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EQUIVALENCE OF THE BLOCKAGE OF URETER AND THE ACTION OF THE URETHANE IN ^{99m}Tc-DMSA BIODISTRIBUTION IN RATS

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ABSTRACT

The indication of the United States Pharmacopoeia (USP) for biological control of ^{99m}Tc-DMSA is the experiment in rats with ureter occlusion. Urethane has a vessel constriction action in the urinary system and keeps the eliminatory mechanism functioning through glomerular filtration. The objective of this work is to show that the use of urethane in animals without blockage of ureteres has total credibility, even if the expressed value of the renal retention does not correspond to 40% injected dose (I.D.) related in the literature. The experiments were performed in 2 groups of 12 rats each, the first using urethane and the second, urethane and blockage of ureter. Four lots of DMSA were labeled with 10 mCi/3 mL of ^{99m}Tc solution, and 300 μCi/0.1 mL was injected intravenously in each animal. After one hour, they were sacrificed and kidneys, bladder, liver, spleen and carcass were taken out for determination of the retained radiation in function of the injected dose. The USP establishes two parameters for the metabolism of ^{99m}Tc-DMSA: renal retention equal or higher than 40% and kidneys/liver plus spleen relation equal or higher than 6. In animals whose ureteres were obstructed, it was clearly observed that the urine was not transferred from kidneys to bladder 0.05 ± 0.35% I.D., while the first group presented 0.50 ± 6.50 % I.D. The kidneys/liver plus spleen relation were above 6 for both. Considering the deviation, all results were in the USP limit of acceptability, and for routine evaluation, urethane can be used without surgical intervention.

1. INTRODUCTION

The technetium-99m-labelled dimercaptosuccinic acid (^{99m}Tc-DMSA) is a largely used radiopharmaceutical for renal studies in Nuclear Medicine for evaluation of the anatomy of the renal cortex and detection of space-occupying lesions such as tumors, cysts, infarcts, hematomas and abcesses [1]. Its uptake correlates with intra-renal blood flow distribution, proximal tubule membrane transport, glomerular filtration rate and creatinine clearance. Its quantitative measurement is therefore a good index for renal function, providing an estimate of the percentage of total function contributed by each kidney [2,3].

For biological control of ^{99m}Tc-DMSA, the United States Pharmacopoeia (USP) recommends the ureter occlusion of rats and the submission of the animals to the effect of an anesthetic and surgical intervention [4].

The urethane anesthetic has a vessel constriction action and keeps the eliminatory mechanism through glomerular filtration and tubular secretion [5].

The objective of this work is to show that the use of urethane in animals without blockage of ureter has credibility, even if the expressed value of the renal retention does not correspond to 40% injected dose (I.D.) related in the literature.

2. EXPERIMENTAL

2.1. Radiolabelling of DMSA, radiochemical purity and intravenous administration

Four different lots of DMSA were used and one vial of each was labeled by addition of 3 mL of a $Na^{99m}Tc$ solution, obtained from a $^{99}Mo/^{99m}Tc$ generator system (IPEN-CNEN/SP, Brazil) containing a radioactive activity of 370 Megabecquerel (MBq) (10 milliCurie). After 15 minutes, the reaction was completed at room temperature and the product was injected in three animals for each lot [4].

Radiochemical purity was determined by paper chromatography with Whatman 3MM paper strip (1 x 8 cm) and thin layer chromatography with silica gel impregnated aluminum strip (TLC-SG Al) (1.5 x 12.5 cm); the solvent was acetone and NaCl 0.9% solution, respectively. The retention factor (Rf) value for $^{99m}TcO_4^-$ was 1.0 and 0.0 for $^{99m}TcO_2$. The Rf value for ^{99m}Tc-DMSA in acetone was 0.0 and in NaCl 0.9% solution was 1.0.

2.2. Biological distribution

Male Wistar rats (IPEN-CNEN/SP, Brazil), 200-250 g weight were used. The experiments were carried out in 2 groups of 3 animals each: rats anesthetized with urethane (Sigma-Aldrich) and rats anesthetized with urethane and blockage of ureter. A dose of approximately 11.1 MBq (300 μCi/0.1 mL) was injected in the caudal vein of each animal. About 1 h after the injection, the rats were sacrificed and samples of kidneys, bladder, liver, spleen and carcass were weighed (Micronal PB 303), the radioactivity was measured in a gamma counter (Capintec) and %I.D. was calculated [4].

2.2. Surgical technique

The occlusion of the ureter ducts was performed in each animal under the urethane anesthetic effect, scraping the fur from the back of the animal in the region of the kidneys, in a sufficient rectangular extension to handle it avoiding contamination. An approximately 4 cm longitudinal incision was made on the vertebral column in the area where the kidneys are localized. The membrane which separates them from other tissues was torn with round tip surgical shears in order to isolate the muscles which involved the cavity.

One of the kidneys was firmly held and a 2 cm lateral cut was made with a scalpel in the direction of the muscular fiber. The region was pressured to display the kidney together with the fatty tissue.

Fig. 1 shows the dorsal incision with one displayed kidney and ureter before the blockage.

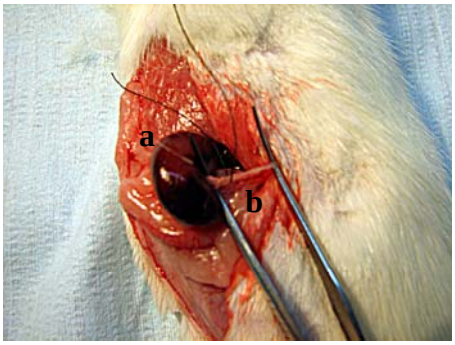

Figure 1. View of one kidney (a) and ureter (b) after dorsal incision.

The ureter (Figure 1, b) was isolated with a clamp and the fatty tissue was separated from the kidney (Figure 1, a). A synthetic wire was used to block two places in the ureter obstructing the urine flow. After the procedure, the clamp was removed, the kidney with the fatty tissue was put back into the place of origin and the muscles were reconstituted to the condition before the surgery. Much care was taken in order to avoid contamination by extravasation of the biological fluid with radioactive product in the peritoneum area.

3. RESULTS AND DISCUSSION

The labeling efficiency of ^{99m}Tc-DMSA was above 90% and the amount of $^{99m}TcO_2$ was lower than 5% in all experiments.

The USP [4] presents the limit of acceptability for release of ^{99m}Tc-DMSA for clinical use not less than 40% of the administered radioactive dose must be found in the kidneys and a ratio of not less than 6:1 of the administered dose in the relation kidneys/ (liver and spleen), in not fewer than two of the animals, when the test is performed in rats with blockage of the ureter ducts.

Table 1 presents the biological distribution in urethane anesthetized animals (Group 1) and urethane anesthetized animals with blockage of ureter (Group 2); both were sacrificed 60 minutes after the administration of ^{99m}Tc-DMSA.

Table 1. Effect of the blockage of ureteres in the biological distribution .

Organs	Group 1 (%I.D.)	Group 2 (%I.D.)
Kidneys	39.88 ± 5.94	50.36 ± 7.25
Bladder	3.36 ± 3.06	0.20 ± 0.15
Liver	3.59 ± 1.40	3.23 ± 1.80
Spleen	0.23 ± 0.18	0.24 ± 0.15
Carcass	41.59 ± 8.40	40.24 ± 8.72

Group 1 – without blockage of ureter

Group 2 – with blockage of ureter

In this study, the %I.D. obtained values in four lots of the labeled product showed lower results than the specification in urethane anesthetized rats without blockage of ureter (Table 1).

In Group 2, it was observed the impediment of the transference of urine from the kidneys to the bladder demonstrated by the accumulated activity of 0.20 ± 0.15% I.D., while in Group 1 was 3.36 ± 3.06% I.D.. The renal retention in the first group was 39.88 ± 5.94 % I.D. and those submitted to the surgical intervention showed higher retention 50.36 ± 7.51 % I.D..

The radioactivity in the carcass was high in all experiments and as ^{99m}Tc-DMSA presented slow blood clearance, 60 minutes was not sufficient to eliminate the product. The presence of maltose in the DMSA formulation might have given a better stability to the product but also contributed to the slow decline of the radioactivity.

The accumulated concentration of ^{99m}Tc-DMSA in the renal structure of the rat, free from the effect of radiation emission of the surrounding structures must be high enough to provide an accurate analysis of the morphology in patients within the USP acceptable limits.

The kidneys, the liver and the spleen are in the abdominal cavity, so the diagnostic image can be influenced by the overlapping of these organs. The ratio of the renal retention and liver plus spleen determines the viability of the product for application in Nuclear Medicine, and the result must be more than 6:1 with 40% I.D. renal retention.

Table 2 shows the results from the relation % I.D. kidneys and the sum of % I.D. liver and % I.D. spleen.

Table 2. % Kidneys / (% Liver + % Spleen) Ratio

	Group 1	Group 2
% Kidneys / (% liver + % spleen)	10,44	14,51

The standard limits were obtained from experiments in rats in anomalous conditions (obstructed ureteres) and express higher values (Table 2 – Group 2) when compared to those with partial restriction of the excretory function due to the action of the anesthetic (Table 2 – Group 1). Therefore the obtained results for both groups were within the acceptable limits.

4. CONCLUSIONS

This study demonstrated that the biological distribution of ^{99m}Tc -DMSA does not need blockage of ureter with urethane anesthetic, though this procedure decreases the renal retention.

The limit of acceptability for biological distribution could be different from the official compendiums without interference in the quality of the product. The suggested limits for ^{99m}Tc-DMSA in rodents are: not less than 33% I.D. renal retention, not more than 5 % I.D. liver retention and % I.D. kidneys/ (% I.D. liver + % I.D. spleen) ratio $\geq$ 6. It was also observed that the presence of radiochemical impurities influenced the biological distribution of the product (data not shown), suggesting that the radiochemical purity should be equal or superior to 90%.

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