Vol. 51, Special Number : pp. 175-180, December 2008
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY

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

Effect of Tripanosomicide Benznidazole (Rochagan®) on the Biodistribution of Sodium Pertechnetate ($Na^{99m}TcO_4$) in Wistar Rats

Vanessa Santos de Arruda Barbosa[1*], Cecília Maria de Carvalho Xavier Holanda[1,2], Roseane Pereira da Silva[1], Daniel Pereira de Oliveira[2], Maurício Ferreira da Silva Júnior[2], Elias Herculano de Oliveira[2], Maria Helena Constantino Spyrides[3] and Aldo Cunha Medeiros[1,4]

[1]Centro de Ciências da Saúde; Universidade Federal do Rio Grande do Norte; Av. Nilo Peçanha, s/n; vambio@oi.com.br; 59012300; Natal - RN - Brasil. [2]Departamento de Microbiologia e Parasitologia; Centro de Biociências; Universidade Federal do Rio Grande do Norte; Av. Salgado Filho, 3000; 59078-970; Natal - RN - Brasil. [3]Departamento de Estatística; Universidade Federal do Rio Grande do Norte; Av. Salgado Filho, 3000; 59078970; Natal - RN - Brasil. [4]Departamento de Cirurgia; Universidade Federal do Rio Grande do Norte; Av. Nilo Peçanha, s/n; 59012300; Natal - RN - Brasil

ABSTRACT

Benznidazole, a drug with specific anti-Trypanosoma cruzi activity, is used in the treatment of Chagas' disease. The radiopharmaceutical sodium pertechnetate ($Na^{99m}TcO_4$) is used to obtain diagnostic images of the stomach, thyroid, parathyroids, salivary glands, brain and in the study of esophageal reflux and blood flow. This study aimed at evaluating in vivo the influence of benznidazole treatment on the sodium pertechnetate biodistribution in Wistar rats. The percentage of radioactivity per gram (%ATI/g) of various organs (brain, heart, esophagus, stomach, small intestine, large intestine, spleen, liver, muscle and blood) was determined. Comparing the treated rats with the controls, we observed that sodium pertechnetate biodistribution did not change when administered to rats treated for thirty days with benznidazole.

Keywords: Antiparasite, Radiopharmaceutical, Technetium-99m, Benznidazole, Biodistribution, *Trypanosoma cruzi*

INTRODUCTION

Chagas' disease is an endemic disorder caused by the flagellate protozoan *Trypanosoma cruzi*. It is a serious medical and social problem in Brazil and several Latin American countries, affecting 18 million individuals, with 300 thousand new cases every year (WHO, 2002). *T. cruzi* can invade multiple host cells, generating megacolon, megaesophagus and chagasic heart disease (Santos et al., 2005; Texeira et al., 2006). Benznidazole (Bz), commercially known as Rochagan®, is a drug with specific anti-*T. cruzi* activity *in vivo* and *in vitro*, available in Brazil since the 1970s (Coura and Castro, 2002). It contains N-benzyl-2-nitro-1-imidazolacetamida, which acts directly on the

* Author for correspondence

macromolecule synthesis by a covalent link with cellular components. It has demonstrated low efficiency and high toxicity, especially in the chronic phase of the disease and its treatment can last for up to 60 days, depending on clinical manifestations and host tolerance (Cançado, 2005).

The most widely used treatment scheme is 10 mg/kg/day in adults and less than 5 mg/kg/day in children (Castro et al., 2000; Cançado, 2002; Urbina and Docampo, 2003; Dias, 2004). Following oral administration, Bz is absorbed by the intestine, and is bound to plasma proteins and red blood cells to be distributed throughout the body. Maximum plasma concentrations are achieved in two to four hours. The half-life of plasma elimination is approximately twelve hours (Morilla et al., 2005).

Among the many diagnostic tools that can be used in tropical diseases, scintigraphy is widely used in the anatomic and functional analyses of organs and systems (Braga, 2002). Technetium-99m (^{99m}Tc), in the form of sodium pertechnetate ($Na^{99m}TcO_4$), is a radionuclide that connects to a wide variety of molecules and cells (Thrall and Ziessman, 2003; Saha, 2004). When injected intravenously, it is distributed through the veins and interstitium and is used to obtain diagnostic images of the stomach, thyroid, parathyroids, salivary glands, brain and in the study of esophageal reflux and blood flow (Saha, 2004; Owunwanne et al., 1995; Thrall and Ziessman, 2003).

Several drugs can interfere with the biological behavior of radiopharmaceuticals used in scyntigraphic examinations. They can change the biological effect of the radiopharmaceutical and their interaction can lead to hypo or hyper uptake of radiopharmaceuticals in a particular organ, causing incorrect diagnosis or misinterpretation of results. Repeated scintigraphy may result in unnecessary radiation for patients (Bernardo-Filho et al., 2005; Gomes et al., 2002). Although Bz is the drug currently recommended by the National Foundation of Health, Brazil, and is used by thousands of people, little is known about its action mechanism, its effects on host cells or its toxicity. Thus, it is important to study the effect of Bz on the biodistribution of the radiopharmaceutical sodium pertechnetate in laboratory animals subjected to chronic treatment with this drug. The aim of this work was to assess *in vivo* the influence of Bz on the biodistribution of sodium pertechnetate in rats.

MATERIALS AND METHODS

Twelve male *Wistar* rats weighing 200-250g from the Centro de Ciências da Saúde, Universidade Federal do Rio Grande do Norte (UFRN), Natal–RN, Brazil were used. The protocol was conducted in accordance with Brazilian College of Animal Experimentation guidelines and was approved by the Research Ethics Committee of Onofre Lopes Hospital-UFRN (08/2007). The animals had free access to water and standard food for rodents (Labina Purina ®) and were randomly allocated to 2 groups: control and treated. The treated group (n=6) received 5 mg/kg/day of Bz diluted with sorbitol, by gavage. The control rats (n=6) received only sorbitol. The animals were treated for 30 days. On the last day of treatment the rats received 0.1 mL of $Na^{99m}TcO_4$ (3.7 MBq) via orbital plexus.

The $Na^{99m}TcO_4$ was eluted in a $^{99}Mo/^{99m}Tc$ generator (Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brazil). After 60 minutes, all animals were quickly killed under anesthesia with xylazine (20 mg/kg) and ketamine (50 mg/kg), by intraperitoneal via. Samples were harvested from the brain, heart, esophagus, stomach, small intestine, large intestine, spleen, liver, muscle and blood. The tissue samples were washed in 0.9% saline, weighed on a precision scale (Mark 160®, Bel equipment, Italy) and the percentage of radioactivity per gram of tissue (%ATI/g) was determined in an automatic gamma counter (Wizard 1470, Perkin-Elmer, Finland). The efficiency of the gamma counter was 86%, as specified by the manufacturer. The biochemical and hematological dosages were performed in automated equipment TermoKonelab 60i, Abbot and Cell-Dyn 3500R, Abbot, respectively. Data were presented as mean ± standard deviation. The percentage of radioactivity per gram (%ATI/g) was determined by dividing the percentage of total radioactivity of each sample by its weight in grams. The ATI%/g was compared using the non-parametric Mann-Whitney test and the biochemical and hematological parameters by Student's t-test, considering the level of statistical significance at $p<0.05$ in both tests. Statistica 6.0 software was used.

RESULTS

Table 1 shows the relationship between the controls and the Bz-treated rats. No statistically significant difference ($p>0.05$) in biodistribution of sodium pertechnetate was observed in any of the organs.

Table 2 shows significant differences in alanine aminotransferase (ALT) dosage and the percentage of neutrophils and lymphocytes, when comparing the treated and control rats. All the other parameters such as iron, total protein, aminotransferase aspartate (AST), creatinine, glucose, red blood cells, hemoglobin, hematocrit, white blood cells and platelets showed no significant differences, when the two groups were compared ($p>0.05$).

Table 1 - Effect of benznidazole treatment on $Na^{99m}TcO_4$ biodistribution in *Wistar* rats.

Organs	% ATI	
	Controls	Treated
Spleen	0.00211 ± 0.00076	0.00199 ± 0.00039
Brain	0.00175 ± 0.00392	0.00020 ± 0.00007
Heart	0.00158 ± 0.00039	0.00192 ± 0.00053
Esophagus	0.00349 ± 0.00123	0.00330 ± 0.00084
Stomach	0.02576 ± 0.00761	0.02276 ± 0.01016
Liver	0.00379 ± 0.00105	0.00402 ± 0.00080
Small Intestine	0.00390 ± 0.00132	0.00299 ± 0.00169
Large Intestine	0.00149 ± 0.00071	0.00163 ± 0.00043
Muscle	0.00050 ± 0.00011	0.00063 ± 0.00014
Blood	0.00683 ± 0.00158	0.00811 ± 0.00165

Mean ± SD. No difference was observed between the two groups ($p>0.05$).

Table 2 - Effect of benznidazole treatment on biochemical and hematological parameters.

Biochemical and hematological dosages	Groups	
	Controls	Treated
Iron (µg/L)	363.0 ± 24.69	308.5 ± 65.56
Total proteins (g/dL)	6.50 ± 0.303	6.55 ± 0.327
ALT (UI/L)	116.0 ± 32.44	98.2 ± 23.60
AST (UI/L)	73.16 ± 8.495	56.66* ± 9.667
Creatinine (mg/dL)	0.375 ± 0.055	0.381 ± 0.040
Glucose (mg/dL)	122.000 ± 19.193	117.666 ± 10.557
Red blood cells (u/mm^3)	6653333 ± 372487.1	6578333 ± 526931.4
Neutrophils (%)	57.5 ± 2.949	34.0* ± 8.786
Lymphocytes (%)	34.5 ± 2.428	58.6* ± 7.890
Hemoglobin (g/%)	12.183 ± 0.598	11.450 ± 0.831
Hematocrit (%)	52.133 ± 3.669	51.616 ± 3,023
Platelets (u/mm^3)	716833.3 ± 14770.47	718333.3 ± 00059.32
Leukocytes (u/mm^3)	3195.0 ± 1126.7	2206.6 ± 646.3

Mean±SD . *, $p<0.05$.

DISCUSSION

The biological behavior of radiopharmaceuticals used for diagnosis in nuclear medicine is well established in the scientific literature. The interaction between a drug and a radiopharmaceutical may alter its biodistribution and result in an unexpected effect (Gomes et al., 2002, Bernardo-Filho et al., 2005).

It is of critical importance to know which drugs can interfere with the normal biodistribution of radiopharmaceuticals, to avoid the misinterpretation of scintigraphic images. Several authors have shown that radiopharmaceutical

biodistribution can be altered by natural and synthetic products, radiotherapy, surgery and diet (Gomes et al., 2002; Xavier-Holanda et al., 2002; Passos *et al*, 2002; Bernardo et al., 2004; Moreno et al., 2005; Santos-Filho and Bernardo-Filho., 2005; Holanda et al., 2006; Araújo-Filho et al., 2007).

Glucantime, an anti-*Leishmania* drug, increased the uptake of the radiopharmaceutical methylene disphosphonic acid, labeled with technetium-99m (^{99m}Tc-MDP) in the spleen, kidney, testicles, heart and liver of rats (Xavier-Holanda et al., 2002). The biodistribution of ^{99m}Tc-MDP was also changed in various organs of rats treated with the antimalarials artemisinin and mefloquine (Holanda et al., 2006). Araujo-Filho et al. (2007) reported changes in sodium pertechnetate biodistribution in the thyroid, lung, pancreas, spleen and muscle after biliopancreatic bypass surgery with duodenal switch in rats. Moreno et al. (2005) showed that *Ginkgo biloba* extract can change the sodium pertechnetate biodistribution in the kidneys, liver and duodenum. Santos-Filho and Bernardo-Filho. (2005) showed that *Hypericum perforatum* extract reduced the uptake of the radiopharmaceutical in bone, muscle and thyroid.

Some studies show that Bz can have adverse effects on adrenal cortex, esophagus and colon cells in rats and that it exerts a mutagenic and carcinogenic effect (Diaz, 2000, Castro et al., 2006, de Castro et al., 2003). Because of its antigenic and toxic effect, Bz can cause several undesirable reactions, mainly in the nervous system and gastrointestinal tract (Cançado, 2002).

During benznidazole treatment (30 to 60 consecutive days)**,** skin reactions, gastrointestinal disorders, nausea, paresthesia, or symptoms of peripheral polyneuritis may occur, especially after prolonged treatment or with excessive doses of Bz. The depression of the bone marrow with neutropenia, thrombocytopenic purple and hepatotoxicity are serious effects of Bz that demand caution when they are being used (Cançado, 2005). In our study, neutropenia was found in Bz-treated rats, but no hepatic and intestinal mucous membrane damage was observed. Although Bz can cause these several undesirable reactions, our results were shown positive in relation to interaction Bz-radiopharmaceutical. Both Bz and sodium pertechnetate are bound to plasma proteins, but it seems that there is no competition between them for the same connection sites (Coura and Castro, 2002; Owunwanne et al., 1995). Bz also, probably, does not interfere in the intestinal mobility and does not alter blood flow. The alteration of these factors could modify the biodistribution of the radiopharmaceutical (Owunwanne et al., 1995). Data from biochemical and hematological parameters also lead us to believe that Bz, administered in equivalent dose to treat Chagas' disease in humans, had no toxic effect on the organs studied, nor did it cause enough tissue damage to promote a change in the uptake of $Na^{99m}TcO_4$.

In this study we demonstrated that the administration of benznidazole didn't alter sodium pertechnetate biodistribution to important target organs, what demonstrate that this result is satisfactory for its use in patients with Chagas' disease. In spite of, this experimental were performed in W*istar* rats.

ACKNOWLEDGEMENTS

The authors thank the Liga Norteriograndense against Cancer for the support, Michael Germain from Canada, for the revision of English language and Italo Medeiros Azevedo for his help with the experiments.

RESUMO

O benzonidazol é um quimioterápico com atividade específica anti-*T. cruzi* utilizado por milhares de pessoas para tratamento da doença de Chagas. O radiofármaco pertecnetato de sódio ($Na^{99m}TcO_4$) é utilizado na obtenção de imagens diagnósticas do estômago, tireóide, paratireóides, glândulas salivares, plexo coróide, cérebro e de estudos de refluxo esofágico e de fluxo sanguíneo. Esse trabalho objetivou avaliar *in vivo* a influência do tratamento crônico com o benzonidazol na biodistribuição do radiofármaco pertecnetato de sódio em ratos *Wistar*. O percentual de radioatividade por grama (%ATI/g) de vários órgãos (cérebro, coração, esôfago, estômago, intestino delgado, intestino grosso, baço, fígado, músculo e sangue) foi determinado. Comparando-se o grupo controle e o tratado observou-se que o pertecnetato de sódio não possui sua biodistribuição alterada quando administrado em ratos tratados por trinta dias com a droga

benzonidazol, mostrando não prejudicar a interpretação de diagnósticos por imagem.

REFERENCES

Araújo-Filho, I.; Rego A. C. M.; Brandão-Neto J.; Villarim-Neto A.; Egito E. S. T.; Azevedo I. M.; Medeiros A. C. (2007), Biodistribution of the Radiopharmaceutical Sodium Pertechnetate after Biliopancreatic Bypass with a Duodenal Switch. *Braz Arch Biol Technol*., **50**, 189-197.

Bernardo, L. C., Santos, A. E. O.; Mendes, D. C.; Ribeiro, C. K.; Gomes, M. L.; Diré, G.; Jesus, L. M.; Abreu, P. R. C. ;Pereira, R.; Frydman, J. N. G.; Moura, R. S.; Bernardo-Filho, M. (2004), Biodistribution Study of the Radiopharmaceutical Sodium Pertechnetate in Wistar Rat Treated with Rutin. *Pak J Biol Sci*., **7**, 518-520.

Bernardo-Filho, M.; Santos-Filho, S. D.; Moura, E. G.; Maiworm, A. I.; Orlando, M. M. C.; Penas, M. E. et al. (2005), Drug Interaction with Radiopharmaceuticals: a Review. *Braz Arch Biol Technol.*, **48**, 13-27.

Braga, F. J. H. N. (2002), Nuclear Medicine in Tropical Diseases. *Braz Arch Biol Technol.*, **45**, 1-7.

Cançado, J. R. (2002), Long term evalution of etiological treatment of chagas disease with benznidazole. *Rev Inst Med Trop S Paulo*, **44**, 29-37.

Cançado, J. R. (2005), *Tratamento Específico da Doença de Chagas nas Fases Aguda e Crônica. In-Dinâmica das Doenças Infecciosas e Parasitárias*, ed. Guanabara koogan, Rio de Janeiro, pp.667-676.

Castro, J. A.; de Mecca, M. M.; Bartel L. C. (2006), Toxic side effects of drugs used to treat Chagas' disease (American trypanosomiasis). *Hum Exp Toxicol.*, **25**, 471-9.

Castro, S. L.; Santa-Rita, R. M.; Einicker-Lamas, M. (2000), In-*Doença de Chagas: Manual de experimentação animal*. FIOCRUZ, Rio de Janeiro, pp. 111-121.

Coura J. R.; Castro S. L. (2002), A critical rewiew on Chagas disease chemotherapy. *Mem Inst Oswaldo Cruz*, **97**, 3-24.

de Castro, C. R., Montalto de Mecca, M.; Fanelli, S. L.; de Ferrevra, E. C.; Diaz, E. G.; Castro, J. A. (2003), Benznidazole-induced ultrastructural and biochemical alterations in rat esophagus. *Toxicology*, **191**, 189-98.

Dias J. C. P. (2004), *Doença de Chagas Aguda. Manual Prático de Subsídio à Notificação Obrigatória no SINAN*. Ministério da Saúde, Brasil, pp. 1-20.

Diaz, E. G.; de Castro R. C.; Montalto de Mecca, M.; Castro, J. A. (2000), Benznidazole-induced ultrastructural and biochemical alterations in rat colon. *Acta Pharmacol Sin*, **21**, 961-6.

Gomes ML, Oliveira MBN, Bernardo-Filho M. (2002), Drug interaction with radiopharmaceuticals: effect on the labeling of red blood cells with technetium-99m and on the bioavailability of radiopharmaceuticals. *Braz Arch Biol Technol*, **45**, 143-149

Moreno, S. R. F.; Carvalho J. J.; Nascimento, A. L.; Pereira, M.; Rocha E. K.; Diré, G.; Arnobio1, A.; Caldas, L. Q. A.; Bernardo-Filho, M. (2005), Bioavailability of the Sodium Pertechnetate and Morphometry of Organs Isolated from Rats: Study of Possible Pharmacokinetic Interactions of a *Ginkgo biloba* Extract. *Braz Arch Biol Technol*., **48**, 73-78.

Morilla, M. J.; Montanari, J. A.; Prieto, M. J.; Lopez, M. O.; Petray, P. B.; Romero, E. L. (2005), Intravenous lipossomal benznidazole as trypanocidal agent: incresing drug delivery to liver is not enough. *Int J Pharm*., **278**, 311-318.

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

Passos, M. C.; Ramos, C. F.; Dutra, S. C.; bernardo-Filho, M.; Moura, E. G. (2002), Biodistribution of 99mTc-O4Na changes in adult rats whose mothers were malnourished during lactation. *J Nucl Med*., **43**, 89-91.

Saha, G. B. (2004), *Fundamentals of Nuclear Pharmacy*. Spring-Verlag, New York.

Santos, C. D.; Caldeira, J. C.; Toldo, M. P. A.; Prado, J. C. (2005), *Trypanosoma cruzi*: Effects of repetitive stress during the development of experimental infection. *Experimental Parasitol.*, **110**, 96-101.

Santos-Filho' S. D.; Bernardo-Filho, M. (2005), Efeito de um extrato de Hipérico (*Hypericum perforatum*) na marcação *in vitro* de elementos sangüíneos com tecnécio-99m e na biodisponibilidade do radiofármaco pertecnetato de sódio em ratos *Wistar*. *Acta Cir Bras*., **20**, 76-80.

Teixeira, A. R. L.; Nascimento, R. J.; Sturm, N. R. (2006), Evolution and pathology in Chagas disease - A Review. *Mem Inst Oswaldo Cruz*, **101**, 463-491.

Thrall, J. H,; Ziessman, H. A. (2003), *Medicina Nuclear*. Guanabara Koogan, Rio de Janeiro.

Urbina, J. A.; Docampo, R. (2003), Specific chemotherapy of Chagas disease: controversies and advances. *Trends Parasitol*., **19**, 495-501.

Xavier-Holanda C. M. C., Jales, R. L. C.; Catanho, M. T. J. A.; Holanda-Leite, R. C.; Brito L. M. L.; Jales-Junior, L. H.; Brandão, K. C.; Amorim, L. F.; Brito, G. G. B.; Gomes, M. L.; Bernardo-Filho, M. (2002), Effects of the glucantime on the kinetic of biodistribution of radiopharmaceuticals in wistar rats. *Cell Mol Biol*., **48**, 761-765.

Holanda, C. M. C.; Holanda-Leite, R. C.; Nunes, R. A. S. N.; Oliveira, H. A.; Catanho, M. T. J. A.; Souza, G. M. L.; Bernardo-Filho, M. (2006), Effect of antimalarial drugs on the bioavailability of the methylene diphosphonic acid labeled with technetium99m (99mTc-MDP) in *wistar* rats. *Braz Arch Biol Technol.*, **49**, 207-214.

World Health Organization. (2002), *Control of Chagas Disease*. Technical Reports Serie, **905**, 1-109.

Received: August 12, 2008;
Revised: September 02, 2008;
Accepted: September 04, 2008