

Nota Técnica

Recebida em 13/12/2002 e aceita em 17/05/2004

**Study of the influence
of the bioactive extract of
Vellozia pusilla Pohl on the
 ^{99m}Tc biodistribution in mice**

*Estudo da influência do extrato
bioativo de Vellozia pusilla Pohl
na biodistribuição do ^{99m}Tc em ratos*

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Abstract

Technetium-99m (^{99m}Tc) has been the most utilized radionuclide in clinical nuclear medicine procedures for diagnosis. It has also been used in basic research to study the influence of drugs on the labeling of red blood cells and plasma proteins and in the biodistribution of this radionuclide in vivo. The methanol extract of *Vellozia pusilla* showed an antitumoral activity through the inhibition of the enzyme Topoisomerase I when analyzed by an *in vitro* bioassay employing DNA repair or recombination deficient mutants of the yeast *Saccharomyces cerevisiae*. This type of activity is similar to compounds like Camptothecin and Topotecan, both used in chemotherapy for solid tumors. In this work we have evaluated the effect of *Vellozia pusilla* extract on the labeling of RBC, plasma and cellular proteins with ^{99m}Tc . Blood of mice was treated with ^{99m}Tc tracer solutions. The percentage of radioactivity (%ATI) bound Plasma (P) and blood cells (BC) was determined. The %ATI in insoluble fraction of plasma was also evaluated. The analysis of the results shows that there is a decrease in %ATI in insoluble fraction of plasma (IF) that represents the plasmatic proteins. A simultaneous study on the effect of the biodistribution of ^{99m}Tc in sodium pertechnetate chemical form was performed. The uptake in the organs was quantified and the %ATI calculated. The results indicated a strong decrease of %ATI in the thyroid and a little increase of %ATI in the stomach and lungs. Plasmatic proteins play a very important role in thyroid metabolism and the results observed in the two experiments suggest a significant competition between ^{99m}Tc and the chemical compounds present in the extract.

Keywords: ^{99m}Tc radionuclide, Antitumoral activity, Biodistribution, Velloziaceae, *Vellozia pusilla* Pohl.

Resumo

O radionuclídeo ^{99m}Tc tem sido amplamente utilizado em procedimentos de diagnóstico em medicina nuclear. Também tem sido utilizado em pesquisa básica para estudar a interação de drogas sobre as células vermelhas e proteínas plasmáticas além do estudo da sua biodistribuição in vivo. O extrato metanólico de *Vellozia pusilla* Pohl (Velloziaceae) mostrou atividade antitumoral através da inibição da enzima Topoisomerase I quando analisado em bioensaio in vitro que utiliza cepas mutantes de *Saccharomyces cerevisiae* que apresentam deficiência na reparação ou recombinação do DNA. Esse tipo de atividade é similar ao observado em compostos como Camptotecina e Topotecan, ambos utilizados em

quimioterapia para tratamento de tumores sólidos. No presente trabalho foi avaliado o efeito do extrato metanólico de *Vellozia pusilla* sobre células vermelhas, plasma e proteínas celulares, em sangue de rato, com a utilização do radionuclídeo ^{99m}Tc , avaliando-se o percentual de atividade (%ATI) em cada uma das frações estudadas. Foi realizado também um estudo sobre o efeito do extrato na biodistribuição do ^{99m}Tc sob forma química de pertecnato de sódio. Os resultados mostraram uma redução no %ATI na fração correspondente as proteínas plasmáticas. Em relação a biodistribuição, os resultados mostraram uma forte redução no %ATI associado ao órgão tireóide e um pequeno aumento no %ATI no estômago e pulmões. As proteínas plasmáticas apresentam um importante papel no metabolismo tireoidiano e os resultados observados nos dois experimentos sugerem uma significativa competição entre o radionuclídeo ^{99m}Tc e os compostos químicos presentes no extrato bioativo de *Vellozia pusilla*. **Palavras-chave:** Atividade antitumoral, Biodistribuição, Radionuclídeo ^{99m}Tc , *Vellozia pusilla* Pohl, Velloziaceae

Introduction

Technetium-99m (^{99m}Tc) has been the most utilized radionuclide in clinical nuclear medicine procedures for diagnosis. It has also been used in basic research to study the influence of drugs on the labeling of red blood cells and plasma proteins and in the biodistribution of this radionuclide in vivo (Braga *et al.*, 2000; Gomes *et al.*, 2001). *Vellozia pusilla* Pohl is a species of the botanic family Velloziaceae that occur in the subtropical regions of the South America, specially in the state of Minas Gerais and although lives under conditions of high solar irradiation and low water availability shows great longevity (Valente *et al.*, 1997, Pinto *et al.*, 1988). The extract of roots, stem and leaf sheaths of this species was obtained based on optimized method previously described by Valente (1982). The methanol extract of *Vellozia pusilla* showed an antitumoral activity through the inhibition of the enzyme Topoisomerase I when analyzed by an *in vitro* bioassay employing DNA repair or recombination deficient mutants of the yeast *Saccharomyces cerevisiae* (Gunatilaka *et al.*, 1994; Dantas, 1997). This type of activity is similar to compounds like Camptothecin and Topotecan, both used in chemotherapy for solid tumor and hematologic malignancies (Chen *et al.* 1997; Mc Donald and Brown, 1998; Cohen *et al.*, 1999). The

DNA Topoisomerase enzymes playing fundamental processes in the cells including replication and recombination and are an important therapeutic target in the cancer chemotherapy (Fukuda *et al.*, 1996). In this work we have evaluated the effect of methanol extract of *Vellozia pusilla* on the labeling of RBC, plasma and cellular proteins with ^{99m}Tc and the effect of this extract on the biodistribution of the ^{99m}Tc radiopharmaceutical.

Materials and Methods

The triplicate samples of blood of mice were incubated with the solutions of methanol extract of *Vellozia pusilla* in NaCl 0.9 %. The concentrations of the extract were 100; 50.0; 25.0; 12.5 and 6.25 % of the solution in 5.0 mg/mL. Stannous chloride (SnCl_2) 1.2 ppm and ^{99m}Tc tracer solutions (3.7 MBq) were added. Plasma (P) and blood cells (BC) were separated. The radioactivity of ^{99m}Tc was counted in a well counter NaI (TI) (Automatic Gamma Counter, 1272 Clinigamma, LKB, Wallac, Finland). The percentage of radioactivity (%ATI) bound to P and BC was determined. The %ATI in the plasma and cellular proteins was also evaluated by precipitation of P and BC samples with trichloroacetic acid 5.0 % (TCA) and isolation of soluble (SF) and insoluble (IF) fractions. A simultaneous study on the effect of the biodistribution of ^{99m}Tc in sodium pertechetate chemical form was performed. Three doses of *Vellozia pusilla* extract were administered to mice. One hour after the last dose, 0.3 mL of pertechetate was also administered in the treated group (N = 4) and in the control group (N = 4). After 20 min. the animals were sacrificed, the organs were isolated, the uptake in the organs quantified and the (%ATI) calculated by dividing the activity in each organ by the total activity administered.

Results and Discussion

The analysis of the results of the influence of drugs on the labeling of red blood cells and plasma proteins shows that there is a decrease in %ATI in IF of plasma, that represents the plasmatic proteins, for all values of

Table 1. Distribution of the radioactivity on labeling of blood cells (Cell), in the fixation of ^{99m}Tc on insoluble fraction of plasma (IFP) and in fixation of ^{99m}Tc in insoluble fraction of cell (IFC), treated with different concentration of methanol extract of *Vellozia pusilla* Pohl

Fraction	Control	100%	50%	25%	12,5%	6,25%
Cell	94 ± 2	89 ± 4	94 ± 1	96 ± 2	96 ± 4	85 ± 23
IFP	71 ± 4	51 ± 11	60 ± 9	48 ± 10	63 ± 2	49 ± 12
IFC	90 ± 2	89 ± 4	94 ± 2	92 ± 1	91 ± 3	89 ± 5

the concentrations of extract solutions (Table 1). Probably the minor concentration was sufficient to alter the value from about 70 to 55 %. These preliminary results indicated an interaction between the chemical compounds of the methanol extract and the plasmatic proteins blocking the bonds with ^{99m}Tc . There are no interaction with the red blood cells and the compounds of the extract as showed in the values of (BC).

The results observed in the biodistribution of ^{99m}Tc radionuclide *in vivo* indicated a strong decrease of %ATI in the thyroid and a slight increase of %ATI in the stomach and lungs (Table 2). Applying the Student t-test, it was concluded that there is no difference between the values measured in the stomach. On the other hand results of %ATI obtained for thyroid and lung are statically different with a significance of 0.05.

Plasmatic proteins play a very important role in thyroid metabolism and the results observed in the two experiments suggest a significant competition between ^{99m}Tc and the chemical compounds present in the extract. The activity of the extract through the inhibition of the enzyme Topoisomerase I in the *in vitro* bioassay and the preliminary results observed in this study suggested an interaction with proteins. Some interactions between ^{99m}Tc and methanol extract may also be playing an important role in the process. Drug and radiopharmaceutical interactions may arise as a result of the pharmacological action of the drug,

physicochemical interactions between the drug and the tracer and drug-induced disease (Hung, James & Hammes, 1996). Future works will be performed with compounds like camptothecin with the aim to correlate these results."

Acknowledgements

This work was performed at the State University of Rio de Janeiro. The authors are grateful to Dr. Bernardo M. Dantas for revising the manuscript. A. L. A. Dantas thanks CNPq for the financial support through a fellowship.

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Table 2. Effect of metanol extract of *Vellozia pusilla* on the biodistribution of radioactivity (%ATI) of ^{99m}Tc (sodium pertechnetate chemical form) in mice

Organs and blood	% ATI / g	
	Control group (N = 4)	Experimental group (N = 4)
Blood	2.6 ± 0.2	2.7 ± 0.2
Thyroid	25.8 ± 8.6	2.9 ± 1.3
Kidney	1.6 ± 0.2	1.4 ± 0.3
Lung	2.1 ± 1.5	6.0 ± 1.8
Pancreas	2.1 ± 0.8	1.2 ± 0.5
Testicle	0.4 ± 0.1	0.3 ± 0.1
Bone	0.5 ± 0.1	0.4 ± 0.1
Muscle	0.5 ± 0.4	1.1 ± 0.9
Liver	22.7 ± 5.2	24.3 ± 2.3
Stomach	4.8 ± 0.9	7.5 ± 2.1
Heart	0.8 ± 0.1	0.8 ± 0.1
Brain	0.1 ± 0.1	0.1 ± 0.1
Spleen	1.1 ± 0.1	0.5 ± 0.1

Bold data indicate significant differences ($p < 0,05$)

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