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The ingestion of a *Nectandra membranacea* extract changes the bioavailability of technetium-99m radiobiocomplex in rat organs

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ABSTRACT

The radiobiocomplexes labeled with technetium-99m (Tc-99m) have been widely used in nuclear medicine in single photon emission computed tomography and in basic research. The aim of this study was to assess the influence of a *Nectandra membranacea* extract on the bioavailability of the sodium pertechnetate ($Na^{99m}TcO_4$) radiobiocomplex in rat organs. The animals were treated with a *N. membranacea* extract (30 mg/ml), for 6 days. $Na^{99m}TcO_4$ was injected, the organs were isolated and weighed, and the radioactivity was determined in each organ (%ATI/organ). The %ATI/organ was divided by the mass of each organ to calculate the %ATI/g. A significant increase of the %ATI/organ of $Na^{99m}TcO_4$ was observed in muscle and thyroid as well as in the %ATI/g in the heart, kidney and thyroid. These findings could result from the interaction between components of the plant extract and the radiobiocomplex which may influence the uptake $Na^{99m}TcO_4$ in rat organs. Therefore, precaution is suggested in the interpretation of nuclear medicine results in patients using this herb.

Key terms: Nectandra membranacea*, bioavailability, sodium pertechnetate, radiobiocomplex*.

INTRODUCTION

Nectandra membranacea, which receives the popular name of white cinnamon, is a lauraceous plant from South America. Plants from this genus have been used in the treatment of several clinical disorders in humans (Silva-Filho, 2004). It has been demonstrated that *Nectandra* plants have potential analgesic, antiinflammatory, febrifuge, energetic and hypotensive activities and are commonly used as berberine sulphate (Le Quesne et al., 1980, Silva-Filho, 2004). *Nectandra* also has been investigated as a possible antitumoral agent and the presence of neolignans was suggested as potential chemotherapeutics (Silva-Filho, 2004). Crude extracts of *Nectandra* contain alkaloids and lignans such as nectandrin A and nectandrin B as well as tannin, berberine and sipirine (Le Quesne et al., 1980). Some authors have postulated that tannins play important roles as antioxidant compounds in the scavenging of free radicals (Fenglin et al., 2004). Slish et al. (1999) reported that an extract of *N.*

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salicifolia has potent relaxant activity on vascular smooth muscle (Slish et al. 1999). Researchers of the entire world agree that pre-clinical large studies on herbal medicine are important and urgent, specially high-quality clinical and pre-clinical trials (Rotblat et al., 2002).

Radiopharmaceuticals, recently called radiobiocomplexes (Moreno et al., 2005), are radioactive tracers used in nuclear medicine useful to study morphological and physiological changes in blood flow, absorption and metabolism in target organs (Chandra, 1998; Gomes et al., 2002). The incorporation of a radionuclide into a drug formulation allows to determine the bioavailability and the release sites of a drug (Hladik III et al., 1987; Early and Sodee, 1996). Technetium-99m (Tc-99m) has been widely used to label various radiobiocomplexes used in nuclear medicine mainly in single photon emission computed tomography (SPECT). Tc-99m presents optimal physical characteristics since its half-life and photonic energy allow to obtain images with high efficiency using low doses (Hladik III et al., 1987; Early and Sodee, 1996). Radiobiocomplexes as sodium pertechnetate ($Na^{99m}TcO_4$) are tracers widely utilized for scintigraphic studies mainly for thyroid, brain and stomach. $Na^{99m}TcO_4$ has also been used to label blood constituents (Hladik III et al, 1987; Early and Sodee, 1996; Moreno et al., 2004; Saha, 2004).

Studies have demonstrated that many natural and synthetic products may affect the bioavailability of radiobiocomplexes (Diré et al., 2003; Gomes et al., 2002, Capriles et al., 2002; Moreno et al., 2005, Jankovic and Djokic, 2005). It has been observed with eggplant, chayotte and *Ginkgo biloba* extracts (Diré et al., 2003; Capriles et al., 2002; Moreno et al., 2005).

Considering that the underlying action mechanisms of several plants extracts remain unknown and that there are persons using *N. membranacea* that may need a nuclear medicine procedure, we decided to evaluate the effect of a *N. membranacea* extract on the bioavailability of the $Na^{99m}TcO_4$ radiobiocomplex using rats as experimental model.

MATERIAL AND METHODS

Plant material

Plants were collected in the Maciço of Pedra Branca Natural Reserve, in Rio de Janeiro, State of Rio de Janeiro, Brazil, in July 2005. They were identified by Professor Rogério Oliveira (Botanist) and deposited at the Herbarium Alberto Castellanos (Fundação Estadual de Engenharia do Meio Ambiente - FEEMA), in Rio de Janeiro, Brazil, under the number 85743-8.

Ethanolic extraction and aqueous preparation

N. membranacea leaves (1300g) were submitted to percolation using 5000 ml of cold ethanol (95%). The solvent was evaporated in a rotative evaporator with reduced pressure and a dried fraction of the *N. membranacea* leaves was obtained (85g). This dried fraction (300mg), was almost totally homogenized with 10 ml of NaCl 0.9% with vigorous shaking in a Vortex for 20 seconds. This preparation (30mg/ml) was centrifuged at 1500 rpm for 5 minutes and the supernatant (aqueous solution) was considered as 100%.

Animals

Female Wistar rats (180-220 g) were obtained from the Laboratório de Radiofarmácia Experimental (Departamento de Biofísica e Biometria, Universidade do Estado do Rio de Janeiro, UERJ, RJ, Brazil). The animals were used after an acclimatization period of 7 days and the experiments were conducted in accordance with the Department Committee of Animal Care of the Institution. The animals (treated and control) were maintained under controlled room conditions corresponding to 22±5º C, 12 h of light/dark cycle with water and a normal diet *ad libitum* during the experimental period.

Experimental protocol

One mililiter of the *N. membranacea* aqueous extract (30 mg/ml) was

administered to Wistar rats (n=5) by intragastric via, once a day, during 6 days. The control group received NaCl 0.9 %. The radiobiocomplex $Na^{99m}TcO_4$ (0.3 mL, 3.7MBq) was obtained from Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo, Brazil and was injected in the ocular plexus. The animals were sacrificed 10 minutes after the treatment with the radiobiocomplex. The organs (brain, liver, duodenum, heart, kidney, spleen, stomach, pancreas, ovary, blood, bone, lung, muscle and thyroid) were isolated and weighed in a clinical balance. The organs were put in glass tubes and the radioactivity was determined in a well counter using an Automatic Gamma Counter (Packard, USA). The percentage of radioactivity per organ (%ATI/organ) was determined dividing the activity in each organ by the total activity administered to the animals. The percentage of radioactivity per gram of tissue (%ATI/g) was calculated dividing the %ATI/organ by the mass of each organ.

The Student's t-test was used for statistical analysis and $p<0.05$ was considered to indicate statistical significance of the difference between experimental and control groups.

RESULTS

The results in table 1 show the relationship between the percentage of radioactivity per organ (%ATI/organ) of the radiobiocomplex $Na^{99m}TcO_4$ in the experimental group treated with *N. membranacea* extract and the control group. Results indicate a significant increase in the uptake of the $Na^{99m}TcO_4$ in thyroid, from 0.475±0.050 (control) to 1.187±0.387 (treated, p=0.010), and in muscle from 0.039±0.017 (control) to 0.110±0.030 (treated, p=0.004) ($p<0.05$). No significant changes in the uptake of this radiobiocomplex in the brain, liver, duodenum, heart, kidney, spleen, stomach, pancreas, lung, ovary, blood and bone (%ATI/organ) were found.

TABLE 1

Percentage of radioactivity in rat organs after the treatment with *N. membranacea* extract.

Organs	Control	Treated
1. Brain	0.047±0.016	0.030±0.008
2. Liver	0.267±0.031	0.297±0.116
3. Duodenum	0.066±0.029	0.112±0.039
4. Heart	0.163±0.019	0.134±0.053
5. Kidney	0.109±0.101	0.213±0.028
6. Spleen	0.053±0.032	0.105±0.041
7. Lung	0.312±0.148	0.330±0.190
8. Stomach	1.249±0.600	1.687±0.540
9. Pancreas	0.079±0.032	0.111±0.039
10. Blood	1.106±0.499	1.321±0.583
11. Bone	0.041±0.025	0.141±0.099
12. Muscle	0.039±0.017	0.110±0.030*
13. Thyroid	0.475±0.055	1.187±0.387*
14. Ovary	0.056±0.013	0.076±0.015

Female *Wistar* rats were treated with 1 ml of *N. membranacea* extracts (30mg/ml) for 6 days and injected with 0.3ml of Tc-99m. Animals organs were isolated, weighed and the percentage of radioactivity in each organ was determined. In the case of blood, 1ml was considered as 1 gram. Asterisks indicate significant differences ($p<0.05$).

Table 2 shows the percentage of radioactivity per gram of tissue (%ATI/g) of the radiobiocomplex $Na^{99m}TcO_4$ in the treated animals with *N. membranacea* extract and in the control group. Significant increases in the uptake of the $Na^{99m}TcO_4$ in the heart, from 0.440±0.070 (control) to 0.823±0.240 (treated, p=0.023), in the kidney from 0.355±0.236 (control) to 1.087±0.270 (treated, p=0.006), and in the thyroid from 3.476±0.876 (control) to 11.810±2.863 (treated, p=0.001) were observed.

DISCUSSION

This study intends to contribute on the possible effects of natural products on radiobiocomplex absorption in animals. Consequently, a dependable use of these products by the population would be possible. Moreover, the development of experimental models allowing to identify biological properties of natural products extracts is highly desired (Rotblatt et al, 2002).

Some authors have described effects of natural and synthetic products on the bioavailability of radiobiocomplexes using animal models (Capriles et al., 2002; Diré et al., 2003; Moreno et al., 2005; Santos-Filho and Bernardo-Filho, 2005). Several studies have been performed to analyze the action of substances on the bioavailability of the radiobiocomplex $Na^{99m}TcO_4$. Capriles et al. (2002) and Diré et al. (2003) demonstrated that natural products, such as, eggplant and chayotte extracts, respectively, are able to promote changes on the bioavailability of the $Na^{99m}TcO_4$. Santos-Filho and Bernardo-Filho (2005) have observed that *Hypericum perforatum* extract decreases the bioavailability of the $Na^{99m}TcO_4$ in the bone, muscle and thyroid (Santos-Filho and Bernardo-Filho, 2005). Moreno et al. (2005) have reported that *Ginkgo biloba* extract altered the uptake of $Na^{99m}TcO_4$ in several organs, such as kidney, liver and duodenum. Results obtained by other authors and those of the present study indicate that the interaction herb extract-radiobiocomplex depends on the phytocomplex used, on the experimental conditions utilized and on the radiobiocomplex selected for the study.

TABLE 2

Percentage of radioactivity per gram of tissue in rat organs after the treatment with *N. membranacea* extract.

Organs	Control	Treated
1. Brain	0.213±0.033	0.118±0.090
2. Liver	6.414±1.448	6.989±1.830
3. Duodenum	0.234±0.154	0.346±0.255
4. Heart	0.443±0.070	0.823±0.247*
5. Kidney	0.355±0.236	1.087±0.271*
6. Spleen	0.210± 0.090	0.293±0.200
7. Lung	1.120±0.355	1.581±0.247
8. Stomach	9.957±3.574	13.340±3.886
9. Pancreas	0.215±0.164	0.408±0.181
10. Blood	1.104±0.490	1.215±0.583
11. Bone	0.224±0.059	0.356±0.110
12. Muscle	0.105±0.084	0.225±0.074
13. Thyroid	3.476±0.876	11.810±2.863*
14. Ovary	0.740±0.230	1.031±0.339

See table 1 (comments).

The treatment with *N. membranacea* extract increased the uptake of $Na^{99m}TcO_4$ by muscle and thyroid (%ATI/organ) and by heart, kidney and thyroid (%ATI/gram of tissue). It is possible to suggest that these findings would be relevant to: (i) increase information about the possible interaction of this plant extract with pharmaceuticals, (ii) develop an experimental model to study the interaction betwen an herb extract and a radiobiocomplex.

These data suggest that the metabolization of the *N. membranacea* extract in rats could generate active metabolites able to influence the bioavailability of $Na^{99m}TcO_4$ radiobiocomplex. This radiobiocomplex has been used to evaluate some functions of the brain, thyroid gland, stomach and heart. Some of these findings could be due to a possible relaxant action in the vascular smooth muscle, as described by Slish et al (1999) for *N. salicifolia* extract.

In conclusion, this work showed an alteration in the radioactivity uptake in heart, thyroid, kidney and muscle due to the treatment with *N. membranacea* extract. Therefore, precaution is suggested in the interpretation of nuclear medicine results when patients are using this herb. Moreover, in order to evaluate the behavior of the *N. membranacea* extract and consequently the mechanism of action of this phytocomplex in the uptake of sodium pertechenetate by organs, other experiments with scavengers of free radicals are being carried out.

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