diltiazem and 36.1±0.6 %/g for ^{99m}Tc-nifedipine) with negligible uptake by other organs in mice. Chromatographic analyses of urine samples indicated excretion of a soluble metabolite of ^{99m}Tc-diltiazem in high quantity (89.6±3.6 % of the total radioactivity in the urine). Because of high liver accumulation and no selective uptake in myocardium (max. 0.72±0.01 %/g for ^{99m}Tc-diltiazem and 0.312±0.224 %/g for ^{99m}Tc-nifedipine) the labeled compounds cannot be used for the scintigraphic visualization of myocardium.

Key Words: ^{99m}Tc-diltiazem, ^{99m}Tc-nifedipine, ITLC, scintigraphy.

INTRODUCTION

Several radio pharmaceuticals have been introduced for the scintigraphic visualization of myocardium, including the much favored thallous chloride ($^{201}TlCl$) and some cationic ^{99m}Tc complexes (6). Commercially available methoxyisobutyl isonitrile (MIBI) kits for labeling with ^{99m}Tc and $^{201}TlCl$ are too expensive for routine use in most countries. There is a need for a radio pharmaceutical which can be prepared in-house with a simple and rapid procedure and at low cost. In addition such an agent should accumulate in the myocardium at a high concentration to give high target-to-non target ratios.

Certain drugs called Ca channel antagonists alter the cellular function of calcium by selective inhibition of the influx of extra cellular Ca^{++}. They are more potent in smooth and cardiac muscle than in skeletal muscle. The main target organs for the therapeutic use of Ca-antagonists is the cardiovascular system. They are mostly vasodilators and used for the therapy of hypertension and angina pectoris. Diltiazem and nifedipine have primarily cardiac effects (5,8) and nimodipine is claimed to penetrate into the central nervous system with selective effect on cerebral vessels (2). The purpose of the present investigation was to label diltiazem and nifedipine with ^{99m}Tc and to determine their affinity to cardiac muscle by both biodistribution and scintigraphic studies in experimental animals.

MATERIALS AND METHODS

Diltiazem was obtained from Bayer Türk Kimya Sanayii Ltd. Sti., Istanbul and nifedipine from Fako Ilaçlari A.S., Istanbul. ^{99m}Tc as pertechnetate was obtained from a generator (Amersham International, U.K.).

Labeling with ^{99m}Tc: 10 mg diltiazem or nifedipine was placed in a glass vial. 1 ml distilled water and 1 ml 95% ethyl alcohol were added. The mixture was stirred until all the active com-

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pounds were dissolved. The pH of the solution was adjusted to 4 with 1 N HCl. 0.2 ml (0.2 mg) $SnCl_2.2H_2O$ was added. The mixture was passed through a Millipore filter (pore size: 0.22 μm) into a sterile vial. 1 mCi (37 MBq) ^{99m}Tc as pertechnetate ion in saline solution was aseptically injected into the vial. The vial was shaken for 1-2 min.

Chromatographic quality control: The labeling efficiency was determined with Impregnated-Thin-Layer-Chromatography with ITLC-SG ready plates obtained from Gelman Scientific Company, U.S.A. They contain silica gel on fiber glass support medium. For solvents methyl ethyl ketone (MEK) and water were used separately. The radiochemical impurity, pertechnetate ($^{99m}TcO_4$-), moved with the solvent front and the labeled compounds remained at origin in both solvents.

Animal experiments: Animal studies were carried out in accordance with the British animal protection practice (4). Swiss albino mice of either sex were injected with 370 kBq in 0.2 ml of either ^{99m}Tc-diltiazem or ^{99m}Tc-nifedipine through the tail vein. They were sacrificed at 5, 30 min, 1, 3, 5 and 24 hours in groups of three by ether anesthesia and cardiac puncture. The following organs were taken out: liver, lungs, heart, kidneys, brain, pancreas, stomach, adrenals, intestines, duodenum, spleen, some muscle and some blood. Urine samples were also obtained at the same time intervals when possible for chromatographic analysis by the method described above. The organs, blood and urine samples were weighed. Their radio activities were measured in a well type gamma counter (Model: BF 5300, Berthold, Germany) against a standard prepared from 1/100 dilution of the injected solution. % uptake and %/g tissue were calculated for each organ or tissue. The mean values with standard deviations were also computed.

Table 1: In vitro stability of ^{99m}Tc-diltiazem and ^{99m}Tc-nifedipine at room temperature.

	% free pertechnetate		
	5-15 min	30 min	4h
^{99m}Tc-diltiazem	0.96±0.65	0.38±0.17	0.062±0.05
^{99m}Tc-nifedipine	0.82±0.50	0.39±0.14	0.42±0.08

Three mice were injected with 3.7 MBq in 0.2 ml of ^{99m}Tc-diltiazem and 3 mice with ^{99m}Tc-nifedipine. Scintigrams (AP view) were obtained at 1h post injection by the use of a gamma camera (Toshiba GCA 60 1E). Two rabbits were also i.v. injected through the ear vein with 37 MBq in 0.2 ml of either of the agents and scintigrams were obtained at 30 min intervals up to 3 hours by the same camera.

RESULTS

Both of the compounds were labeled with an efficiency of greater than 99%. The labeled compounds were stable up to 4 hours of testing. The amount of $^{99m}TcO_4^-$ was always less than 1% (Table 1). Since the animal experiments were carried out within 1 h of preparation the injected amount of $^{99m}TcO_4^-$ was negligible. The biodistribution of both of the labeled compounds were almost identical, and consequently only the results for ^{99m}Tc-diltiazem

Table 2: Biodistribution of ^{99m}Tc-diltiazem in normal mice, expressed as %/g tissue (mean±SD).

Organ	15 min	30 min	Time 1 hr.	3hr.	5hr.	24 hr.
Liver	43.80±1.3	53.80±1.80	49.70±4.30	46.40±4.70	61.4±10.0	34.60±5.0
Spleen	4.16±0.51	4.01±1.00	7.71±2.11	4.76±1.07	5.95±2.67	4.26±3.75
Brain	0.13±0.02	0.11±0.03	0.06±0.03	0.03±0.00	0.04±0.01	0.05±0.01
Heart	0.66±0.16	0.72±0.19	0.72±0.19	0.40±0.05	0.33±0.09	0.25±0.06
Kidneys	0.69±0.06	0.93±0.16	1.05±0.04	1.15±0.10	1.53±0.49	1.43±0.14
Lungs	2.17±0.40	3.00±1.73	4.17±2.80	1.25±0.19	3.15±0.84	1.37±0.47
Pancreas	0.20±0.01	0.37±0.07	0.23±0.06	0.19±0.03	0.39±0.16	0.43±0.31
Stomach	0.22±0.07	0.33±0.09	0.58±0.01	0.56±0.31	1.00±0.55	0.19±0.02
Duodenum	0.17±0.05	0.50±0.26	0.50±0.19	0.23±0.01	0.21±0.04	0.15±0.05
Adrenal	1.12±0.48	0.57±0.28	0.39±0.14	0.37±0.07	0.70±0.37	0.40±0.05
Intestine	0.14±0.02	0.25±0.05	0.22±0.03	0.37±0.04	0.51±0.17	0.30±0.13
Muscle	0.11±0.01	0.23±0.01	0.11±0.01	0.09±0.01	0.10±0.03	0.10±0.03
Blood	0.65±0.03	0.83±0.08	0.75±0.01	0.46±0.15	0.59±0.18	0.47±0.07
Urine	4.01±0.06		28.40±3.60	13.80±9.50	30.7±15.40	9.65±3.35

Table 3: Results of Chromatographic analysis of urine samples of mice after ^{99m}Tc-diltiazem administration.

	Amount (%)		
No of observations	Pertechnetate	^{99m}Tc-diltiazem	^{99m}Tc-metabolite
11	2.88±1.34	7.47±4.74	89.6±3.6

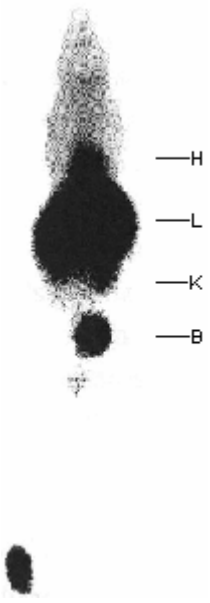
are presented in Table 2. The highest accumulation was observed in liver for both compounds (max. 61.4±10.0 %/g for ^{99m}Tc-diltiazem and 36.1±0.6 %/g for ^{99m}Tc-nifedipine). The rest of the organs had negligible uptakes with no selective uptake in the myocardium (max. 0.72±0.01 %/g for ^{99m}Tc-diltiazem and 0.312±0.224 %/g for ^{99m}Tc-nifedipine) to justify further studies with these agents in this context. The blood clearance was fast for both agents. Some radioactivity was excreted via kidneys as indicated by urine counts (Table 2). The chromatographic analysis of the urine samples at different time periods showed similar results, consequently they were combined and one mean value was obtained (Table 3). The amount of $^{99m}TcO_4^-$ was calculated from the runs made in MEK as % migrated. In water 92.5±3.6 % migration was observed. 2.88% of this was due to pertechnetate, but the rest (89.6±3.6%) might be a soluble metabolite. The amount which remained at origin in water (7.47±4.74%) is intact ^{99m}Tc-diltiazem which is not water soluble.

Scintigraphic studies confirmed the biodistribution data. Liver was the major organ that was visualized. There was radioactivity accumulation in the cardiac blood pool, the kidneys and the urinary bladder, indicating excretion via kidneys. Figure 1 displays the whole-body scintigrams of a mouse injected with ^{99m}Tc-diltiazem. Similar results were obtained in rabbits.

DISCUSSION

Both the biodistribution (Table 2) and scintigraphic (Figure 1) studies with ^{99m}Tc-diltiazem indicated a very high accumulation in the liver with negligible uptake by other organs. The liver uptake increased up to 5 h post-injection and showed a decline at 24 h. In a similar fashion the kidney uptake reached a max. at 5 h. High levels of radioactivity in urine indicated excretion via kidneys. These findings are in agreement with the reported pharmacokinetics of unlabelled diltiazem and nifedipine (3,7). It is known that nifedipine undergoes almost complete hepatic oxidation to three pharmacologically inactive metabolites which are excreted in the urine. Diltiazem is extensively metabolized in the liver with 60% of the dose being excreted in the bile and 35-40% in the urine with 2-4% as unchanged diltiazem. Our urine analysis gave a similar figure of 7.47±4.74% of unchanged diltiazem and 89.6±3.6% of a soluble metabolite (Table 3). After labeling with radio nuclides some differences in the biological behavior are expected due to electrostatic and structural changes introduced into the molecules. The high liver and spleen uptakes also indicated that a colloidal suspension might have formed in the blood of animals after administration of the complexes (Table 2). Both diltiazem and nifedipine are slightly soluble in water. Because of this the labeling with ^{99m}Tc was carried out in ethanolic solutions to facilitate dissolution. However, after injection into the blood stream small colloidal particles of labeled compounds might have formed and cleared by the liver, spleen and macrophages in the lungs. This may account for some but not all of the liver uptake, because liver is in fact the target organ for metabolism of these compounds (7).

Figure 1: Scintigram of a mouse obtained at 1 hour post-injection of 3.7 MBq of ^{99m}Tc-diltiazem. Note accumulation of radio activity in the cardiac blood pool (H), the liver (L), the kidneys (K) and the urinary bladder (B).



The uptake by heart is similar to blood radioactivity levels, which indicates that it is due to cardiac blood pool rather than active uptake by the myocardium (Table 2). The uptake values (<1%) obtained in this study are lower than those reported for well established radio pharmaceuticals such as ^{201}TlCl (5%) and ^{99m}Tc-MIBI (3-4%) (1). A higher uptake by the myocardium is desired but not attained so far by any of the proposed ^{99m}Tc complexes. The imaging properties of ^{201}Tl, a potassium analogue, are not ideal, because of lower energy gamma emissions of 65-83 keV and long physical half-life of 73.1 hr. In addition, the high cost of ^{201}Tl, a cyclotron product, should be considered. ^{99m}Tc complexes are more attractive due to ideal physical characteristics of the radio nuclide ($T_{1/2}$=6 h, E$\gamma\pm$140 keV) and easy availability in all nuclear medicine departments. MIBI kit is commercially available, but it is too expensive. The search for new ^{99m}Tc complexes for myocardial imaging will continue until ideal agents are obtained. In this study two new compounds were labeled with ^{99m}Tc and evaluated in experimental animals for the purpose of imaging myocardium, however, the myocardial uptakes were not sufficient for scintigraphic visualization.

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