

Biodistribution of Sodium Pertechnetate and Light Microscopy of Organs Isolated from the Rats: Study of the Effects of a *Ginkgo biloba* Extract

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Abstract: *Ginkgo biloba* extract (EGb) is a phytoterapeutic used in the treatment of hypoxic conditions. This medicinal plant has several effects, specially, vasodilator, anti-inflammatory and anti-coagulant properties. It has antioxidant characteristics. Many substances have been reported to affect the biodistribution of different radiopharmaceuticals. We evaluated the influence of an EGb on: (i) the biodistribution of the technetium-99m (Tc-99m) and (ii) on the morphology of the organs from *Wistar* rats. The animals were treated (6 days, intra gastric via) with EGb (40 and 400 mg/ml). After that, Tc-99m was injected and the animals were sacrificed. The organs were isolated and counted in a well counter. The percentages of radioactivity per organ (%ATI/organ) and radioactivity per gram (%ATI/gram) of each organ were calculated. Histological preparations were carried out with the pieces of organs (kidney, liver, duodena) withdrawn from the treated animals (400mg/ml EGb). The results showed that EGb altered (not significant, $p > 0.05$) the biodistribution of the Tc-99m in the kidneys and liver. A significant ($P < 0.05$) decrease in the uptake of this radiopharmaceutical in the duodena after the treatment with EGb 40 mg/ml and with EGb 400 mg/ml was observed (%ATI/organ and %ATI/gram). However, this decrease was not capable to alter the optical microscopy of the duodena. Morphological alterations on kidney and liver due to treatment (*in vivo*) were found. We suggest that the action of EGb could generate metabolites capable to promote modifications in the organs, such as, kidney and liver and to alter the biodistribution of the Tc-99m in the treated animals.

Key words: *Ginkgo biloba*, biodistribution, technetium-99m, morphology

Introduction

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; Hesslewood and Leung, 1994; Jacobs and Browner, 2000; Moreno *et al.*, 2001; Pietta, 1999; Yucheng *et al.*, 1996).

In nuclear medicine, the radiopharmaceuticals are labeled with technetium-99m (Tc-99m), as sodium pertechnetate and widely used as the imaging agents (Hladik III *et al.*, 1987). The biodistribution of radiopharmaceuticals can be recognizably altered by a wide variety of conditions, such as, drug therapy, radiation therapy, diseases and medicinal plant therapy (Dire *et al.*, 2003; Early and Sodee, 1999; Lima *et al.*, 2002; Lima-Filho *et al.*, 2003; Mattos *et al.*, 2001; Oliveira *et al.*, 2002; Sampson, 1996). The altered biologic behavior helps a physician to make a diagnosis (Lima

et al., 2001).

The purpose of this work was to study the influence of a *Ginkgo biloba* extract on the biodistribution of the radiopharmaceutical sodium pertechnetate and on the morphology of the some organs from the *Wistar* rats.

Materials and Methods

Commercial solution *Ginkgo biloba* extract (China Jiangsu Medicines and Health Products Lot GB 001128) containing 24% of the extract was prepared in 0.9% NaCl. From this solution (crude extract), saline dilutions containing 40 and 400 mg/ml of the commercial extract were made. These preparations were administrated to female *Wistar* rats ($n = 5$) during 6 days (intra gastric via). The control group has received a solution of 0.9% NaCl. After that, Tc-99m (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, duodena, heart, kidney, spleen, stomach, pancreas, lung, ovary, blood, bone,

Table 1: Effect of a *Ginkgo biloba* crude extract (40 and 400mg/ml) on biodistribution of Tc-99m: %ATI/organ

Organs	Control	40mg/ml	400mg/ml
Brain	0.07±0.03	0.09±0.02	0.07±0.02
Liver	4.08±1.74	5.02±2.05	5.53±2.77
Duodena	0.59±0.18	0.38±0.06	0.26±0.19
Heart	0.42±0.12	0.43±0.20	0.34±0.10
Kidney	0.79±0.43	2.82±3.10	0.53±0.24
Spleen	0.23±0.04	0.15±0.06	0.20±0.08
Lung	0.91±0.60	1.06±0.57	0.79±0.37
Stomach	9.16±5.31	6.53±3.00	5.20±1.73
Pancreas	0.12±0.10	0.14±0.18	0.05±0.05
Blood	3.43±1.50	2.34±0.85	3.34±0.54
Bony	0.36±0.23	0.33±0.09	0.24±0.03
Muscle	0.21±0.15	0.65±0.36	0.16±0.10
Thyroid	0.99±0.47	1.62±0.68	0.80±0.49
Ovary	0.09±0.05	0.15±0.08	0.09±0.03

After 6 days of treatment with a 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/organ determined. Blood (1ml).

Table 2: Effect of a *Ginkgo biloba* crude extract (40 and 400mg/ml) on biodistribution of Tc-99m: %ATI/gram of tissue

Organs	Control	40mg/ml	400mg/ml
Brain	0.04±0.02	0.04±0.01	0.06±0.02
Liver	0.64±0.32	0.66±0.29	0.73±0.36
Duodena	1.01±0.37	0.55±0.09	0.51±0.36
Heart	0.42±0.17	0.30±0.17	0.35±0.08
Kidney	0.56±0.18	1.37±1.36	0.56±0.27
Spleen	0.33±0.12	0.20±0.11	0.27±0.07
Lung	0.61±0.35	0.53±0.38	0.55±0.19
Stomach	5.55±3.30	3.71±1.97	3.38±1.01
Pancreas	0.21±0.15	0.22±0.24	0.09±0.05
Blood	3.43±1.50	2.34±0.85	3.34±0.54
Bony	0.28±0.14	0.34±0.16	0.22±0.04
Muscle	0.12±0.07	0.13±0.13	0.09±0.06
Thyroid	2.72±1.18	1.62±0.68	1.10±1.14
Ovary	0.24±0.14	0.15±0.08	0.24±0.02

After 6 days of treatment with a 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 (1ml was considered to be 1 gram).

muscle and thyroid) and counted in a well counter (Automatic Gamma Counter Packard, Canada). The percentages of radioactivity per organ (%ATI/organ) and per gram of each organ (%ATI/gram) were calculated. A statistical analysis of the results (ANOVA test, with Dunnet test, p<0.05) was performed.

Histological preparations were carried out with some organs of interest (liver and kidney) from the animals that have received EGb (400mg/ml) per intra gastric via. The pieces of rats organs (treated and control) were fixed in 2.5% glutaraldehyde (Riedel-de Haen) in 0.1M cacodylate buffer (pH 7.2). The fixative was added with

either 0.25% tannic acid (Merck). The postfixation was in 1% osmium tetroxide (Sigma) (OsO₄), 0.8% potassium ferricyanide and 5mM calcium chloride (CaCl₂) in 0.1M cacodylate buffer. The tissues were dehydrated in acetone and embedded in Epon (Embed-812). Thin sections (2µm) were stained with toluidine blue (Vetec) and observed in light microscopy (Olympus BH2-RFCA).

Results

The Table 1 and 2 show the effect of the *Ginkgo biloba* crude extract on the biodistribution of Tc-99m (% ATI/organ and %ATI/gram) in the female *Wistar* rats which had received (40 and 400 mg/ml) or not (control group) the extract. The EGb altered (not significant, n = 5, p>0.05) the uptake of the Tc-99m in the kidneys and liver. A significant (n = 5, p<0.05) decrease in the uptake of this radiopharmaceutical in the duodena after the treatment with EGb 40 mg/ml from 0.59±0.18 to 0.38±0.06 (% ATI/organ) and with EGb 400 mg/ml from 0.59±0.18 to 0.26±0.19% (ATI/organ) was found (Table 1). Similar results in %ATI/gram with EGb 40 mg/ml from 1.01± 0.37 to 0.55±0.09 (%ATI/gram) and with EGb 400 mg/ml from 1.01±0.37 to 0.51±0.36 (%ATI/gram) were observed (Table 2).

Morphological alterations on kidney and liver due to treatment (*in vivo*) were found (Fig. 1 and 2). The Fig. 1B shows treated kidney where the glomerular capillary was replete of blood cells and with reduced diameter. The renal corpuscles and Bowman's space were increased, suggesting that the glomerular filtration rate could be increased. These alterations were not observed in the control kidney (Fig. 1A). In the Fig. 2B the treated liver exhibits enlargement of hepatocytes with abundant cytoplasmic contents and dilated hepatic sinusoidal spaces. These modifications were not found in the control liver (Fig. 2A). The decrease of the uptake of sodium perthecnetate by duodena was not capable to alter the optical microscopy of this organ (Fig. 3A and 3B).

Discussion

We can suggest that the *in vivo* treatment with EGb could generate metabolites with direct action on the morphology of organs and on biodistribution of Tc-99m. In order to elucidate the action mechanism of the *Ginkgo biloba* extract in the uptake of Tc-99m by organs, experiments with scavengers of reactive oxygen and chelating agents are now in progress. Histological preparations also are now in progress with others organs withdrawn from the animals that received EGb per intra gastric via.

In conclusion, the metabolization of the *Ginkgo biloba* extract (*in vivo*) could generate active metabolites that could modify the biodistribution of the treated animals.

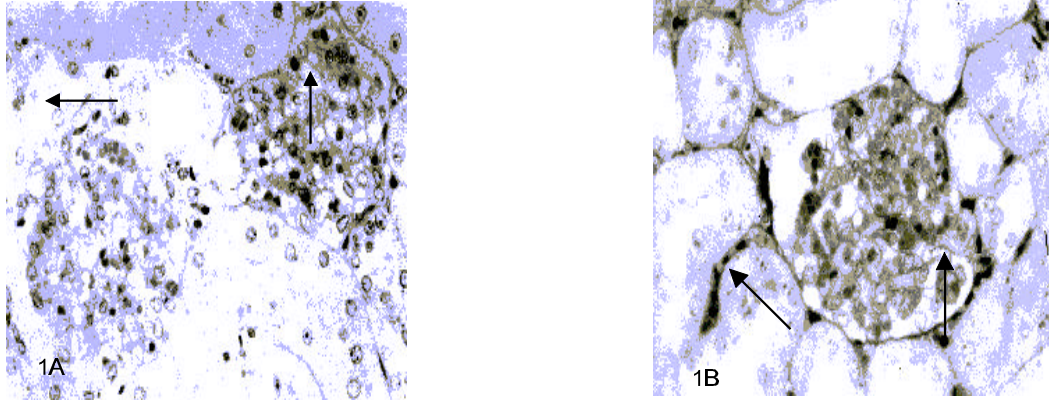


Fig. 1: Photomicrography of control (1A) and treated (1B) kidney with glomerular capillary (arrows), Bowman's space (stars). X800

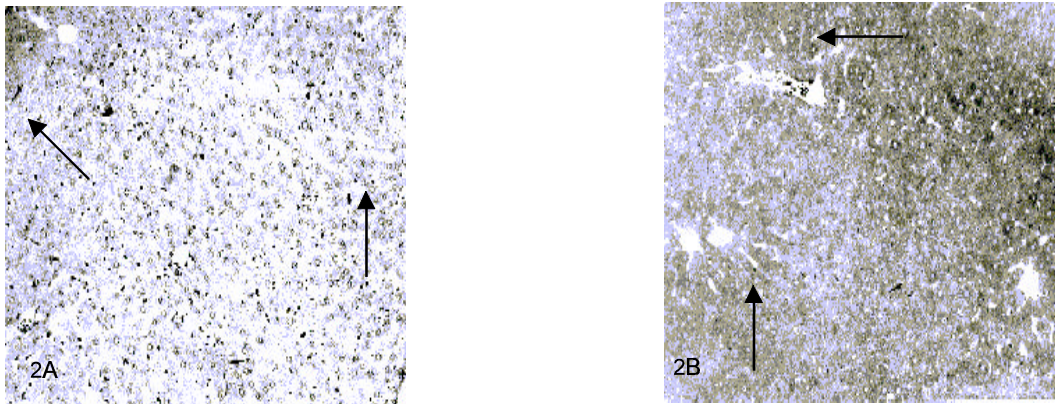


Fig. 2: Photomicrography of control (2A) and treated (2B) liver with hepatic sinusoidal spaces (arrows). X800

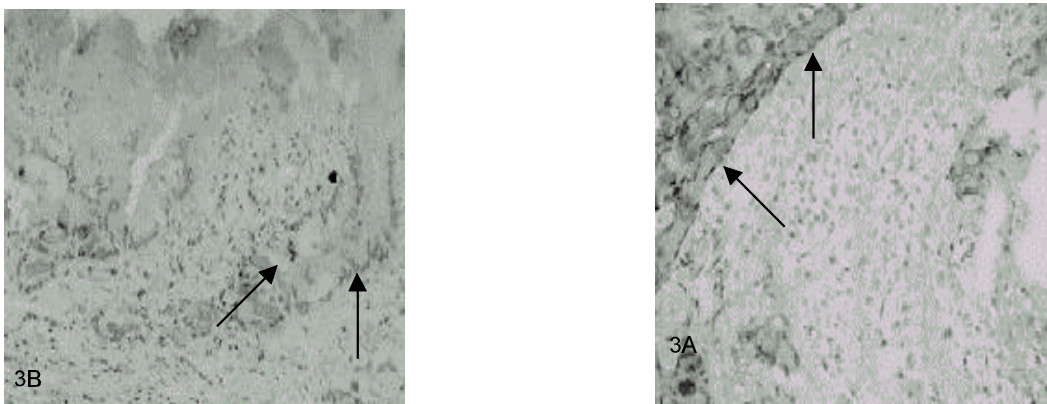


Fig. 3: Photomicrography of control (3A) and treated (3B) duodena with mucous cells. X800

The morphological alterations observed in kidney and liver such as, increase of the glomerular filtration and of fluid in Bowman's space, enlargement of the hepatocytes and of hepatic sinusoidal spaces could be explained by the metabolization of the *Ginkgo biloba* extract and its products with vasodilator and

anticoagulant properties.

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