

Preparation of *Crotalus* Venom Radiolabeled with Technetium-^{99m} as a Tool for Biodistribution Study

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ABSTRACT

Technetium-99m (^{99m}Tc) has been the radionuclide of choice for nuclear medicine procedures and experimental research. Because of its optimal nuclear properties, ^{99m}Tc is suitable for high efficiency detection with the advantage of reduced radiological waste. *Crotalus* venom (CV) has been shown to reduce tumors in clinical studies and tissue distribution studies are very important for clinical use. The goal of this work was to obtain CV labeled with ^{99m}Tc which preserves its biological activity. After labeling, biological activity was assessed by hemolytic activity evaluation. Labeled and crude venom caused indirect hemolysis provided that the incubation medium contained an exogenous source of lecithin. High yield radiolabeled-CV was obtained and biological activity was preserved. The results suggest that ^{99m}Tc-CV can be a useful tool for biodistribution studies.

Key words: Technetium-99m, labeling, hemolytic activity

INTRODUCTION

Technetium-99m (^{99m}Tc) has been selected as a radiotracer of choice for several experimental studies such as morphologic and functional imaging of different organs. Over 80% of the radiopharmaceuticals currently being used make use of this short-lived, metastable radionuclide, which has reigned as the workhorse of diagnostic nuclear medicine. The preeminence of ^{99m}Tc is attributable to its optimal nuclear properties of a short half-life and a gamma photon emission of 140 keV, which is suitable for high-efficiency detection and which results in low radiation exposure (Banerjee et al, 2001).

Snake venoms are complex mixtures of toxins, enzymes and bioactive molecules. Although the South American Rattlesnake is a predatory animal

capable of killing a human being, the proteins that can be extracted from the venom have been used in laboratories to improve health care. Many individual components have already been characterized, and some have been used as bases for synthetic drugs against hypertension. Other potential uses for snake venom fractions include a biocompatible glue for plastic surgery, inhibitors of cancer cell growth and activators of the complement system.

Crotalus venom (CV) produces neurotoxicity, coagulation disorders, systemic myotoxicity and acute renal failure, with possible additional heart and liver damage (Monteiro et al, 2001 and Barraviera et al, 1995). This venom contains enzymes, toxins (crotoxin, crotamine, gyroxin, convulxin) and several peptides (Barraviera et al, 1995). Pharmacokinetic and tissue distribution

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studies are very important for clinical use. Although crotoxin has been labeled with ^{125}I for membrane interaction analysis (Krizaj et al, 1996), radiolabeling of snake venom with $^{99\text{m}}\text{Tc}$ has not been reported, neither has the utility of $^{99\text{m}}\text{Tc}$ -based snake venom for *in vivo* studies been assessed.

The goal of the present work was to label *Crotalus durissus terrificus* venom with $^{99\text{m}}\text{Tc}$. The crude and labeled venom were then subjected to a hemolytic activity study to verify that labeling did not alter the biological activities.

MATERIALS AND METHODS

Reagents

Unless otherwise noted, all chemicals and media were purchased from Sigma (St. Louis, MO). $^{99\text{m}}\text{Tc}$ radionuclide was extracted from a $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator (obtained from IPEN, São Paulo, Brazil), using solvent extraction procedures.

CV labelling

Crude venom saline extract was labeled with $^{99\text{m}}\text{Tc}$ using stannous chloride and sodium borohydride as reducing agents according to Pauwels et al (Pauwels et al, 1993). Preliminary studies were done to establish the optimum conditions for obtaining the highest yield of labeled venom. Briefly, stannous chloride (2-4 μg) and sodium borohydride (20 μg) were transferred to a vial containing CV (100-1500 μg) for reduction of pertechnetate anions. The pH was adjusted to 7.0 with hydrochloric acid and sodium hydroxide. $\text{Na}^{99\text{m}}\text{TcO}_4$ (varying from 3.7 to 37 MBq), freshly eluted from a $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator, was added to the reaction vial. The mixture was incubated for 20 min at room temperature under vacuum condition.

Labeling yield

Radiochemical analyses were performed by the method adapted from United States Pharmacopeia- USP 21 (United States Pharmacopeia, 1995) using

two chromatographic systems: ascending chromatography in silica gel 60 (Merck) developed in saturated sodium chloride solvent and descending chromatography in Whatman paper n° 1 developed in acetone. Any unbound hydrophilic $^{99\text{m}}\text{Tc}$ -impurities such as $^{99\text{m}}\text{Tc}$ -pertechnetate ($^{99\text{m}}\text{TcO}_4^-$) migrate to RF 1.0, and $^{99\text{m}}\text{Tc}$ -Crtx remains at RF 0.0 in ITLC-SG chromatograms developed in acetone solvent. In paper chromatography developed in saline solution, $^{99\text{m}}\text{Tc}$ -Crtx migrates to RF 1.0 whereas any insoluble $^{99\text{m}}\text{Tc}$ ($^{99\text{m}}\text{TcO}_2$) species remain at RF 0.0. Radiochemical contaminants, mainly under TcO_2 form, were eliminated by filtration.

Biological activity of $^{99\text{m}}\text{Tc}$ -CV

Biological activity of the *Crotalus* venom after the labeling process was assessed by indirect hemolytic activity. Hemolytic activity of crude and labeled venom was assayed as described by Cadillo et al (Cadillo et al, 1992) and activity was expressed as absorbance at 540 nm.

RESULTS AND DISCUSSION

Radiochromatographic analyses showed radiochemical purity of ~60% for $^{99\text{m}}\text{Tc}$ -CV. The amount of labeled compound obtained by the described procedure is enough for carrying out experiments dealing with toxin or venom target sites and biodistribution pathways.

The phospholipase A_2 activity of *Crotalus* venom is well known and this activity is responsible for breakdown of phospholipid membranes leading to hemolysis (Zwaal et al, 1975). To assess biological activity of CV, its hemolytic activity was measured. Crude and labeled CV did not cause direct hemolysis (not shown). An indirect hemolytic activity evaluation did not show any significant difference between unlabeled (IC₅₀ 301.5 + 73 $\mu\text{g}/\text{mL}$) and labeled CV (IC₅₀ 247.7 + 49 $\mu\text{g}/\text{mL}$) (Fig.1).

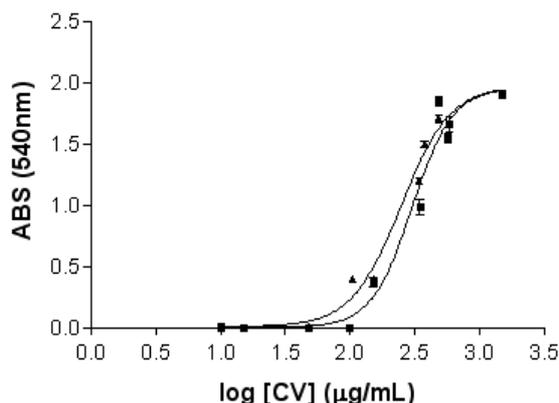


Figure 1 - ^{99m}Tc-CV kept its hemolytic activity. (■) Curve dose-response of the indirect hemolytic (phospholipase activity) of crude venom ($IC_{50} = 301.5 + 73 \mu\text{g/mL}$) and (▲) ^{99m}Tc-CV ($IC_{50} = 247.7 + 49 \mu\text{g/mL}$). Red cells were incubated at 37 °C for one hour with 0.2 mL of a mixture containing a well-shaken suspension of an egg yolk (6.4%) and CV at different concentrations. After centrifuging at low speed for 10 minutes, the absorbance of the supernatant was read for released haemoglobin at 540 nm.

DISCUSSION

Crotoxin (Ctx), the main proteic component of *Crotalus* venom, displays cytotoxic activity against a variety of murine (Zwaal et al, 1975) and human (Corin et al, 1993) tumor cells in vitro. Antitumor efficacy in vivo has been demonstrated on lung carcinoma (Rudd et al, 1994), suggesting good specificity toward solid tumors. Pharmacokinetic studies are very important for clinical trials and radioisotope-based drugs are very convenient for these approaches.

Although Ctx has been labeled with ¹²⁵I (Newman et al, 1993), this low gamma emitter (30 keV) is not ideal for detection using the gamma camera for non-invasive imaging of the organ. On the other hand, ^{99m}Tc is a pure gamma emitter radionuclide (140 keV), which is ideal for gamma camera imaging. Because ^{99m}Tc does not emit any particulate radiation, the dose delivered to the organs of interest may be extremely negligible compared to ¹²⁵I.

Crotalus durissus terrificus (South American rattlesnake) venom possesses phospholipase A₂ activity, which can lead to hemolysis, and neurotoxic activity, both of which are also expressed by crotoxin. The biological activity evaluation showed that ^{99m}Tc labeling did not alter the activity of the CV.

In the present study, labeling of CV was successfully achieved with ^{99m}Tc using direct tin

reduction procedures. High yield ^{99m}Tc-CV was produced in sufficient quantity for imaging studies.

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RESUMO

Tecnécio-99m tem sido o radioisótopo de escolha para procedimentos médicos e pesquisas experimentais. Em decorrência de suas propriedades nucleares, ^{99m}Tc é adequado para detecção de alta eficiência com a vantagem do baixo risco radiológico. O veneno de *Crotalus* (CV) apresentou propriedades antitumorais em estudos clínicos e estudos de biodistribuição são fundamentais em pesquisas clínicas. Esse trabalho teve como objetivo obter um análogo de veneno de *Crotalus* marcado com ^{99m}Tc que preservasse sua atividade biológica. Após a marcação, a atividade biológica foi avaliada através do ensaio de atividade hemolítica. Veneno nativo e marcado apresentaram atividade hemolítica indireta quando incubados em um meio contendo uma fonte

exógena de lecitina. Obteve-se um alto rendimento de marcação e a atividade biológica das moléculas foi preservada. Nossos resultados sugerem que $^{99m}\text{Tc-CV}$ pode representar uma ferramenta muito útil para estudos de biodistribuição.

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