Effect of an extract of Centella asiatica on the biodistribution of sodium pertechnetate ($Na^{99m}TcO_4$) and on the fixation of radioactivity on blood constituents

Journal Item

Vol. 51, Special Number : pp. 215-219 , 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 an Extract of *Centella asiatica* on the Biodistribution of Sodium Pertechnetate ($Na^{99m}TcO_4$) and on the Fixation of Radioactivity on Blood Constituents

Claudia Leite Diniz[1*], Fernanda Santos do Carmo[1], Danielle Santos de Almeida[1], Sebastião David Santos-Filho[1], Sotiris Missailidis[3], Adenilson de Souza da Fonseca[1,2] and Mario Bernardo Filho[1,4]

[1]Laboratório de Radiofarmácia Experimental; Departamento de Biofísica e Biometria; Instituto de Biologia Roberto Alcantara Gomes; Universidade do Estado do Rio de Janeiro; Av. 28 de setembro, 87; 20551030; claudiadiniz@uerj.br; Rio de Janeiro - RJ - Brasil. [2]Centro de Ciências da Saúde; Centro Universitário Serra dos Órgãos; Avenida Alberto Torres, 111; 25964004; Teresópolis - RJ - Rio de Janeiro - Brasil. [3]Department of Chemistry and Analytical Sciences; The Open University; Walton Hall;; MK6 7AA; Milton Keynes - United Kingdom. [4]Instituto Nacional do Câncer, Praça Cruz Vermelha, 23; 20230130; Rio de Janeiro - RJ - Brasil

ABSTRACT

This study evaluates the effects of an acute treatment with a Centella asiatica (CA) extract on the biodistribution of the radiopharmaceutical $Na^{99m}TcO_4$ and on the fixation of technetium-99m on blood constituents. Wistar rats were treated with CA extract and, 1 hour after, $Na^{99m}TcO_4$ was administered; organs/tissues were withdrawn and weighted. The radioactivity was counted to calculate the percentage of activity per gram (%ATI/g). Also, blood samples were withdrawn, plasma (P), blood cells (BC), insoluble fraction (IF) and soluble fractions of P and BC were isolated and the radioactivity was counted to calculate the percentage of activity (%ATI). Data indicated that the acute treatment with CA extract changed significantly ($p<0.05$) the %ATI/g in several organs/tissues (spleen, heart, duodenum, stomach, liver, muscle, kidney, testis and blood) and the %ATI on the blood constituents (P, BC, IF-P and IF-BC). These results indicate that the substances or metabolites of the CA extract would change the biodistribution of $Na^{99m}TcO_4$ and the fixation of the technetium-99m on blood constituents in an acute treatment.

Keywords: Biodistribution; *Centella asiatica*; Blood; Plasma; Technetium-99m

INTRODUCTION

Centella asiatica (CA) has been used in *Ayurveda* (Indian system of medicine). This medicinal plant is known as *Brahmi* in India, belonging to Umbeliferas family and is native of tropical Asia, growing in wet places at an altitude up to 2000 m. In South America, CA is easily found, particularly in the Southern region of Brazil. CA is a rasayan (general tonic), used in *Ayurveda* in preparations either with the whole plant, or with fresh leave extract for the treatment of skin disorders and as a moderator of the central nervous system. Parts of the plant contain triterpene saponins, asiatic acid and madecassic acid and triterpene derivatives

* Author for correspondence

(ester glucosyl asiaticoside and madecassoside) (Inamdar et al., 1996; Shobi et al., 2001).

Taking into account their different applications, the CA extract has been tested with respect to its radioprotective properties against the effects of gamma radiation, mainly due to antioxidant properties of the extract (Sharma et al., 2002; Jayashree et al., 2003).

CA contains antioxidant molecules like carotenoids, ascorbic acid and terpenoids (Padma et al., 1998). Antioxidants may scavenge the free radicals produced by ionizing radiation, thus protecting cells against free radical-induced cytotoxity and genotoxity (Goel et al., 1996).

Shobi et al. (2001) demonstrated that the administration of an aqueous extract of CA protects rats against the effects of gamma-rays, such as weight loss and damage to the gastrointestinal epithelium, which are also observed in cancer patients undergoing radiotherapy.

Gamma-radiation emitting radionuclides are also utilized to label cellular and molecular structures and are used in nuclear medicine for medical imaging (*single photon emission computed tomography* – SPECT) (Saha, 2004, Bernardo-Filho et al., 2005).

Sodium pertechnetate ($Na^{99m}TcO_4$), after intravenous administration, presents a preferential uptake by thyroid, stomach, intestinal tract and salivary glands (Owunwanne et al., 1995). Red blood cells labeled with technetium-99m (^{99m}Tc) are also employed in diagnostic procedures (Saha, 2004).

Drugs (natural and synthetic) could alter the biodistribution of radiopharmaceuticals and the labeling of blood constituents with ^{99m}Tc (Bernardo-Filho et al., 2005; Fonseca et al., 2007, Benarroz et al., 2008). This study evaluates biological effects of a CA extract on the biodistribution of the radiopharmaceutical $Na^{99m}TcO_4$ and on the fixation of the radioactivity on blood constituents.

MATERIALS AND METHODS

Experimental protocols followed in this study were approved by the Ethical Committee of the *Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro* (CEA/212/2007), Rio de Janeiro, RJ, Brazil.

For the preparation of the extract, 1g of CA was added to 10 mL of 0.9% NaCl solution and agitated in vortex for 1 minute. The preparation was centrifuged in a clinical centrifuge (2000 rpm, 15 minutes) and the supernatant was considered to be 100 mg/mL. To standardize the preparation of the extract, an absorbance spectrum (400 to 700 nm) of the extract was obtained in a spectrophotometer (Analyser, 800M, São Paulo, Brazil). An absorbance peak of 0.22±0.02 was obtained at 460 nm, and this was used as a marker of a quality control for the preparation of the extract and a mean of standardizing the concentrations of the extracts.

For the biodistribution assay, male *Wistar* rats (3-4 months old, 250-350 g, n = 10) were treated with CA (6.25 mg/kg, oral via, 1 h) or with 0.9% NaCl, (saline solution) as control. After that, the animals were anesthetized with sodium thiopental (50 mg/kg) and sodium pertechnetate (0.3 mL, 3.7 MBq, 10 minutes) was administered into the orbital plexus. Animals were sacrificed, organs/tissues were withdrawn, weighed, the radioactivity was counted (Automatic Gamma Counter, Packard Instrument Co. Illinois, USA), and the percentage of radioactivity per gram of tissue/organ (% ATI/g) was determined.

For the radiolabeling assay, samples of heparinized blood were withdrawn by cardiac puncture from male *Wistar* rats (3-4 months, 250-350 g) treated with CA extract (6.25 mg/kg) or with 0.9% NaCl, as control. These samples were incubated with ^{99m}Tc, as sodium pertechnetate (10 minutes). Blood samples were centrifuged and plasma (P) and blood cells (BC) were separated. Aliquots of P and BC were also precipitated by trichloroacetic acid (5%) and insoluble (IF) and soluble fractions were separated after centrifugation (1500 rpm, 5 minutes). Radioactivity was counted (Automatic Gamma Counter, Packard Instrument Co. Illinois, USA). The data are expressed as mean ± standard deviation of %ATI/g and %ATI. The values were analyzed by one-way variance analysis (ANOVA) with a $p<0.05$ as significant level followed by Bonferroni post-test. Statistical analysis was performed using InStat Graphpad software (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego, California, USA).

RESULTS

Our results demonstrate the effect CA extract had effect on the radioactivity uptake of various organs in the body, as well as on the fixation of the ^{99m}Tc on the blood constituents with interesting findings. Table 1 represents the values obtained for the %ATI/g in animals treated with CA extracts. Data presented in this table indicate that the acute treatment with CA extract significantly ($p<0.05$) modify the %ATI/g of several organs/tissues, and in particular spleen, heart, duodenum, stomach, liver, muscle, kidney and testis. It is interesting to note that, the treatment with CA extract has generally resulted in reduced uptake of radiation in the various organs, with the exception of kidneys, where the %ATI/g has nearly tripled.

Table 2 represents the values obtained for the %ATI of plasma, blood cells and insoluble fractions of plasma and blood cells from *Wistar* rats treated with CA extract. Data reveal that CA extract alter the fixations of the ^{99m}Tc on the blood constituents, particularly with regards to the difference between plasma and blood cells.

DISCUSSION

Data obtained in this work indicate that animals acutely treated with CA extract could present an altered biodistribution of the radiopharmaceutical sodium pertechnetate (Table 1).

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

Organ/tissue	%ATI Control	Treated
Spleen	0.23±0.08	0.14±0.06*
Brain	0.03±0.02	0.03±0.02
Heart	0.21±0.03	0.14±0.05**
Duodenum	0.35±0.12	0.18±0.09*
Stomach	1.19±0.24	0.34±0.22***
Liver	0.45±0.05	0.28±0.14**
Muscle	0.08±0.02	0.05±0.01***
Bone	0.13±0.04	0.11±0.02
Pancreas	0.32±0.10	0.26±0.09
Lung	0.45±0.08	0.38±0.13
Kidney	0.57±0.25	1.55±0.83**
Testis	0.09±0.02	0.06±0.01**
Thyroid	2.06±0.50	1.29±0.95

(*) $p<0.05$, (**) $p<0.01$, (***) $p<0.001$.

Also, this extract, at the same conditions, was capable of altering the radiolabeling of blood constituents (Table 2). It has been described that extracts of medicinal plants could interfere with the biodistribution of sodium pertechnetate (Moreno et al., 2007; Rebello et al., 2008). In those studies, alterations on the uptake of radiopharmaceutical were observed after repeated doses of extracts (about one week). Our data were obtained 1 hour after a unique treatment (acute treatment).

Interference on the fixation of the ^{99m}Tc on the blood constituents was obtained after *in vivo* chronic treatment (four weeks) with an extract of eggplant (Capriles et al., 2002).

Table 2 - Effect of the CA extract on the labeling of blood constituents with ^{99m}Tc.

	%ATI			
	P	**BC**	**IF-P**	**IF-BC**
Control	40.50±6.55	59.50±6.55	72.11±4.06	56.34±8.56
CA (6.25mg/mL)	$16.72±7.16^{***}$	$83.28±7.16^{***}$	$77.44±5.44^{***}$	$64.67±6.85^{***}$

(*) $p< 0.05$, (**) $p< 0.01$, (***) $p< 0.001$.

Similarly, our data suggest that CA extract would interfere on the radiolabeling of blood constituents in an acute treatment schedule.

It is possible that substances in the extract, as well as its metabolites, would interact with the sodium pertechnetate or with structures in organ/tissues where these substance/metabolites had high uptake.

For the this radiolabeling of blood constituents, substances in CA extract or their metabolites would interfere with the cell membrane or present redox action, altering the radiolabeling process. In fact, saponins have the ability to form complexes with steroids, proteins and phospholipids of membranes (Simões et al., 1999) and they could alter the entry of pertechnetate ions into cells.

Although the results were obtained with animals, they are of great importance at the time of interpretation of scintigraphic images, because consumption of CA extract immediately before treatment could influence the diagnostic procedures in nuclear medicine that use sodium pertechnetate (thyroid scintigraphy), red blood cells labeled with ^{99m}Tc (blood volume or gastrointestinal hemorrhage) or plasma proteins (albumin) labeled with ^{99m}Tc (lung perfusion or reticuloendotelial system). This may also be relevant in therapeutic applications against cancer using radiopharmaceuticals, where the radioactivty uptake in the various organs can be reduced, thus conferring radioprotection from radiation damage.

In conclusion, our data indicate that acute treatment with *Centella asiatica* extracts could interfere with the biodistribution of sodium pertechnetate and the fixation of ^{99m}Tc on blood constituents.

ACKNOWLEDGEMENTS

This research was supported by *Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro* (FAPERJ), *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Universidade do Estado do Rio de Janeiro* (UERJ) and *The Open University*. We are grateful to Analu Custódio, Gabrielle de Souza Rocha, Cristiano Viana and Raphael Sancho Sisley de Souza for technical support.

RESUMO

Este estudo avalia o efeito do tratamento agudo com extrato de *Centella asiatica* (CA) na biodistribuição do radiofármaco $Na^{99m}TcO_4$ e na fixação do tecnécio-99m pelos constituintes sanguíneos. Ratos *Wistar* foram tratados com extrato de CA e, 1 hora após, $Na^{99m}TcO_4$ foi administrado, órgãos e tecidos foram retirados e pesados. A radioatividade foi contada para calcular a porcentagem de atividade por grama (%ATI/g). Amostras de sangue foram retiradas, plasma (P), células sanguíneas, frações insolúveis (FI) e solúveis de P e C foram isoladas e a radioatividade foi contada para calcular a porcentagem de atividade (%ATI). Os dados revelam que o tratamento agudo com o extrato de CA alterou significativamente (p<0,05) a %ATI/g em diversos órgãos e tecidos (baço, coração, duodeno, estômago, fígado, músculo, rim, testículo e sangue) e a %ATI de constituintes sanguíneos (P, CS, FI-P e FI-CS). Esses resultados indicam que substâncias ou metabólitos do extrato de CA poderiam alterar a biodistribuição do $Na^{99m}TcO_4$ e a fixação do tecnécio-99m nos constituintes sanguíneos em um tratamento agudo.

Palavras chave: ьiodistribuição, *Centella asiatica*, sangue, plasma, tecnécio-99m

REFERENCES

Benarroz, M. O., Fonseca, A. S., Rocha, G. S., Frydman, J. N., Rocha, V. C., Pereira, M. O., Bernardo-Filho, M. (2008), *Cinnamomum zeylanicum* extract on the radiolabelling of blood constituents and the morphometry of red blood cells: In vitro assay. Appl Radiat Isot., **66,** 139-146.

Bernardo-Filho, M., Santos-Filho, S. D., Moura, E. G., Maiworm, A. I., Orlando, M. M. C., Penas, M. E., Cardoso, V. N., Bernardo, L. C., Brito, L. C. (2005), Drug interaction with radiopharmaceuticals: a review. *Braz Arch Biol Technol.*, **48**, 13-27.

Capriles, P. V., Dias, A. P., Costa, T. E., Oliveira, M.B., Faria, M.V., Moura, E. G., Abreu, B. A., Bernardo-Filho. M. (2002), Effect of eggplant (Solanum melongena) extract on the in vitro labeling of blood elements with technetium-99m and on the biodistribution of sodium pertechnetate in rats. *Cell Mol Biol (Noisy-le-grand).*, **48**, 771-776.

Fonseca, A. S., Frydman, J. N., Rocha, V. C., Bernardo-Filho, M. (2007), Acetylsalicylic acid decreases the labeling of blood constituents with technetium-99m. *Acta Biol Hung*., **58**, 187-198.

Frydman, J. N. G., Rocha, V. C., Benarroz, M. O., Rocha, G. S., Pereira, M. O., Fonseca, A. S., Bernardo-Filho, M. (2008), Assessment of effects of a *Cordia salicifolia* extract on the radiolabeling of blood constituents and on the morphology of red blood cells. *J Med Food*, in press.

Goel, H. C., Ganguly, S. K., Prasad, J., Jain, V. (1996), Radioprotective effects of diltiazem on cytogenetic damage and survival in gamma ray exposed mice. *Indian J Exp Biol*., **34**, 194-200.

Inamdar, P. K., Yeole, R. D., Ghogare, A. B., Souza, N. J. (1996), Determination of biologically active constituents in *Centella asiatica. J Chromatogr*., **742**,127-130.

Jayashree, G., Muraleedhara, G. K., Sudarslal, S., Jacob, V. B. (2003), Anti-oxidant activity of *Centella asiatica* on lymphoma-bearing mice. *Fitoterapia*, **74**, 431-434.

Moreno, S. R., Silva, A. L., Diré, G., Honeycut, H., Carvalho, J. J., Nascimento, A. L., Pereira, M., Rocha, E. K., Oliveira-Timóteo, M., Arnobio, A., Olej, B.; Bernardo-Filho, M.; Caldas, L. Q. (2007) Effect of oral ingestion of an extract of the herb Uncaria tomentosa on the biodistribution of sodium pertechnetate in rats. *Braz J Med Biol Res.*, **40**, 77-80.

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

Padma, P. R., Bhuvaneswari, V., Silambuchelvi, K. (1998), The activities of enzymatic antioxidants in selected green leaves. Indian J Nutr Diet., **35**, 1-3.

Rebello, B. M., Moreno, S. R. F., Ribeiro, B. M., Neves, R. F., Fonseca, A. S., Caldas, L. Q. A., Bernardo-Filho, M., Medeiros, A. C. (2007), Effect of a Peel Passion Fruit Flour (*Passiflora edulis f. flavicarpa*) extract on the labeling of blood constituents with technetium-99m and on the morphology of red blood cells. *Braz Arch Biol Technol.*, **50**, 153-159.

Saha, G. B. (2004), *Fundamentals of nuclear pharmacy*. Springer-Verlag, New York.

Sharma, J., Sharma, R. (2002), Radioprotection of swiss albino mouse by *Centella asiatica* Extract. *Phytother Res.*, **16**, 785-786.

Shobi, V., Goel, H. C. (2001), Protection against radiation-induced conditioned taste aversion by *Centella asiatica. Physiol Behav.*, **73**, 19-23

Simões, C. M. O., Schenkel, E. P., Gosmann, G., Mello, J. C. P., Mentz, L. A., Petrovick, P. R. (1999), *Farmacognosia: da planta ao medicamento*. UFRGS/UFSC, Porto Alegre. pp. 617-626.

Received: August 18, 2008;
Revised: September 01, 2008;
Accepted: September 04, 2008.