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Effect of Verapamil on Kidney Function Using Radionuclide Imaging

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Keywords
Verapamil · Calcium channel blockers · Technetium-99m mercaptoacetyltriglycine · Renogram

Abstract
Background and Objective: Calcium channel blockers (CCBs) are among the most widely used prescribed drugs for the treatment of cardiovascular diseases. The present study investigates the effect of verapamil, which is most commonly used as a CCB, on kidney function using radionuclide imaging. ***Methods:*** Ten New Zealand white rabbits were used in vitro (4) and in vivo (6) studies. Isometric tensions were recorded for isolated renal artery ring segments, while renographic studies were performed using Technetium-99m mercaptoacetyltriglycine and Gamma camera. Time to peak activity (T_{max}) and time from peak to 50% activity ($T_{1/2}$), were calculated from the renograms for control and treated rabbits with verapamil. ***Results:*** In vitro, verapamil shifted the curve of phenylephrine concentration-dependent contraction on renal artery to the right, and decrease the highest contraction by 30 ± 3%. In vivo, the average values of T_{max} for control and treated rabbits were 2.8 ± 0.1 and 2.2 ± 0.2 min respectively. The $T_{1/2}$ for control and treated rabbits were 4.7 ± 0.05 and 4.2 ± 0.08 min respectively. The differences were statistically significant: $p < 0.05$. There is 30 ± 4% decrease in the 2 values. This indicates that there is a rapid renal uptake of the tracer and clearance of the radioactivity after verapamil. ***Conclusion:*** Verapamil dilates the renal artery and accelerates both the T_{max} and $T_{1/2}$ in the renogram. It increases renal blood perfusion and protects kidney function and therefore improves its work. However, verapamil should not be used while performing renograms to avoid misleading results.

Introduction

Calcium channel blockers (CCBs) are widely used in clinical practice. These drugs have been prescribed with increasing frequency because of their valuable therapeutic effects. They are used for the treatment of cardiovascular diseases such as hypertension, angina pectoris, cardiac arrhythmias, and other disorders [1–3]. All CCBs inhibit the L-type calcium channels, preventing the entry of calcium, which has a vital role in the cell. Verapamil is one of the most commonly used drug in this group [4–7]. CCBs exert also important vascular and tubular effects on the kidney [8–10]. Experimental studies in animals and humans showed the potential therapeutic uses of CCBs in decreasing the course of acute renal failure and reducing the progression of chronic renal failure. They also assist in preserving renal function in renal transplantation.

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A Nuclear Medicine Renogram is performed using a specific radioactive substance; Technetium-99m (^{99m}Tc), which when injected into the blood circulation shows the kidney blood supply and filtering action of the kidneys. The radioactive substance is usually bound to other non-radioactive materials. These combined stuff is called "radionuclides", Technetium-99m mercaptoacetyltriglycine (^{99m}Tc-MAG3). The radionuclides emit energy called "photons". Once a radionuclide is distributed in the kidney, the photon energy is gathered by a "Gamma Camera". The Gamma Camera detects the pattern of distribution of the radionuclide in the urinary system and sends this information to a computer. The computer handles the information and displays the collected information in the form of a picture called renogram.

Different suitable radionuclide investigations have been used as tools to investigate physiological, pathological, and pharmacological studies for different body organs such as brain, lung, and kidney [11–21]. Renogram determines the renal function by measuring radiotracer uptake and excretion by the kidney. The images of renogram include initial cortical uptake of the radiotracer, cortical retention, first visualization of collecting system, and time to peak cortical activity and half clearance [22].

The present study investigates the effect of CCBs, verapamil, on the renal artery and on the function of the kidney using the renographic imaging technique.

Materials and Methods

Experimental Animals

Ten adult, male New Zealand white rabbits weighing 3.5–4 kg and of the same age (10 weeks) were used, 4 rabbits for in vitro experiments and 6 rabbits for in vivo imaging. The animals were handled according to the guidelines of the Institutional Animal Care and Use Committee of Kuwait University.

In vitro Experiments

Four rabbits were anesthetized with sodium pentobarbital (50-mg/kg, i.v) via the marginal ear vein. Then they were exsanguinated by cutting the carotid artery. The abdominal cavity was opened and the renal artery was isolated and placed in Krebs' solution with the following composition (in mM): NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.2, KH_2PO_4 1.2, $NaHCO_3$ 26 and glucose 11.1, at pH 7.4. The renal arteries were cut into ring segments 4 mm in length, which were mounted on triangular wire supports and attached in 10-mL organ baths containing Krebs' solution, maintained at 37 °C and gassed continuously with 95% O_2 and 5% CO_2 mixture. Care was taken not to injure the endothelium throughout the preparation. Isometric tension of the renal artery was recorded using UF1 dynamometer transducers connected to the Multi Trace2 Lectromid recorder (Lectromid Limited, UK). The tissues were initially loaded to a tension of 1 g, which had been formerly determined as an optimal load, and allowed to equilibrate for 60 min, during which time they were washed twice. Cumulative concentration curves for phenylephrine were established and then repeated after incubating the segments with verapamil (10 μmol/L) for 30 min. The responses were calculated as % to the maximum response.

In vivo Experiments

Six adult male New-Zealand white rabbits were used for this study. All the animals were given sufficient water and food in our animal house. Butterfly needles were connected to the marginal veins in their ears. Each rabbit was anaesthetized with ketamine (40 mg/kg, i.v) given via the marginal ear vein. Furthermore, 60 mL of normal saline was given intravenously, 30 min prior to the administration of the radiopharmaceutical to confirm adequate and consistent hydration. Each rabbit served as its control and rabbits administered verapamil were referred to as treated animals.

Radionuclide Imaging

Baseline imaging studies were performed in each rabbit following injection of 48 MBq (1.3 mCi) of ^{99m}Tc-MAG3. Two days later, the same rabbit was given a single i.v dose of verapamil 2 mg and the ^{99m}Tc-MAG3 renogram repeated 20 min later. Dynamic images were acquired using Gamma camera (Meridian System, T55B-1473) equipped with a low-energy, high-resolution, parallel hole collimator interfaced with a dedicated computer. Rabbits were positioned after anesthesia in the supine position. The dynamic images were acquired in the posterior projection for 2 s frames for the first 1 min (flow phase) and every 30 s for the next 30 min (sequential functional phase) using a matrix of 64 x 64. Regions of interest were drawn over the whole kidneys manually. Radioactivity-time curves (renograms) were automatically generated and later corrected for background radioactivity for both kidneys. Curves were drawn using the Xeloris workstation (GE Medical system, version 1.06). The time to peak activity (T_{max}), time from peak to 50% activity ($T_{1/2}$) and the uptake slope of each kidney were automatically calculated from the renograms.

Drugs

Verapamil hydrochloride and phenylephrine hydrochloride were obtained from Sigma Chemicals (St Louis, MO, USA). Verapamil hydrochloride injection (Isoptin 2.5 mg/mL ampule) from Abbott Laboratories, Ireland.

Verapamil hydrochloride was dissolved in ethanol for in vitro experiments.

Statistical Analysis

Values are presented as mean ± (SEM) of number of rabbits (n) used in the studies. Differences between mean values were compared using Student t test paired. The difference was considered significant where $p < 0.05$.

Results

In vitro Experiments

To study the vasodilator effect of verapamil, the tone of renal artery should be elevated by a vasoconstrictor substance as phenylephrine, an α-adrenoceptor agent.

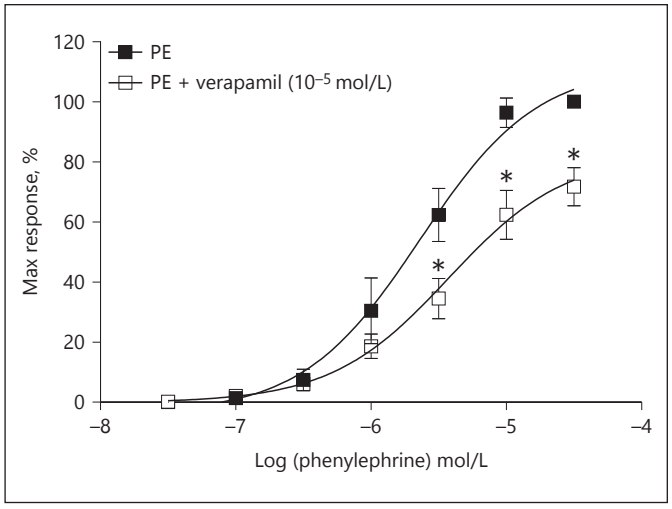

Fig. 1. Cumulative concentration-response curves of phenylephrine, PE, (0.05–50 μmol/L) on renal artery ring segments, (■) before; and (□) with verapamil (10 μmol/L). Each point on the graph represents mean ± SE of 4 rabbits, * $p < 0.05$.

Table 1. Calculated mean values of time to T_{max} and $T_{1/2}$ for the control and rabbits treated with verapamil

	^{99m}Tc-MAG3	
	T_{max}	$T_{1/2}$
Control	2.8±0.1	4.7±0.05
Verapamil	2.2±0.2*	4.2±0.08*

* $p < 0.05$ for the comparison between control and after verapamil.

T_{max}, time to peak activity (min); $T_{1/2}$, time from peak to 50% activity (min); 99mTc-MAG3, technetium-99m mercaptoacetyltriglycine.

Cumulative concentration-response curves to phenylephrine were established. Phenylephrine (0.05–50 μmol/L) produced concentration-dependent contractions of renal artery segments. The response to each concentration was allowed to reach a plateau before adding the next concentration to the bath. This was continued until the maximum response was obtained. After obtaining control responses, the tissues were washed, allowed to return to the baseline, and left to equilibrate for 60 min. Then the tissues were incubated for 30 min in the presence of CCB, verapamil 10 μmol/L. Concentration-response curves of phenylephrine were repeated. The responses were calculated as percentage to the maximum contraction response. Verapamil shifted the curve to the right and the maximal response decreased by 30 ± 3% as shown in Figure 1.

Radionuclide Imaging

In the present study, the data collected by the gamma camera were analyzed by a computer and plotted on time graphs, renograms. Counts (how much ^{99m}Tc-MAG3 is in the kidneys) is shown on the Y axis, and time from injection is shown on the X axis. The response of each kidney is plotted separately.

Both kidneys take up the radiotracer rapidly so the curves are steeply rising between 1 and 3 min. The concentration of ^{99m}Tc-MAG3 peaks at 3–5 min and then starts to fall. The radiotracer is almost excreted and drained from the kidneys within 30 min. Renograms were normal, with time to peak of T_{max} for control and treated rabbits were 2.8 ± 0.1 and 2.2 ± 0.2 min respectively. The $T_{1/2}$ for control and treated rabbits were 4.7 ± 0.05 and 4.2 ± 0.08 min respectively. The differences were statistically significant; $p < 0.05$. After verapamil administration, the overall function increased with acceleration in cortical clearance, and the renograms were shifted to the left. There were decrease in T_{max} and $T_{1/2}$ in all treated rabbits than control and there is 30 ± 4% decrease in the 2 values as shown in Table 1.

The renograms using ^{99m}Tc-MAG3 before and after verapamil administration are shown in Figure 2a and b. After verapamil treatment, the curves shifted to the left of the control curves indicating that there was slightly increase in renal uptake of ^{99m}Tc-MAG3 and acceleration in the clearance of radioactivity. The retention of radiotracer ^{99m}Tc-MAG3 in left kidney and right kidney after 2–3 min was greater after verapamil treatment. The radiotracer did not appear in the urinary bladder within 2–3 min, while it was clearly shown after verapamil treatment, indicating fast clearance, as represented in Figure 3a and b. The sequential functional images of the same rabbit before and after verapamil administration are shown in Figure 4a and b. Evidently, acceleration in the appearance of the bladder and in the clearance of renal activity can be seen. This indicates that the blood flow to the kidney is increased by verapamil administration. Both right and left kidneys have the same results in all renograms.

Discussion

The results of this study showed that verapamil promoted renal artery vasodilatation. Therefore, induced afferent arteriolar vasodilatation, which opposes vaso-

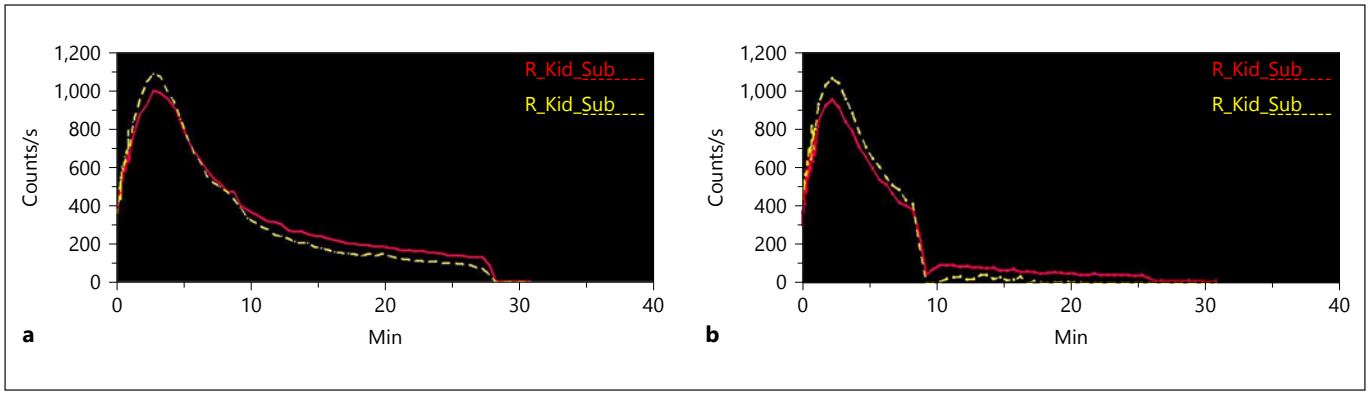

Fig. 2. Time activities curves (renograms) for (**a**) control rabbit; and (**b**) after verapamil treatment using ^{99m}Tc-MAG3. Note the acceleration for the peak and the clearance of activity from kidneys after verapamil.

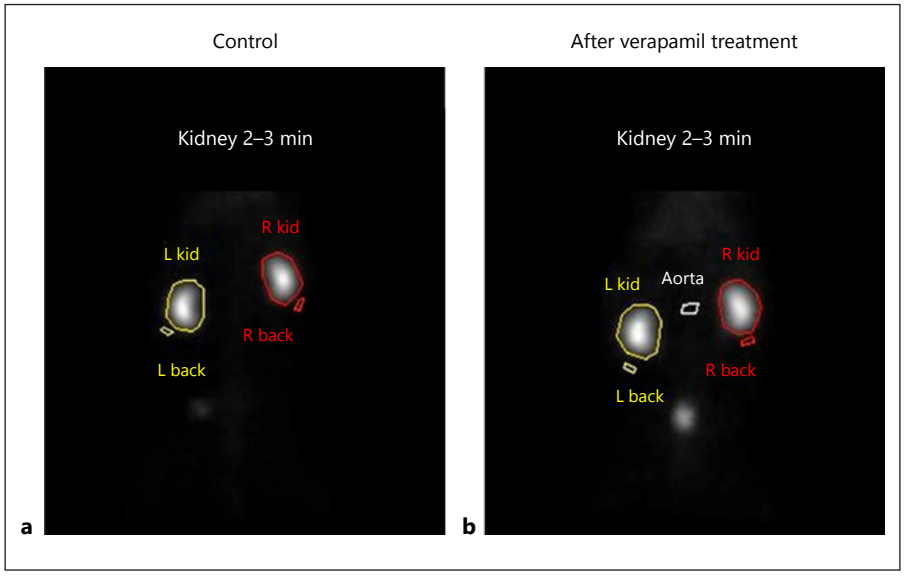

Fig. 3. The retention of radiotracer ^{99m}Tc-MAG3 in L Kid and R Kid after 2–3 min (**a**) control rabbit; and (**b**) after verapamil treatment. The urinary bladder did not showed radioactivity in control while there is retention of radioactivity in the bladder after verapamil treatment indicating the start of clearance. L kid, left kidney; R kid, right kidney.

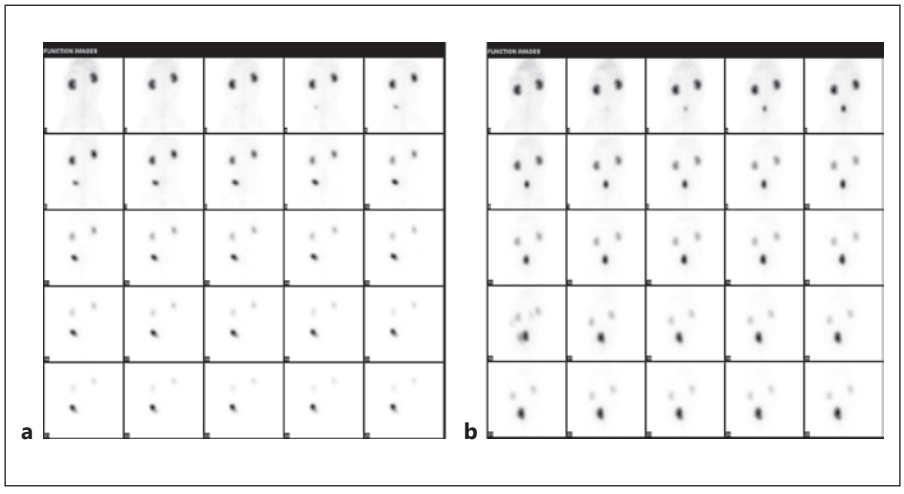

Fig. 4. Sequential functional images obtained (the dynamic images acquired for every 30 s frames for the first 30 min after administration of ^{99m}Tc-MAG3 show more retention of radioactivity in verapamil-treated rabbit (**b**) than in control rabbit (**a**), reflecting the more clearance by the kidney.

constriction due to endogenous vasoconstrictor agents, increases vascular permeability. This will lead to an increase in the glomerular filtration rate (GFR) resulting in increased urine output. It is also known that the main effects of CBBs, as used therapeutically are on cardiac and vascular smooth muscle. They cause generalized arterial dilatation, thereby reducing blood pressure.

Their effectiveness for the treatment of essential hypertension with normal maintenance of renal function has been proven [23, 24]. In this respect, there are some reports indicating that calcium blockers are particularly effective in a subgroup of patients with essential hypertension who exhibit subtle but detectable alterations in calcium metabolism [25]. CBBs have other effects in normal animals and humans such as diuretic and natriuretic properties and inhibition of aldosterone secretion [26].

The renal artery enters the kidney and branches; one of the branches is the afferent arteriole. The afferent arteriole branches into the glomerular capillaries and the distal glomerular capillaries merge to form the efferent arteriole. Efferent arterioles subdivide to form peritubular capillaries in the cortex. The tone of the afferent and efferent glomerular arterioles can modulate glomerular capillary hydrostatic pressure. Increased tone in the afferent arteriole or decreased tone in the efferent arteriole increases the capillary hydrostatic pressure and GFR. The high hydrostatic pressure in the glomerular capillaries causes a rapid fluid filtration in the glomeruli.

Angiotensin II increases the afferent arteriolar and glomerular capillary hydrostatic pressure [27]. Angiotensin II type 1 receptors are localized in both afferent and efferent arterioles [28]. The increase in the tone of efferent arteriole decreases the hydrostatic pressure in peritubular capillaries and increases sodium and water reabsorption [29]. Renin-angiotensin system antagonists play an important role in blood pressure and renal function. The present study showed that verapamil dilates the afferent and efferent arterioles, since they need calcium to contract; therefore, it increases hydrostatic pressure in peritubular capillaries. The high hydrostatic pressure is mainly due to the difference between the afferent and efferent arterioles diameters.

Radionuclide renography plays a crucial role in assessing perfusion and kidney function. It is used for evaluating many kidney diseases and transplanted kidneys [30–34]. The cortical function is the interval between administration of the radiotracer and its excretion to the collecting system. Delayed appearance of the collecting system is related to the renal insufficiency. The interval between radiotracer administration and maximum cortical activity is another parameter of function. It can be measured from the renogram (time-activity curve). The renogram consists of 3 phases; the first phase is the perfusion phase and renal peak activity. The second phase is the cortical or tubular concentration phase of initial parenchymal transit. This phase occurs during 1–5 min and contains the peak of the curve. The initial uptake slope closely relates with the effective renal plasma flow values. The third phase is the excretion or clearance phase that represents the down slope of the curve and is produced by excretion of the radiotracer from the kidney and clearance from the collecting system. A renogram is performed to evaluate the blood supply, function, and excretion of urine from the kidneys.

The cortical retention of ^{99m}Tc-MAG3, measured by calculating renal counts on the renogram for 20–30 min as a percentage of the peak uptake, is a measure of the quickness with which the radiotracer is excreted by the kidney. The present renogram showed almost complete excretion within 30 min for ^{99m}Tc-MAG3. Generally, as renal function improved, the percentage retained decreases.

Conclusions

Verapamil dilates the renal artery and accelerates T_{max} and $T_{1/2}$ in the renogram. It increases renal blood perfusion and GFR, protects kidney function, and improves its work. Based on the findings, it is suggested not to use CCBs when the patients have to perform renogram to avoid any misleading results of their kidney condition.

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Disclosure Statement

There are no possible conflicts of interest for all authors.

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