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URETHANE INFLUENCE IN THE URINE FORMATION IN SWISS RATS AND SYRIAN HAMSTER

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ABSTRACT

Urethane is an anaesthetic agent with minimal cardiovascular and respiratory system depression with long-lasting (6-10h) effects. Its carcinogenic potential avoids it from veterinary use. Either, the knowledge of its effects over the circulating catecholamines (cortisone and corticosterone), with reflects in the muscles physiology, it is widely used in pharmacological studies in laboratory species. At the first minutes, Urethane induces a hyperglycaemia condition due the insulin concentration decrease, later than, the insulin concentration and the condition becomes in hypoglycaemia, but the Urethane interfering in the urine production mechanisms has not been described. It is accepted that the glycolic level would not interferes in the kidney function, except in chronic states, notably associated with insulin related diseases. The relative high biological half-life of ^{177}Lu-Dotatate allows its use in biodistribution studies among small animals whose metabolic rates are so fast that would be impossible observe them with the most part of the labeled molecules. During the performance of a cross-species extrapolation study using Urethane as anaesthesia and ^{177}Lu-Dotate as metabolic tracer, was observed the Urethane influence over urine formation in Swiss rats and Syrian hamster (Mesocricetus auratus). The objective of this work is only describes the Urethane action over the urine production. Firstly, four male inbread Wistar Swiss rats (±250g), are anaesthetized, with around 1200mg/kg, i.p., in groups of two. One rat from each group get ahead to the injection of ^{177}Lu-Dotatate and Gamma camera *in vivo* study, the second ones, anaesthetized, waited under warming lights until more than one hour to initiate the biodistribution study. The scintillographical images shown the radiopeptide stopped at the kidneys and the urinary empty in the animals who attempt more than one hour before enter to radiopharmaceutical injection and Gamma camera imaging procedures. The rates of transfer from the tail to the whole body were also significantly different in the two rat groups. A new set of experiments performed with five male inter outbread, Syrian hamsters (±50g), anaesthetized one to one, each time, shown hamster sensibility in relation of Urethane concentration. The minimal dose that induced anaesthetic state in hamsters and followed the complete biodistribution study was 1000mg/kg, i. p.. Whereas, higher recommended dose (1200 - 2000mg/kg, i. v.) injection, caused the same blockage of urine production effect observed in longer post anaesthesia time in the rats. Considering the CNS (Central Nervous System) influence over the blood pressure and kidney function, these results were a sign that urine production rate could be related with the Urethane dose and the time after its injection.

1. INTRODUCTION

All anaesthetic agents have undesirable side-effects upon physiological responses. However, to capture *in vivo* biodistribution scintillographical images it is absolutely necessary that the animal remains immobile. Therefore, the knowledge of potential interference of the

anaesthetic in the physiological variables is important to assessing the relevance of data obtained under that condition [2-6].
Urethane has been currently used in pharmacology research mainly for the reason that its ability to induce surgical level of anaesthesia without affecting neurotransmission in various subcortical areas and peripheral nervous system [1, 2, 6].

The information about the Urethane anaesthesia effects on the renal function is limited. In 1986, Maggi and Meli [2-4] published a series of three articles about the suitability of its use in pharmacological research, which is until now the only review about the subject. Many studies about Urethane effects on the endocrine systems have been performed by using a) larger doses than the usual to obtain the surgical level of anaesthesia, b) more than a pharmacological agent, c) vivisection or isolated organs in artificial conditions and d) rats species.

Urethane used to be compared with other anaesthetics on basis of the physiological undesirable disturbing side-effects degree. In the same way, as all drugs, the dose-related effects should be considered [2, 5, 6].

The lessons took from Maggi and Meli [2-4] should be applied in the ^{177}Lu-Dotatate biodistribution studies among small animals to set a map and a procedure to analyze the Urethane effects over the results.

The relative high biological half-life of ^{177}Lu-Dotatate allows its use in biodistribution studies among small animals whose metabolic rates are so fast that would be impossible observe them with the most part of the labeled molecules. By the imaging, it is possible “to see” the urine production process: the liquid volume increasing in the kidneys and urinary bladder and still the organ region, lumen or cortex where the process developed.

This paper describes the gamma camera images observation of urine production processes using a radioactive tracer. The quality of the images permits to trail the ^{177}Lu pathways from the injection site to the urinary excretion. The ROI analysis follows the conversion of images in numerical results.

2. SUITABILITY OF URETHANE ANAESTESIA FOR PHARMACOLOGICAL STUDIES AND PHARMACOKINETICS

Urethane is an anaesthetic agent with minimal cardiovascular and respiratory system depression with long-lasting (6-10h) effects. Its carcinogenic potential avoids it from veterinary use. Either, the knowledge of its effects over the circulating catecholamines (cortisone and corticosterone), with reflects in the muscles physiology, it is widely used in pharmacological studies in laboratory species [6].

At the first minutes, Urethane induces a hyperglycaemia condition due the insulin concentration decrease, later than, the insulin concentration and the condition becomes in hypoglycaemia, but the Urethane interfering in the urine production mechanisms has not been completely described [6].

Urethane concentration is observed in similar levels in blood and organs and it is largely and slowly metabolized by the liver and excreted by the urine as ethanol and carbamic acid [3].

Antunes-Rodrigues and collaborators assume all anaesthetic, acting in the CNS leads to systemic consequences, because **CNS** structures involve integrative responses. Sympathetic nervous system and humoral factors control renal functions as renal blood flow and urinary sodium production, glomerular filtration rate, solute and water transport, and hormonal production and secretion [1].

Maggi and Meli do not believe Urethane doses higher than 1200mg/g active the CNS sympathetic structures leading to increased cathecolamine secretion from the adrenal medulla and hyperglycemia. They suppose the effect is due the lesion and consequent blood outflow caused by the intraperitoneal (i. p.) injection. The damage of the mesenteric vasculature is the responsible by the substantial leakage of plasma to peritoneal cavity that reduces the glomerular filtration rate. So the changes in the renal function would be independent of the Urethane per se since in intravenous injection there is not fluid leakage but there is a rapid osmotic diuresis [3].

Bibby and Grimble did not make considerations about the i. p. injection and the damage of peritoneal region [5].

Urethane effect on micturition and smooth muscle of lower urinary tract has been described for *in vivo* studies with cats, dogs, rats, guinea pigs, hamsters and mice using activation or inactivation of the sensory pathways by drugs or nerves section. These studies with subcutaneous (1200mg/kg, s. c.) administration showed that firstly there is a decreasing of the rat urinary bladder rhythmic contractions with inhibitory of the colo-vesical reflex and lastly, a suppression of the rat urinary bladder rhythmic contractions with inhibitory of the somato-vesical reflex [3].

Despite all the interferences in many biological systems, Maggi and Meli [4] concluded that Urethane is suitable for studies at cardiovascular, respiratory, gastrointestinal and urinary system level.

2.1. Urethane Anaesthetized Rats and Hamsters *In Vivo* Studies

Four male inbread Wistar Swiss rats (±250g), are anaesthetized, with around 1200mg/kg, i.p., in groups of two. One rat from each group get ahead to the injection of ^{177}Lu-Dotatate and Gamma camera *in vivo* study, the second ones, anaesthetized, waited under warming lights until more than one hour to go ahead to the biodistribution study.

Scintilographical images were taken in the static mode each 3min to trace the ^{177}Lu-Dotatate biodistribution until the micturition or unchangeable images in the kidney and urinary bladder uptakes (approximately 1-2 hours).

To observe the hamster sensibility in relation of Urethane concentration, five male inter outbread, Syrian hamsters (*Mesocricetus auratus*), (±50g), anaesthetized one to one. The first one, anaesthetized with 1200mg/kg, i. p., dead before the radiopeptide injection and the

following four ones received 1000mg/kg, i. p., minimal dose that induced adequate anaesthetic state to perform one biodistribution study for 1-2 hours as described before. After this time period, the animals started to move.
The anaesthetic agent volume administrated i.p by each animal, rat and hamster, was 100µl and the ^{177}Lu-Dotatate volume administrated i.v., other 100µl.

All collected images were analyzed using the ROI tolls of the Gamma camera as shown in the Figure 1. Kidneys and urinary bladder uptakes results from these analyses allow composing the graphics presented in the Figures 4 and 5.

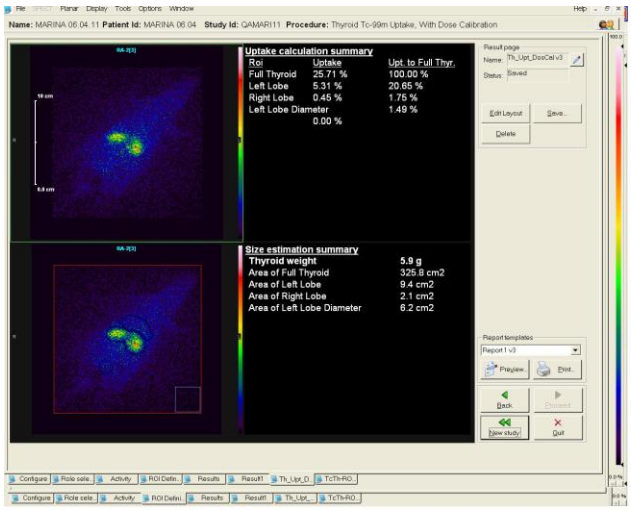

Figure 1. Gamma camera ROI tools to calculate the concentration of activity (Rat A). The equipment parameters fixed to ^{99m}Tc studies, would be interpreted as: full thyroid (whole body), left lobe (kidneys), right lobe (liver) and left lobe diameter (urinary bladder).

3. RESULTS

The scintilographical images did not demonstrate that Urethane i. p. injection damaged the mesenteric vasculature in an extent enough to promote a substantial leakage of plasma to peritoneal cavity and consequent blood outflow

The images show the radiopeptide stopped at the kidneys and the urinary empty in the rats which attempt more than one hour before receive radiopharmaceutical injection and to inicialize Gamma camera imaging procedure.

The Figures 2 and 3 are examples of full rat biodistribution studies with two diverse responses. Figure 2, from radiopeptide injection until micturition and Figure 3, from radiopeptide injection until urine production blockage.

The visual analyze indicates the contrasting response between two animals, genetically identical, while inbread, same age, same weight, under the same experimental environmental conditions. The graphics construction as presented in the Figure 4 confirms that difference.

In the hamsters case the interpretation of results is more complex, because they were outbread. By the other hand, when grouped for the tests carrying out, the responses were very similar.

Other aspect to considerer, all of studied hamsters presented natural micturition always they were manipulated. Thus, it was assumed, they had the urinary bladder empty. However, it seems the total volume of injected liquid was bigger than the species urinary bladder capability and the glomerular filtration very fast.

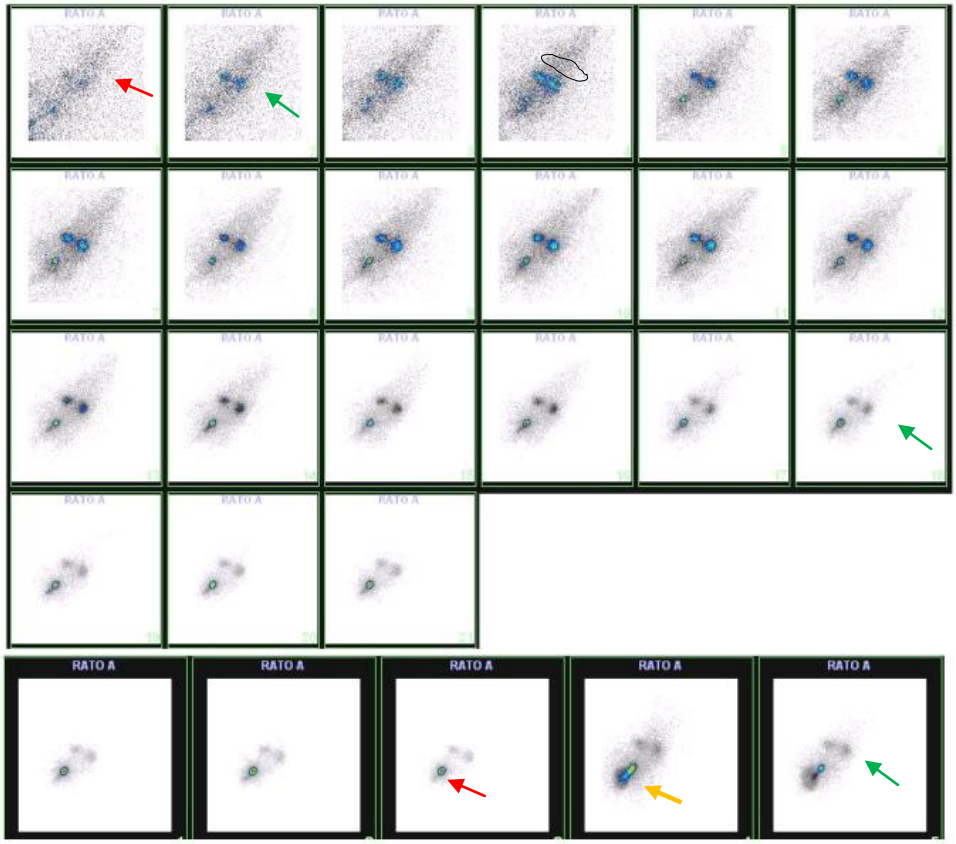

Figure 2. Wistar Swiss rat (Rat A) ^{177}Lu-Dotatate dynamic study showing the urine production in spite of Urethane anesthesia. It is possible to trace the mounting ^{177}Lu-Dotatate uptake by liver (black circle), kidneys (green arrow) and urinary bladder (red arrow) each 3 minutes. A thoughtful visual examination of the five last images shows the complete filled urinary bladder and the two final images captures the micturition moment and the urine spot (yellow arrow).

Comparative analyzes between graphics results presented in the Figures 3 and 4 demonstrated the rats and hamsters without urine production blockage presented renal uptake in compatible

amounts. The same consideration should be taken in relation with the velocity to full the urinary bladder in the two species.

The kidney uptake in rats and hamsters with urine production blockage presented diverse comportment. In rats, the amounts raising until more than 30% of total injected activity, meanwhile in hamsters, that concentration did not arrive to 9%.

The urinary bladder behavior in rats and hamsters with urine blockage were also diverse. In rats, it is observed that less than 1% of total injected activity is filtered to the urinary bladder, meanwhile in hamsters, that concentration arrived to 3%.

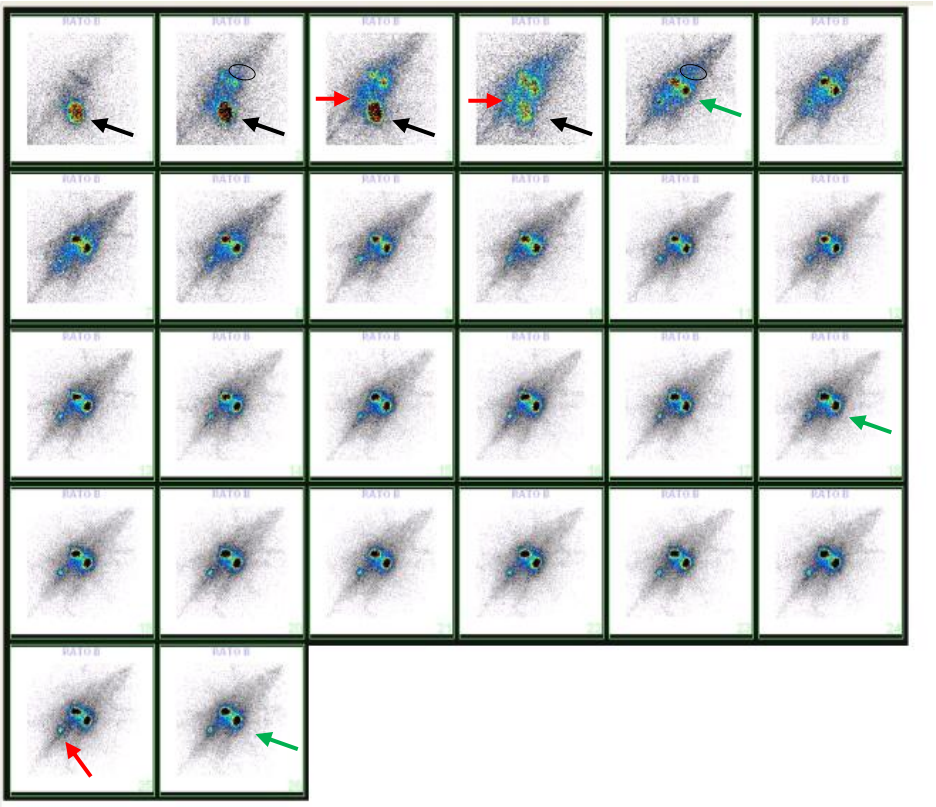

Figure 3. Wistar Swiss rat (Rat B) ^{177}Lu-Dotatate dynamic study showing the urine production blockage due Urethane anesthesia later effect. In the initial four images the black arrow indicates a spot due to animal urination. In spite of the urine stain, it is possible to trace the mounting ^{177}Lu-Dotatate uptake by liver (black circle), kidneys (green arrow) and urinary bladder (red arrow). A thoughtful visual examination of the twenty-two following images shows no considerate changes in the concentration of activity or volume rising in the urinary bladder.

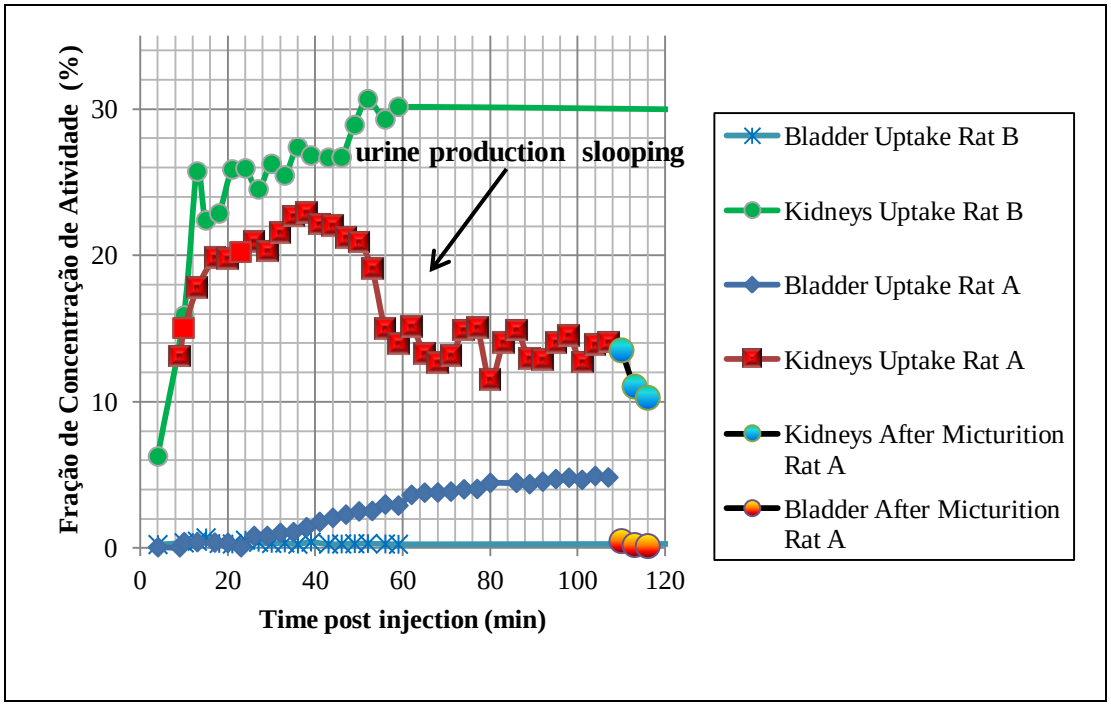

Figure 4. Wistar Swiss rat (Rats A and B) ^{177}Lu- Dotatate biodistribution studies data showing diverse renal response. Rat A: Micturition and rat B: Urine production inhibition.

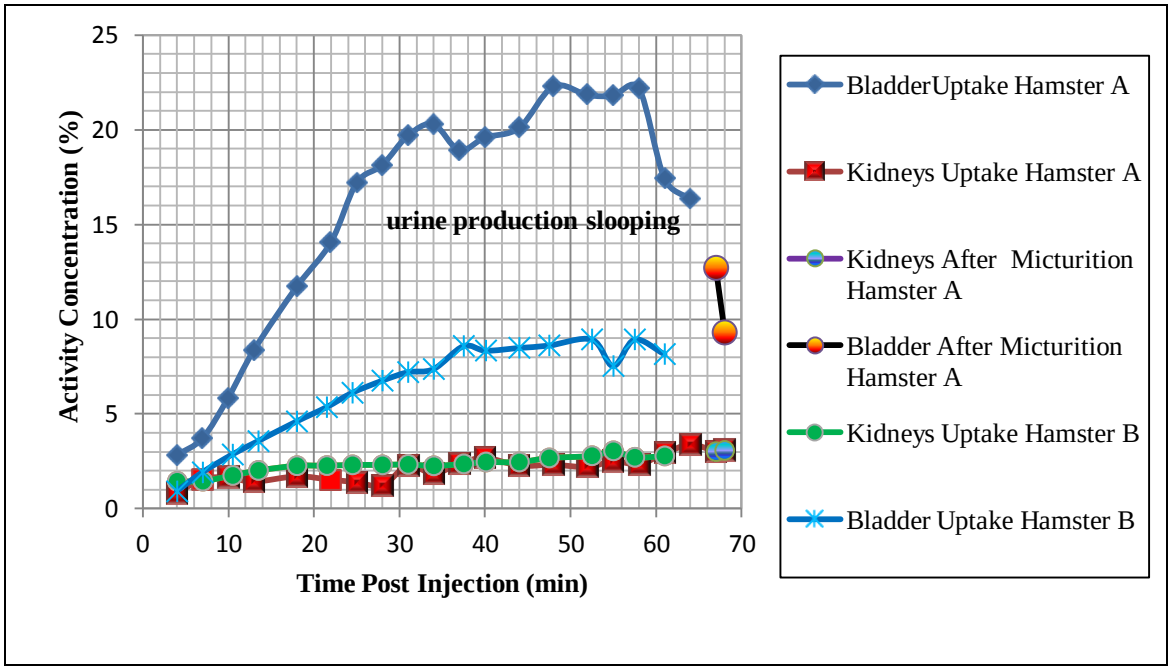

Figure 5. Siberian hamsters (Hamsters A and B) ^{177}Lu- Dotatate biodistribution studies data showing diverse renal response. Hamster A: Micturition and Hamster B: Small Urine production.

3. CONCLUSIONS

These results indicated that the i.p. anaesthetic injection do not cause massive damage in the mesenteric vasculature. If the process has been remarkable, it would be visible in the biodistribution images, since ^{177}Lu-Dotatate is a Somastatin simile with high affinity with the prostaglandins always found in damaged tissues. Actually, in the radiopeptide injection site, it is possible to observe a slight rising in the activity concentration, due the organism physiological reparation process, during *in vivo* studies during more than one hour.

Considering the CNS influence over the blood pressure and kidney function, these results were a sign that urine production rate could be related with the Urethane dose and the time after its injection in the studied species: Wistar Swiss rat and Syrian hamsters. Also considering that the injection of high liquid volume probably modified the blood pressure, consequently the glomerular filtration rate.

The knowledge of Urethane interference in the animal physiological response, until incomplete have to be mapped with very standardized procedures to allow comparative analyzes between different species and working methods (p. ex. *in vivo* or *ex vivo*, visual or mathematical determination). By the same way, the species characteristics as described in comparative physiology and allometry handbooks are vital to arrive to the best interpretation of results.

This work is part of cross species study in a development stage involving other laboratory animals. The Urethane effect over the urine formation will study in other small rodent as mouse, guinea pig and hamster sub species to corroborate these previous conclusions.

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