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A N I N T E R N A T I O N A L J O U R N A L

Biodistribution of ^{99}Mo in Rats

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ABSTRACT

The modification of ^{99}Mo standard metabolism in the presence of MDP would alter the dosimetry of this radionuclide in nuclear medicine patients. Therefore, the objective of this work is to evaluate the influence of MDP in the biodistribution of ^{99}Mo. Wistar rats were divided in two groups of six animals, being inoculated respectively ^{99}Molibdate and ^{99}Mo+MDP via plex ocular. The biodistribution study was carried out after 10 and 120 minutes respectively. The organs were counted with a NaI(Tl) detector. The uptake values did not present significant differences among the groups. An in vitro study through planar chromatography was carried out to determine the affinity between molybdenum and MDP. The results show that ^{99}Mo has low affinity both to propanone and NaCl-0.9% solution. However, ^{99}Mo in the presence of MDP presented affinity to NaCl-0.9% solution and low affinity to propanone suggesting that ^{99}Mo was bound to MDP under the conditions of the experiment.

Keywords: *Wistar*, Molybdenum, Nuclear Medicine.

INTRODUCTION

About 90% of the diagnostic procedures in nuclear medicine use ^{99m}Tc, which is obtained from the elution of ^{99}Mo generators.

Molybdenum is a chemical element of the family 6B of the periodic table. ^{99}Mo is produced by neutron irradiation of stable ^{98}Mo. (Owunwanne, 1995). Molybdenum decays by beta emission to ^{99m}Tc (87%) and ^{99}Tc (13%), emitting photons of 740 and 778 keV and has a half-life of 66 h (LBNL, 2005).

^{99}Mo-^{99m}Tc generators contain a column made of aluminum oxide where molybdate ($^{99}MoO_4^-$) and pertechnetate ($^{99m}TcO_4^-$) ions are adsorbed. This device is easily operated and is based on the relative difference in the affinity of $^{99}MoO_4^-$ and $^{99m}TcO_4^-$(Owunwanne, 1995).

The ^{99m}Tc eluate is expected to be free of contaminants but, under certain conditions (generator malfunction, damage during transportation, inadequate storage conditions and handling problems), ^{99}Mo may occur in ^{99m}Tc eluates as a radionuclidic impurity causing unnecessary dose to the patient and possible degradation of the organ image. According to the IAEA, the acceptable limit of ^{99}Mo in ^{99m}Tc eluates in 0.015% (IAEA, 1999).

MDP (Diphosphonate pyrophosphate) is one of the most used pharmaceuticals in nuclear medicine associated to ^{99m}Tc for bone scintigraphy. The suitable properties of ^{99m}Tc-MDP for radionuclide

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imaging include its rapid clearance from plasma and its high urinary excretion, which contribute to the high contrast between bone and soft tissue. Immediately after intravenous injection, protein 'binding is 25 to 30%, increasing from 45 to 55% and from 60 to 70% in about 4 and 24 hours respectively. (Francis et al., 1969; Glen et al., 2001)

This study aims to evaluate the influence of the presence of the radiopharmaceutical MDP on molybdenum biodistribution in rats and their possible chemical affinity.

MATERIALS AND METHODS

Animals

The animals used in this study were male Wistar Rats (*Rattus norvegicus*), aging 4 to 6 month and masses of 311 ± 21g, obtained in the vivarium of the Faculty of Medical Sciences of the Federal University of The State of Rio de Janeiro (UNIRIO). The animals were kept under the same feeding protocol (food and water *ad libitum*), temperature of 22±5ºC, humidity in the range of 45 to 65% and 12 hours cycles of clear and dark.

Biodistribution

Twelve Wistar rats divided in two groups were inoculated ^{99}Mo in the form of molibdate and ^{99}Mo+MDP. Sodium thiopental (6.7%) was used as anesthetic by intra-peritoneal injection. A volume o 0.3 mL of the radiopharmaceutical was injected in the animals via plex ocular. The animals were sacrificed in a CO_2 chamber after reference times based on the ones applied to ^{99m}Tc, i.e., 10 and 120 minutes for sodium molibdate and molibdate+MDP respectively. The organs were separated, weighted at 0.01g precision and counted with a NaI(Tl)8x4 scintillation detector installed in the shielded room of the In Vivo Monitoring Laboratory of IRD (Souza et al., 2007).

Planar Chromatography

Solutions of NaCl 0.9% and propanone were used as mobile phases and a filter paper Whatman number 1 was used as stationary phase. The chromatography was carried out for the solutions of sodium molibdate and ^{99}Molibdate+MDP. After each chromatographic run filters were cut in 10 pieces of 1 cm and counted with a NaI(Tl) 8x4 scintillation detector.

Statistical analysis

Arithmetic mean and standard deviations of each experiment were calculated by using the software GraphPad-Instat version 3.00 for Windows 95/NT. Statistical analysis were performed using ANOVA test ($p<0.05$) to determine the significance of the difference between experimental and control groups.

RESULTS AND DISCUSSION

Figures 1 and 2 show respectively the distribution of free molybdenum and molybdenum combined to MDP in planar chromatography using 0.9% NaCl solution and Propanone as mobile phases.

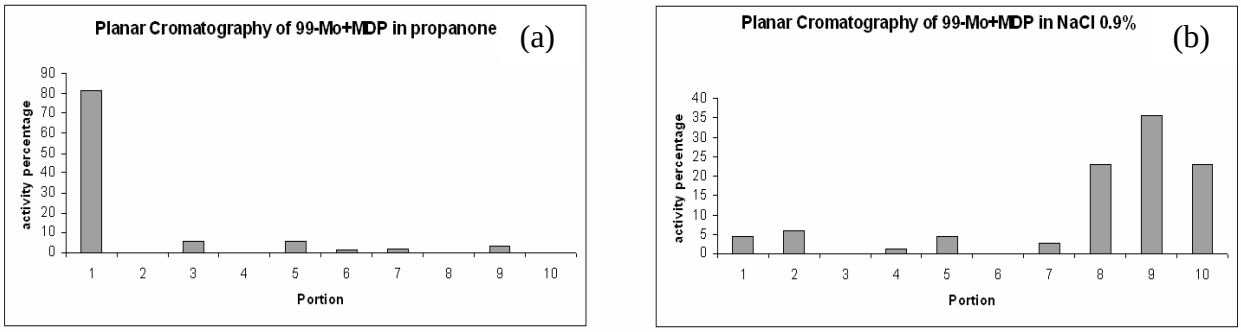

Figure 1 - a) Planar chromatography of molybdenum in Propanone; b) Planar chromatography of molybdenum in NaCl 0.9%.

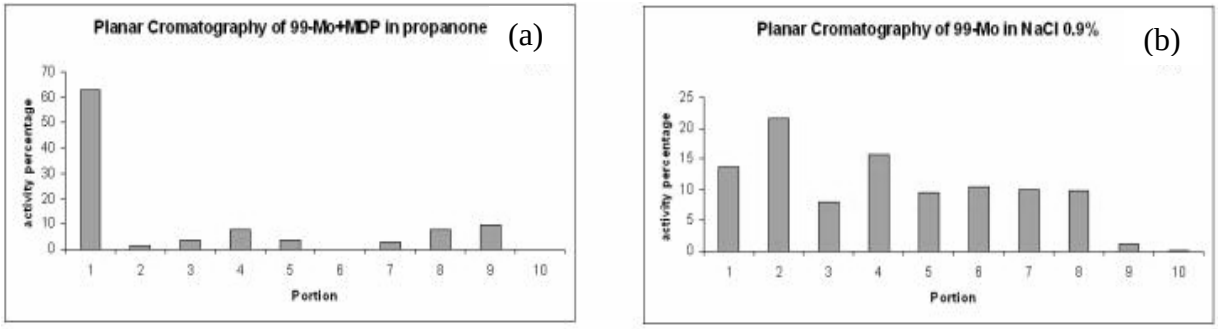

Figure 2 - a) Planar chromatography of molybdenum + MDP in Propanone; b) Planar chromatography of molybdenum + MDP in NaCl 0.9%.

Figure 3 shows a comparison between Molybdenum distribution in Free State and in solution with MDP.

It is known that MDP presents high affinity to NaCl 0.9% solution as well as to technetium in the form of pertechnetate (Owunwanne, 1995). Such characteristic is not observed in molybdenum, which presents higher affinity to the medium. The present experiment suggests that there was a chemical combination of molybdenum and MDP shown by the interaction of the complex with the mobile phase (NaCl 0,9%).

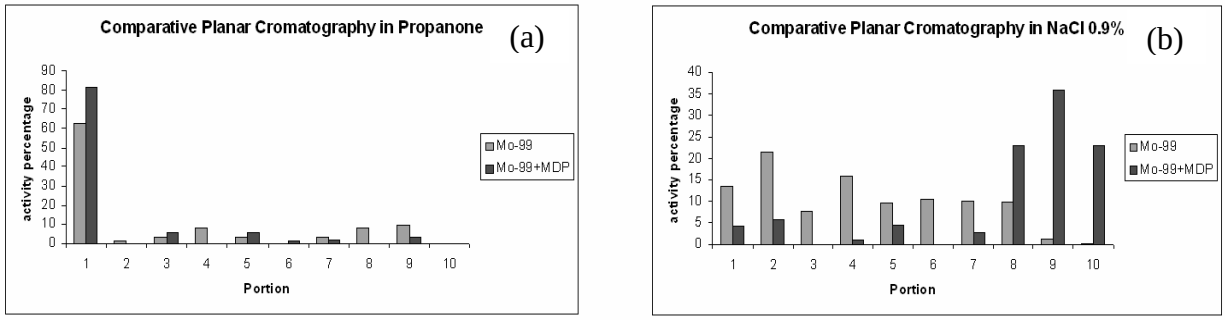

Figure 3 - a) Comparative study by Planar Chromatography of Molybdenum, Molybdenum + MDP in Propanone; b) Comparative study between Planar Chromatography of Molybdenum, Molybdenum + MDP in NaCl 0,9%.

Figure 4 presents the biodistribution of free molybdenum and Mo+MDP in selected organs of Wistar rats after injection of 0.3 mL via plex ocular, sacrificed after 10 and 120 minutes respectively.

The results of this experiment are equivalent to the biokinetic model of molybdenum suggested by the ICRP (1993). According to such model, the highest concentration of molybdenum administered by injection would be found in liver and kidneys. This study provides additional support to the dose estimations performed in a recent study on the exposure of nuclear medicine patients to ^{99}Mo (Silva et al., 2008). In such study it was assumed for dose calculations that molybdenum metabolism is independent of the presence of the radiopharmaceutical MDP and follows the standard biokinetic model available in the literature.

It should be highlighted that although MDP is known to present high affinity to bone tissue, its administration in combination with ^{99}Mo did not modify significantly the expected biodistribution of the radionuclide in Wistar Rats.

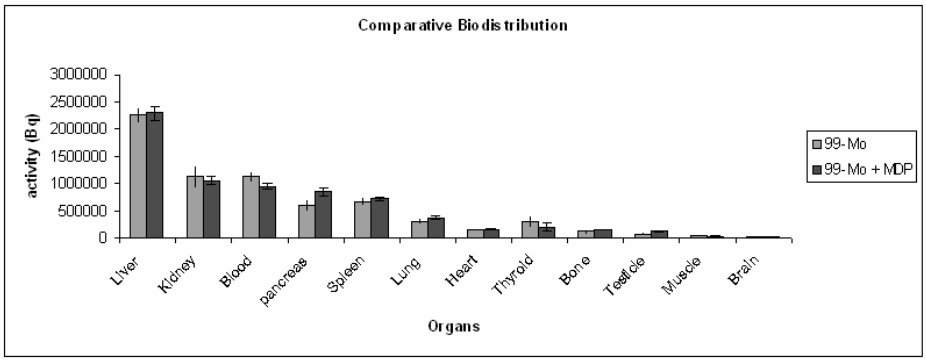

Figure 4 - Biodistributions of free Molybdenum (gray bar) and Mo+MDP (black bar) in Wistar Rats.

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RESUMO

A modificação do metabolismo padrão do ^{99}Mo em presença de MDP levaria a alterações na dosimetria deste radionuclídeo em pacientes de medicina nuclear. Assim, o objetivo deste trabalho é avaliar a influência do MDP na biodistribuição de ^{99}Mo. Ratos Wistar foram divididos em dois grupos de seis animais, sendo inoculados respectivamente com ^{99}Molibdato e ^{99}Molibdato+MDP via plexo ocular. O estudo de biodistribuição foi realizado após 10 e 120 minutos respectivamente. Os órgãos foram contados com detector NaI(Tl). Os valores de uptake não apresentaram diferenças significativas entre os grupos. Foi realizado um estudo in vitro através de cromatografia planar para determinar a afinidade entre o molibdênio e o MDP. Os resultados mostraram que o molibdênio tem baixa afinidade tanto pela propanona quanto pela solução 0.9% de NaCl. Entretanto, o molibdênio em presença de MDP apresentou afinidade pela solução 0.9% de NaCl e baixa afinidade pela propanona, sugering ter ocorrido ligação entre o ^{99}Mo e o MDP nas condições do experimento.

REFERENCES

Francis, M. D; Russell, R. G. G.; Fleisch, H. (1969), Diphosphonates inhibit formation of calcium phosphate crystals in vitro and pathological calcification in vivo. *Science*, **165**, 1264-1266.

Glen, M. B.; So-Jin, Park-Holohan.; Gary, J. R. C.; Fogelman, I. (2001), Quantitative Studies of Bone With the Use of ^{18}Fluoride and ^{99m}Tc-Methylene Diphosphonate. *Semin Nucl Med.*, **1**, 28-49.

International Atomic Energy Agency (IAEA). (1999), Manual de protocolos de calidad de radiofármacos. *Projeto ARCAL XV* - Producción y control de radiofármacos.

International Commission on Radiological Protection (ICRP). (1993), Age-dependent doses to members of the public from intake of radionuclides: Part 2 - Ingestion dose coefficients". *Publication 67*, Pergamon Press, Oxford.

LBNL (2005), Isotopes Project - LUNDS Universitet. Table of Radioactive Isotopes. http://www.ie.lbl.gov/toi/index.asp.

Owunwanne, A.; Patel, M.; Sadek, S. (1995), *The Handbook of Radiopharmaceuticals*. Chapman and Hall Medical, London.

Silva, C. O. A.; Lucena, E. A.; Souza, W. O.; Dantas, A. L. A.; Dantas, B. M. (2008), Estimation of internal exposure to ^{99}Mo in nuclear medicine patients. *Cell Mol Biol., in press.*

Souza, D. E., Senna-Fernandes, V., Carvalho Brito, L.; Souza, R. S. S.; França, D.; Manoel, C. V.; Almeida, M. C.; Bernardo-Filho, M. (2007), Acupuncture stimulation at Sanyinjiao: effect on the sodium pertechnetate bioavailability in rats. *Am J Chin Med.*, **35**, 977-986.

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