

Bioavailability of the Sodium Pertechnetate and Morphometry of Organs Isolated from Rats: Study of Possible Pharmacokinetic Interactions of a Ginkgo Biloba Extract

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ABSTRACT

Many compounds affect the bioavailability of radiobiocomplexes as radiopharmaceuticals. Ginkgo Biloba extract (EGb) has several effects. The influence of an EGb on the bioavailability of the radiobiocomplex sodium pertechnetate ($\text{Na}^{99\text{m}}\text{TcO}_4$) and on the morphometry of the organs was evaluated. Rats were treated with EGb and $\text{Na}^{99\text{m}}\text{TcO}_4$ was injected. The animals were sacrificed; the radioactivity in the organs was counted. The results showed that EGb altered the $\text{Na}^{99\text{m}}\text{TcO}_4$ bioavailability in the kidneys, liver and duodenum. Morphometric analysis of the organs showed significant alterations ($P < 0.05$), probably caused by metabolites generated by EGb and capable of altering the bioavailability of the $\text{Na}^{99\text{m}}\text{TcO}_4$.

Key words: Ginkgo biloba extract, bioavailability, radiobiocomplex, sodium pertechnetate, morphometry

INTRODUCTION

Radiobiocomplexes, known as radiopharmaceuticals, are radioactive tracers employed in nuclear medicine for diagnostic and/or treatment of diseases or to study the (i) blood flow; (ii) morphology of organs; (iii) bioavailability and metabolism of drugs (Chandra, 1998, Braga et al., 2000, Gomes et al., 2002). Radiobiocomplexes may be labeled with technetium-99m (Tc-99m), as sodium

pertechnetate ($^{99\text{m}}\text{TcO}_4\text{Na}$), and used as imaging agents (Hladik III et al., 1987).

The bioavailability of radiobiocomplexes can be recognizably altered by diseases and a wide variety of conditions, such as radiation therapy, drug therapy (medicinal plant or synthetic drugs) and several invasive medical procedures (Diré et al., 2003, Early and Sodee, 1996; Lima et al., 2002; Mattos et al., 2001; Oliveira et al., 2002; Sampson, 1996, Hladik III et al., 1987). The altered biological behavior caused by diseases

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helps the physician to make a diagnosis (Early and Sodee, 1996; Hladik III et al., 1987).

Ginkgo Biloba extract (EGb) is a medicinal plant which comes from the leaves of the ginkgo tree, one of the oldest living plant species (Jacobs and Browner, 2000). This extract has several effects, including: [1] increases the blood flow, [2] acts as platelet activating factor antagonism and [3] prevents the membrane against the damage caused by free radicals (Diamond et al, 2000; Galluzzi et al, 2000; Jacobs and Browner, 2000; Moreno et al, 2001; Pietta, 1999; Yucheng et al, 1996).

Since the study of drug interactions with radiopharmaceuticals is highly relevant and desirable, the purpose of this work was to study the influence of a *Ginkgo biloba* extract on the bioavailability of the radiobiocomplex sodium pertechnetate and on the morphometry of some organs isolated from the *Wistar* rats treated with EGb.

MATERIAL AND METHODS

A solution containing 24% of a commercial *Ginkgo biloba* extract (China Jiangsu Medicines and Health Products Lot GB 001128, w/w) was prepared in 0.9% NaCl. From this solution (crude extract), saline dilutions containing 40 and 400 mg/mL of the commercial extract were prepared. These preparations were administered to female *Wistar* rats (n = 5) during 6 days (intragastric via). The control group received a solution of 0.9% NaCl. Tc-99m, as $^{99m}\text{TcO}_4\text{Na}$, (0.3 mL, 7.4 MBq), recently milked from a Molybdenum-99/Technetium-99m generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo, Brazil), was injected by ocular plexus and the animals were sacrificed after 10 minutes. The organs were isolated (brain, liver, duodenum, heart, kidney, spleen, stomach, pancreas, lung, ovary, blood, bone, muscle and thyroid) and counted in a well counter (Automatic Gamma Counter Packard, USA). The percentages of radioactivity per gram of each organ (%ATI/g) were calculated. A statistical analysis of the results (ANOVA test, with Dunnett test, $p < 0.05$) was performed.

Histological preparations were performed with some organs of interest (liver, kidney and duodenum) from the animals that received EGb (400 mg/mL) by an intragastric route. The pieces of the organs isolated from the rats (treated and

control) were fixed in 2.5% glutaraldehyde (Riedel-de-Haen) in 0.1 M cacodylate buffer (pH 7.2). The fixative was added with 0.25% tannic acid (Merck). The postfixation was in 1% osmium tetroxide (Sigma) (OsO_4), 0.8% potassium ferricyanide and 5 mM calcium chloride (CaCl_2) in 0.1 M cacodylate buffer. The tissues were dehydrated in acetone and embedded in Epon (Embed-812). Thin sections (2 μm) were stained with toluidine blue (Vetec, Brazil) and observed under light microscopy (Olympus BH2-RFCA).

Images of these tissues (glomerulus, hepatocitus and duodenum) were recorded on a computer employing the *Nero Pro plus Image* Program. The Morphometry Analysis with Mann Whitney test ($P < 0.05$) was performed. Perimeter and frequency (number of cells/area) were the parameters considered to compare the glomerulus, hepatocytes and duodenum cells obtained from control animals and treated during 6 days with *Ginkgo biloba* extract (400 mg/ml).

RESULTS AND DISCUSSION

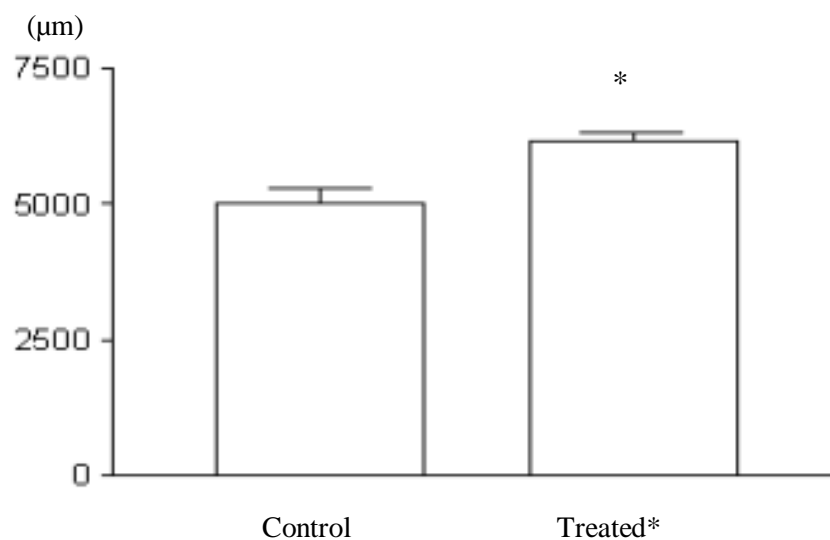
Herbs and drugs usually interact in two general ways: pharmacokinetically and pharmacodynamically. Pharmacokinetic interactions (bioavailability) result in alterations in absorption, distribution, metabolism, or elimination of an agent, quantitatively increasing or decreasing the amount that becomes effective in the body (Rotblat et al, 2002).

The effect of the *Ginkgo biloba* crude extract on the bioavailability of the $^{99m}\text{TcO}_4\text{Na}$ (%ATI/g) in the female *Wistar* rats which had (40 and 400 mg/mL) or had not (control group) received the extract are presented in Table 1. The EGb altered (not significant, n = 5, $p > 0.05$) the uptake of the $^{99m}\text{TcO}_4\text{Na}$ in the kidneys and liver. A significant (n = 5, $p < 0.05$) decrease in the uptake of this radiobiocomplex in the duodenum from 1.01 ± 0.37 to 0.55 ± 0.09 after the treatment with EGb (40 mg/mL) and from 1.01 ± 0.37 to 0.51 ± 0.36 after the treatment with EGb (400 mg/mL) was observed (Table 1).

Morphometric alterations in the kidney and liver due to treatment (*in vivo*) were observed (Figs. 1 and 2) and were considered significant ($p < 0.05$). The perimeter (μm) of the glomerulus from kidney was significantly ($P < 0.05$) increased when compared with the control (Fig. 1).

Table 1 - Effect of a *Ginkgo biloba* crude extract (40 and 400mg/mL) on bioavailability of Tc-99m: %ATI/gram of tissue.

Organs	Control	EGb 40 mg/ml	EGb 400 mg/ml
1. Brain	0.04±0.02	0.04±0.01	0.06±0.02
2. Liver	0.64±0.32	0.66±0.29	0.73±0.36
3. Duodenum	1.01±0.37	0.55±0.09*	0.51±0.36*
4.Heart	0.42±0.17	0.30±0.17	0.35±0.08
5. Kidney	0.56±0.18	1.37±1.36	0.56±0.27
6. Spleen	0,33±0.12	0.20±0.11	0.27±0.07
7. Lung	0.61±0.35	3.22±0.45	0.55±0.19
8. Stomach	5.55±3.30	3.71±1.97	3.38±1.01
9.Pancreas	0.21±0.15	0.22±0.24	0.09± 0.04
10. Blood	3.43±1.50	2.34±0.85	3.34±0.54
11.Bony	0,28±0.14	0.34±0.16	0.21±0.03
12. Muscle	0.12±0.07	0.12±0.12	0.09±0.05
13. Tiroid	2.72±1.18	1.62±0.68	1.10±1.14
14. Ovary	0.24±0.14	0.15±0.08	0.24±0.02

**Figure 1** - Morphometric analysis of glomerulus from animals that received EGb (400mg/ml) and controls animals

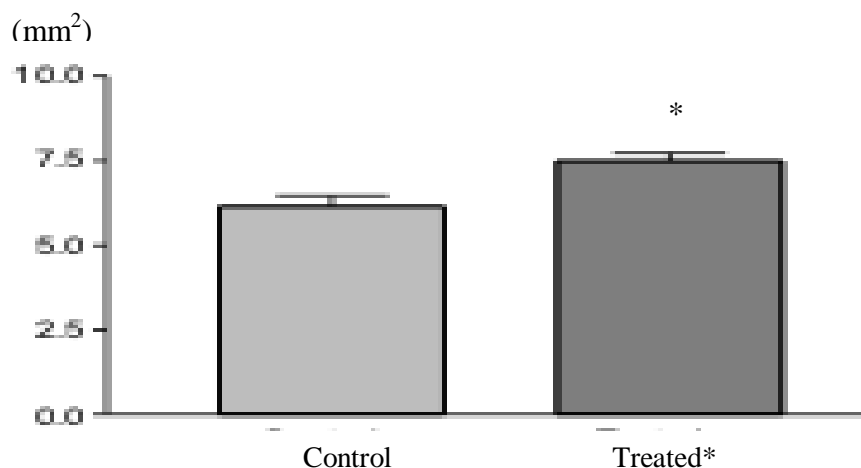


Figure 2 - Morphometric analysis of Hepathocitus number/area from animals that received EGb (400 mg/ml) and control animals.

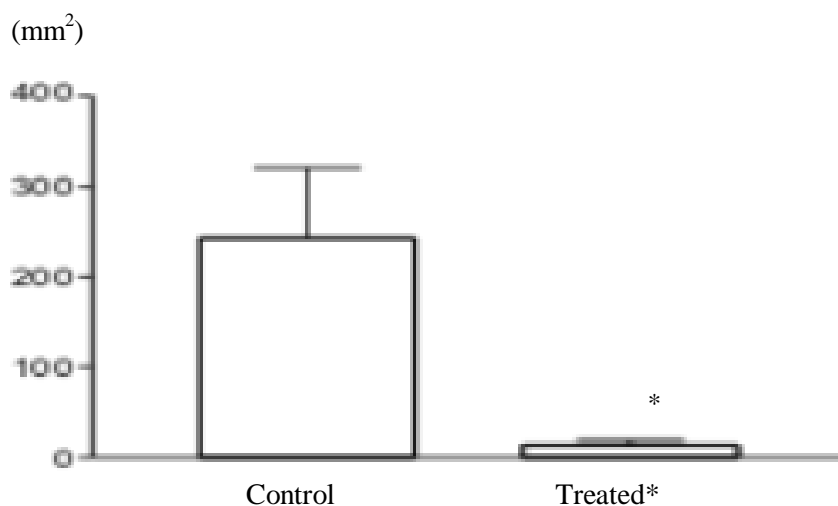


Figure 3 - Morphometric analysis of caliciform cells from animals that received EGb (400 mg/ml) and control animals

The results of the morphometric analysis employing the frequency of cells per area of liver (Fig. 2) have revealed a significant difference ($p < 0.05$) when compared with the control (no treated animals). The frequency of cells/area of liver in the treated animals was higher than in the control animals. The decrease in the uptake of sodium perthecnetate by the duodenum can be justified by the alterations in the caliciform cells, observed by optical microscopy (image not

presented), and the morphometry of this organ (Fig. 3).

The analysis of the results indicates that the *in vivo* treatment with EGb could generate metabolites with action on specific structures leading to alterations in the morphology of organs and in the bioavailability of $\text{Na}^{99\text{m}}\text{TcO}_4$. The morphometric alterations observed in the glomerulus could suggest that the glomerular filtration rate should be increased.

Nevertheless, it is not easy to predict the consequences of the increase in the number of hepatocytes per area in the liver and the reduced number of caliciform cells in the duodenum as a consequence of the metabolization of the *Ginkgo biloba* extract.

In conclusion, the experimental model employed in this study may be a suitable method for evaluating pharmacokinetic interactions with *Ginkgo Biloba* extract. Moreover, to better understand the mechanism of action of the *Ginkgo biloba* extract in the uptake of $\text{Na}^{99\text{m}}\text{TcO}_4$ by organs, experiments with scavengers of reactive oxygen species and chelating agents are now in progress. Electron microscopy studies will also be done to evaluate whether the changes that were found under light microscopy were capable of promoting ultrastructural alterations.

After six days of treatment (intragastric route) with crude EGb (control, 40 and 400 mg/mL), female Wistar rats ($n = 5$) received 0.3 mL of Tc-99m (endovenous via). The animals were sacrificed, the organs isolated and %ATI/gram determined. Blood (1 ml was considered to be 1 gram). * $P < 0.05$.

Morphometry analysis with Mann Whitney test (P value = 0.0079*, $P < 0.05$). Perimeter is the parameter considered for comparison of glomerulus (kidney) obtained from control animals and treated during six days with *Ginkgo biloba* extract (400 g/ml).

Morphometric Analysis with Mann Whitney test (P value = 0.0159*, $P < 0.05$). Number cells/area is the parameter considered for comparison of hepatocitus (liver) obtained from animals controls and treated during 6 days with *Ginkgo biloba* extract (400mg/ml).

Morphometric Analysis with Mann Whitney test (P value= $P < 0.05$. * $P = 0.0079$). Number of cells/area is the parameter considered for comparison of caliciform cells (of duodenum) obtained from control animals and treated during six days with *Ginkgo biloba* extract (400 mg/ml).

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Fluminense (UFF) and Universidade do Estado do Rio de Janeiro (UERJ).

RESUMO

Substâncias podem interferir na biodisponibilidade de radiobiocomplexos, como os radiofármacos. O extrato de *Ginkgo Biloba* (EGb) apresenta efeitos. Avaliou-se a influência de um EGb na biodisponibilidade do pertechnetato de sódio ($^{99\text{m}}\text{TcO}_4\text{Na}$) e na morfometria de órgãos de ratos que foram tratados com EGb. $^{99\text{m}}\text{TcO}_4\text{Na}$ foi injetado, os animais sacrificados e a radioatividade nos órgãos contada. Os resultados mostraram que o EGb alterou a biodisponibilidade do $^{99\text{m}}\text{TcO}_4\text{Na}$ em rins, fígado e duodeno e alterações morfométricas significativas ($p < 0.05$) foram encontradas. Sugere-se que o EGb poderia gerar metabólitos capazes de alterar morfometricamente os órgãos citados e alterar a biodisponibilidade do $^{99\text{m}}\text{TcO}_4\text{Na}$.

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