Effect of *Ginkgo biloba* on the labeling of blood elements with technetium-99m: *in vitro* study

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Abstract

Ginkgo biloba is the phytoterapic most used in popular medicine in the treatment of cerebral senescence. Red blood cells (RBC) labeled with technetium-99m (Tc-99m) is used for several evaluations in nuclear medicine. This labeling depends on a reducing agent, usually the stannous ion. Any drug, which alters the labeling of the tracer, could be expected to modify the disposition of the radiopharmaceutical. We have evaluated the influence of the *Ginkgo biloba* extract on the labeling of RBC and plasma proteins with Tc-99m. Blood was withdrawn and incubated with Ginkgo biloba extract (0; 0.004; 0.04; 0.4; 4; 20 and 40 mg/ml). Stannous chloride (1.2 ml/ml) was added and, then, Tc-99m was added. Plasma (P) and blood cells (RBC) were isolated, also precipitated with trichloroacetic acid and soluble (SF) and insoluble fractions (IF) separated. The analysis of the results shows that there is a decrease in the radioactivity (from 97.7 ± 0.7 to 49.5 ± 3.9%) in RBC with the drug (4 mg/ml). In the labeling process of RBC with Tc-99m, the stannous and pertechnetate ions pass though the membrane, so, we suggest that the *Ginkgo biloba* effect can be explained by (i) an inhibition of the transport of these ions, (ii) damage in membrane, (iii) competition with the cited ions for the same binding sites, or (iv) possible generation of reactive oxygen species that could oxidize the stannous ion.

The use of medicinal plants or natural products has increased in the last decades all over the world. *Ginkgo biloba* is a gymnosperm considered a "living fossil" and is the phytoterapic most used in popular medicine in the treatment of cerebral senescence. *Ginkgo biloba* extract has important antioxidant properties due to probably to the presence of flavonoids[1,2,3].

In nuclear medicine, red blood cells (RBC) are usually labeled with technetium-99m (Tc-99m) and used as radiopharmaceutical in studies of the cardiac function, volemia and detection of gastrointestinal bleeding sites. Plasma proteins are also labeled with Tc-99m and used for evaluation of lung perfusion and location of placenta[4,5]. These labeling techniques involve the pre-tinning of the blood constituents with stannous ions, followed by exposure to Tc-99m, as sodium pertechnetate, which is reduced within of the cell and remains trapped intracellularly by the binding in the beta chain of hemoglobin [4,5, 6].

It is reported that many substances can alter the labeling of blood elements with Tc-99m[7]. There are some studies about the effect of the medicinal plants (*Thuya occidentalis*, *Nicotiana tabacum*, *Peumus boldus*, *Maytenus ilicifolia*) on the labeling of RBC (6,7,8, 9,10). We have studied the effect of *Ginkgo biloba* extract on the labeling of RBC and plasma proteins with Tc-99m.

The analysis of the results (Table 1) indicates that there is a significant decrease ($p<0.05$) on the uptake of Tc-99m by the red blood cells with the concentrations from 0.4 up to 20 mg/ml of the extract of *Ginkgo biloba*. The analysis of the results also indicates that there is a significant decrease ($p<0.05$) in the fixation of Tc-99m in insoluble fractions of the blood cells when the concentrations from 0.4 up to 40 mg/ml of the extract.

Table 1. Effect of *Ginkgo biloba* extract on the labeling of red blood cells (RBC) and on the insoluble fraction of the red blood cells (IF-RBC) and plasma (IF-P) with Tc-99m

Ginkgo biloba concentration (mg/ml)	RBC	IF-RBC	IF-P
0.00 (control)	97.7 ± 0.7	87.1 ± 2.8	73.8 ± 6.6
0.004	97.4 ± 0.3	88.0 ± 3.5	69.0 ± 3.2
0.04	96.3 ± 0.1	88.7 ± 1.8	75.3 ± 0.5
0.4	62.3 ± 3.1	68.7 ± 1.0	27.9 ± 0.7
4.0	49.5 ± 3.9	32.8 ± 10.7	11.6 ± 3.0
20.0	53.7 ± 0.9	32.2 ± 2.9	8.8 ± 0.1
40..0	48.1 ± 15.5	23.5 ± 12.1	8.3 ± 0.7

Samples of heparinized blood were incubated with different concentrations of *Ginkgo biloba* extract (0.004; 0.04; 0.4; 4; 20 and 40 mg/ml). A sample of heparinized whole blood was incubated with saline solution (NaCl 0.9%) as control. Then, stannous chloride (1.2mg/ml) and Tc-99m, as sodium pertechnetate were added. The radioactivity in P, RBC, IF-C and IF-P were determined in a well counter and the percent of radioactivity (% ATI) was calculated. A statistical analysis (ANOVA test, n=10) was used to compare the results.

Although the exact mechanism of the effect of *Ginkgo biloba* (40 - 0.4 mg/ml) on the labeling of RBC, IF-RBC and IF-P is not elucidated, we suggest that it might be explained by (i) a direct inhibition (chelating action) of the referred ions or (ii) by

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damages induced in plasma membrane; (iii) by competition of the cited ions to the same bindings sites; or (iv) by possible generation of reactive oxygen species that could oxidize the stannous ion.

Material and Methods

Decoction of a commercial *Ginkgo biloba* extract (Farmacutis RJ Brazil/ China Jiangsu Medicines and Health Products Lot no GB 001128) with 24% of active principle was prepared with 0.9% NaCl. Heparinized whole blood was withdrawn from Wistar rats. Blood samples (0.5 ml) were incubated, and gently mixed, with 100 ml of different dilutions of the *Ginkgo biloba* extract (0.004; 0.04; 0.4; 4; 20 and 40 mg/ml) for 60 min. After this period of time, 0.5 ml of a recently prepared stannous chloride solution (1.2 mg/ml) was added and the incubation continued for 60 min with this reducing agent. Then, 100 ml of Tc-99m, recently milked from a 99Molibdenium/99mTechnetium generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo, Brazil) were added and the incubation was continued for another 10 min. These samples were centrifuged and plasma (P) and blood cells (RBC) were separated.

Samples (20 ml) of P and RBC were precipitated with 1 ml of trichloroacetic acid (TCA) (5 %) and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity in P, RBC, IF-P, SF-P, IF-RBC and SF-RBC were determined in a well counter (Clinigamma, gamma counter, LKB, Wallac, Finland). After that, the percent of radioactivity (% ATI) was calculated, as previously described[6,9]. A statistical analysis (ANOVA test and Tukey-Kramer test) was employed to compare the experimental data.

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