Journal of Venomous Animals and Toxins
Print version ISSN 0104-7930 *On-line version* ISSN 1678-4936

J. Venom. Anim. Toxins vol. 5 n. 1 Botucatu 1999

http://dx.doi.org/10.1590/S0104-79301999000100003

Tc 99m - SCORPION VENOM: LABELLING, BIODISTRIBUTION AND SCINTIIMAGING

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ABSTRACT: Labelling of scorpion (*Mesobuthus tamulus concanesis* Pocock) venom was successfully achieved with Tc 99m using direct tin reduction procedure. Biodistribution studies were carried out in Wistar rats at different time intervals after i.v. administration of the labelled venom.
Scintiimages were obtained after scorpion envenoming using a large field of view gamma camera to ascertain the pharmacological action of venom in the body. Within 5 min of administration, labelled venom was found in the blood (27.7%), muscle (30.11%), bone (13.3%), kidneys (11.5%), liver (10.4%), and other organs. The level of venom in the kidneys was higher than in the liver. The labelled venom was excreted through renal and hepatobiliary pathways. An immunoreactivity study was carried out in rabbits after i.v. injection of labelled scorpion venom followed by the injection of the species specific antivenom. A threefold increase in uptake by the kidneys was observed compared with that seen with scorpion venom alone. The neutralisation of the venom in the kidneys was higher than in the liver.

KEY WORDS: *Mesobuthus tamulus concanesis,* Tc 99m, scorpion venom.

INTRODUCTION

Scorpion envenoming results in an autonomic storm releasing massive amounts of catecholamines. The pathogenesis of cardiovascular, haemodynamic, electrocardiographic changes, metabolic and hormonal disturbances and pulmonary oedema in the envenoming by scorpion venom is multifactorial**(5,6,17-19,22-32)**. Scorpion envenoming is a syndrome of fuel-energy deficits and an inability to utilise the existing metabolic substrates by vital organs resulting in multi-system-organ-failure (MSOF) and death. Study of the effects of the scorpion (*Mesobuthus tamulus concanesis* Pocock) venom in experimental**(12,17-20,22-27,33)** and scorpion sting victims**(21)** has been reported. However, the *in vivo* study of scorpion venom labelled with Tc 99m has not been reported. Ismail *et al.* **(3,9)** have carried out the pharmacokinetic studies of labelled scorpion venom with I^{125} in rabbits showing an open, two compartment behaviour, namely distribution and elimination phases having half lives of 5.6 min and 6.4 h respectively. In the present study we have made an effort to label the scorpion venom with Tc 99m. This radionuclide possesses an ideal gamma ray energy (140 KeV), which is extremely suitable for mapping the distribution of scorpion venom *in vivo* using a gamma camera computer system.

MATERIALS AND METHODS

Crude lyophilized venom from the scorpion (*Mesobuthus tamulus concanesis* Pocock) was obtained from the Haffkine Institute, Mumbai, India. The species specific anti-scorpion venom serum (AScVS) was obtained from the Haffkine Biopharmaceutical Corporation Ltd., Mumbai, India. Tc 99m radionuclide was extracted from MO-9 (obtained from BRIT, Mumbai, India), using solvent extraction procedures.

Labelling of scorpion venom was carried out with Tc 99m by direct tin reduction procedures. The radiochemical purity and stability were ascertained by paper chromatography. Biodistribution studies were carried out in Wistar rats (n=6) after i.v. administration of labelled scorpion venom. A dose of 1.10 Mbq (0.20 ml) of Tc 99m scorpion venom was administered into the penile vein after ether anaesthesia. The animals were sacrificed at different time

intervals. Organs were dissected and counted on a scintillation detector. Percentage uptake per organ was calculated in comparison with a prepared standard activity.

Tc 99m-scorpion venom (100 mg in 0.5 ml) was injected into the ear vein, and scintiimages of the rabbits (n=6) were obtained at different intervals using the gamma camera. In this study, labelled scorpion venom (1.0 mg) was injected i.v. and the animal was kept under observation. Ten minutes after administration, hypersalivation and many other behavioural changes were observed. The antivenom (2.0 mg) was injected into the other ear vein. Scintiimages were obtained at different time intervals on the gamma camera.

RESULTS

The radiochemical purity of Tc 99m-scorpion venom was 97% and radiochemical stability was greater than 4 h. The results obtained from the study using Tc 99m-scorpion venom are shown in **Table 1** and **Figure 3**. The initial uptake in the blood after 5 min was 29.5% dropping to 6.19% 3 h post-injection. Liver uptake after 5 min was 10.4% dropping to 8.3% 3 h post-injection. The uptake of tagged venom by the kidneys was 11.5% at 5 min and reached a peak uptake of 31.9% at 30 min. The retention of radioactive tagged venom by the kidney was observed up to 24 h (11.7%) after injection. The concentration of venom in the stomach and thyroid was seen as insignificant. The peak uptake in the small intestine was 14.5% 3 h post-injection, and in the large intestine was 13.3% 24 h post-injection.

TABLE 1. Biodistribution of Tc 99m-scorpion venom in Wistar rats. The values represent the percentage of administered dose loacated in each organ at the indicated time (Mean ± S.D.).

ORGANS	5 min n = 6	30 min n = 6	1 h n = 6	3 h n = 6	24 h n = 6
Blood	29.7 ±1.3	11.46 ±1.33	6.19 ±0.3	4.49 ±0.37	1.64 ±0.57
Liver	10.4 ±0.6	12.66 ±0.29	11.52 ±0.3	8.3 ±0.1	4.59 ±0.5
Spleen	0.32 ±0.2	0.18 ±0.06	0.39 ±0.06	0.2 ±0.08	0.15 ±0.02
Kidneys	11.5 ±2.1	31.92 ±1.41	29.08 ±0.97	22.33 ±2.99	11.65 ±0.38
Stomach	0.87 ±0.02	0.86 ±0.28	0.88 ±0.04	1.18 ±0.46	1.07 ±0.84
Small Intestine	3.4 ±0.1	4.23 ±0.69	9.44 ±0.18	14.55 ±5.9	1.83 ±0.78
Large Intestine	1.3 ±0.02	0.7 ±0.01	0.48 ±0.05	0.23 ±0.06	13.26 ±3.94
Heart	0.8 ±0.03	0.2 ±0.07	0.2 ±0.04	0.04 ±0.02	0.05 ±0.03
Lungs	1.9 ±0.2	0.6 ±0.3	0.5 ±0.02	0.3 ±0.02	0.2 ±0.04
Thyroid	0.1 ±0.03	0.08 ±0.05	0.05 ±0.02	0.1 ±0.06	0.04 ±0.01
Muscle	30.1 ±0.7	15.4 ±3.3	6.6 ±2.7	4.5 ±0.9	2.7 ±0.7
Bone	13.3 ±1.6	6.9 ±1.8	6.2 ±2.7	4.6 ±0.4	3.2 ±0.4
Pancreas	0.3 ±0.05	0.13 ±0.01	0.1 ±0.01	0.05 ±0.04	0.03 ±0.0
Adrenals	0.07 ±0.001	0.08 ±0.0	0.08 ±0.0	0.02 ±0.1	0.04 ±0.0
Carcass	55.9 ±2.2	47.6 ±0.03	22.4 ±4.1	14.9 ±0.9	13.2 ±1.05

FIGURE 1. Distribution of Tc 99m-scorpion venom in male rabbit (4kg) a) 30 min after injection, b) 4 h after injection and c) 24 h after injection.

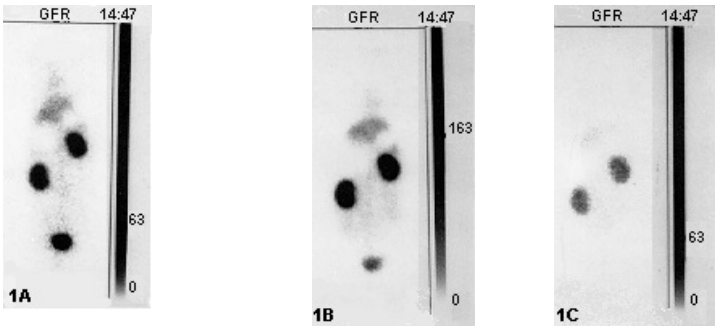


FIGURE 2. Immunoreactivity test in rabbit **a)** 30 min post-injection, **b)** 5 h post-injection and **c)** 20 h post-injection.

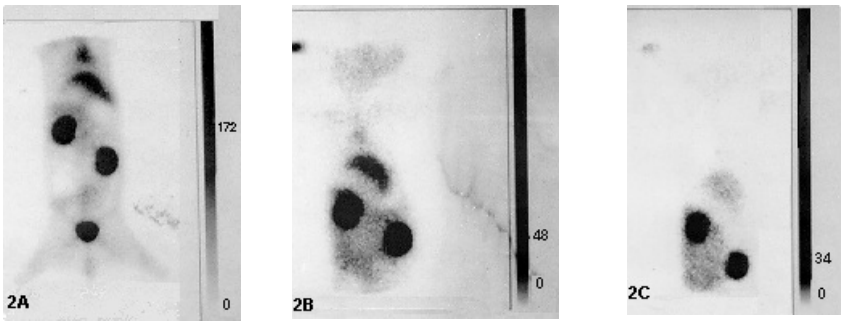
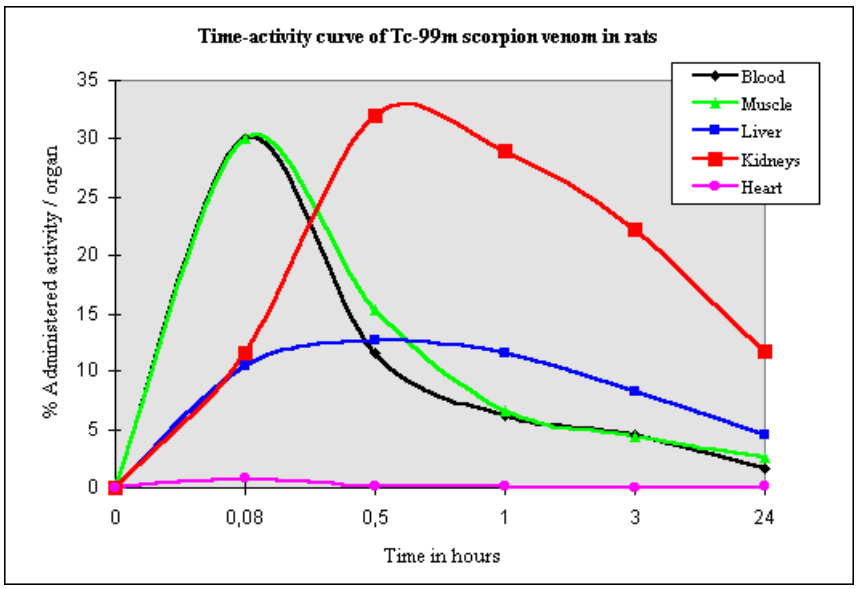


FIGURE 3. Biodistribution of Tc 99m-scorpion venom in blood, muscle, liver, kidneys and heart.



Scintiimages were obtained at different times after Tc 99m-scorpion venom injection. **Figures 1a**, **1b** **and** **1c** show the distribution of radioactively tagged scorpion venom at 30 min, 4 h and 24 h post-injection, respectively.

Tc 99m-tagged venom localized in the kidneys, liver and intestine. An immunoreactivity study was carried out using AScVS against labelled scorpion venom and the results obtained are shown in **Figures 2a**, **2b** **and** **2c** at 30 min, 5 h and 20 h post-injection, respectively. Very avid uptake was seen in the kidneys. A threefold (76%) increase in renal uptake was observed compared to that seen with Tc 99m-scorpion venom (26%) alone.

DISCUSSION

Radiolabelling of scorpion venom with Tc 99m **(2)** has not been reported. In the present study, an attempt was made to label scorpion (*Mesobuthus tamulus concanesis* Pocock) venom with Tc 99m. Ismail *et al.*, **(9)** labelled scorpion venom with I^{125} in order to quantify the uptake in different organs. The isotope I^{125} emits low gamma energy (30 KeV) which is not ideal for detection using the gamma camera for non-invasive imaging of the organ. This radionuclide can only be used for the *in vitro* measurement studies, while Tc 99m is a pure gamma emitter radionuclide (140 KeV), which is ideal for gamma camera imaging so that one can obtain qualitative image as well as quantitative data. Also, Tc 99m is a cheap and easily available radionuclide in all nuclear medicine clinics. Since Tc 99m does not emit any particulate radiation, the dose delivered to the organs of interest may be extremely negligible compared to I^{125}. Thus, one may even use millicurie level of activity of Tc 99m for nuclear imaging.

In the present study, labelled scorpion venom was injected i.v. to Wistar rats. Biodistribution studies showed that the initial high blood level of 27% (5 min) decreased to 11.5% 30 min post-injection, thereby indicating the rapid clearance of scorpion venom from the circulatory compartment.

The initial liver uptake of the labelled venom decreased over the measured time intervals**,** indicating that the uptake was due to hepatocytes and not to the reticuloendothelial cells. The labelled scorpion venom is not colloidal in nature. Therefore, it is taken up by the hepatocytes and not by the reticuloendothelial cells. Radioactively tagged scorpion venom was excreted through the hepatobiliary pathway as an increased uptake was seen in the large intestine 24 h post-injection. No significant accumulation in the thyroid or stomach was observed, suggesting that there was no free pertechnetate in the labelled scorpion venom.

This study showed the uptake of venom by different organs. *In vitro* studies using scorpion venom of different species demonstrated that the venom has toxic effects on heart, stomach, pancreas, lungs and various organs, which may account for myocarditis **(22,24-26)**, changes in gastric secretion **(14)**, acute pancreatitis **(1,23)**, pulmonary oedema **(34)**, and many other clinical manifestations **(7,8,16,21)**. The level of the labelled venom (0.32% at 5 min) dropped to 0.05% after 4 h**,** indicating that the clearance of Tc 99m scorpion venom from the pancreas was very fast. Inhibition of insulin secretion **(10)**, stimulation of glucagon secretion **(11)** and changes in insulin secretion after scorpion envenoming **(12,24,25,28)** indicate the action of scorpion venom on the pancreas, but this could not be demonstrated in this study. A probable explanation could be that the labelled venom acts on the pancreas but that retention may not occur, thereby explaining its non-detection in the pancreas. The increased skeletal muscle uptake (31.0%) could account for the disturbances observed in isolated skeletal muscles *in vitro*, when they were soaked in the venom and failed to respond to stimulation after repeated washing **(38)**.

The maximum renal uptake of 32% (30 min), which dropped to 22% (3h), indicates that the clearance of labelled venom from the kidneys is slow. Scorpion venom releases catecholamines, which in turn act on renal beta adrenergic receptors to release renin causing an elevation of blood pressure **(13,26)**. The avid uptake observed in the kidneys could be receptor mediated. Further experimental study is needed to confirm this.

The immunoreactivity study revealed that the localization of scorpion venom was in the kidneys rather than in other organs such as the liver. It has been demonstrated that radioiodinated meta iodobenzylguanidine (MIBG), a new radiopharmaceutical used for the diagnostic and therapeutic applications of neural crest tumors **(4,15,35-37)**, acts on the sympathetic nervous system. The adrenal uptake obtained 1 h (0.08%) after scorpion venom injection is comparable with the uptake of 131 MIGB **(15)**, thereby suggesting that the scorpion venom acts through the sympathetic nervous system. This study indicates that the kidneys and hepatobiliary pathways are the route of excretion of scorpion venom from the body, which is comparable with the results obtained by Ismail *et al.* **(9)**.

The present study showed the involvement of almost all the organs of the body in scorpion envenoming. Scorpion envenoming results in a massive release of catecholamines, glucagon, angiotensin II, cortisol and simultaneous reduction in insulin levels **(10,11,20,22,24,25,28)**. The rise in the counter regulatory hormones (glucagon, cortisol and catecholamines) oppose the anabolic functions of insulin (secretion is either suppressed or deficient). Under these conditions, essentially scorpion envenoming results in a syndrome of fuel-energy deficits and in an inability to utilise the existing metabolic substrates by the vital organs **c**ausing multi-system-organ-failure (MSOF) and death.

In conclusion, the labelling of scorpion venom is successfully achieved with Tc 99m using direct tin reduction procedures. The biological distribution of scorpion venom in various organs can be observed non-invasively by scintigraphic imaging procedures using gamma camera computer systems. The actions of scorpion venom are targeted more to the kidneys than to any other organs. Further studies using different renal function tests are suggested.

ACKNOWLEDGEMENTS

We thank Mr. R.B. Patkar for his technical assistance. We also thank Dr. R.C. Kankonkar, Haffkine Biopharmaceutical Corporation Ltd., Mumbai for supplying anti-scorpion venom (AScVs).

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