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Artigo

Influence of biflorin on the labelling of red blood cells, plasma protein, cell protein, and lymphocytes with technetium-99m: *in vitro* study

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RESUMO: "Influência da biflorina na marcação do tecnécio-99m em células vermelhas do sangue, proteínas do plasma, proteínas celulares e em linfócitos: estudos *in vitro*". Neste artigo relatam-se os resultados de um estudo *in vitro* envolvendo a influência da biflorina (uma *o*-quinona isolada de *Capraria biflora* L. que possui uma potente atividade antimicrobiana) na marcação do Tc-99m em células vermelhas do sangue, proteínas do plasma, proteínas celulares e em linfócitos. O sangue foi coletado de ratos *Wistar* e incubado com várias concentrações de biflorina, e soluções de cloreto estanoso ($SnCl_2$) adicionando-se Tc-99m. O plasma (P) e as células vermelhas do sangue (CVS) foram isolados, precipitados e centrifugados, isolando-se as frações solúveis (FS) e insolúveis (FI). A maior concentração de biflorina (100%) é capaz de reduzir a captação do Tc-99m (%ATI) nas CVS e a fixação na FI-P. Uma solução de 0,2 mL de linfócitos (2,5 mL; 1.0 x 10^6 células/mL), obtidos por centrifugação de sangue humano tratado com Ficoll-Hypac, foi incubada com biflorina (0,1 mL). Soluções de cloreto estanoso e Tc-99m foram então adicionadas. Os linfócitos foram separados e o %ATI presente nessas células foi avaliado. Uma redução no %ATI (de 97,85 ± 0,99 a 88,86 ± 5) foi observada para CVS e para FI-P (73,24 ± 5,51 a 20,72 ± 6,95). Os resultados não mostraram decréscimo no %ATI para os linfócitos com biflorina.

Unitermos: *Capraria biflora*, radiofarmacêuticos, pertecnetato de sódio.

ABSTRACT: In this paper we report the results of an *in vitro* study involving the influence of biflorin (an *o*-quinone isolated from *Capraria biflora* L. that has potent antimicrobial activity) on the Tc-99m labeling of red blood cells, plasma protein, cells protein, and lymphocytes. Blood was withdrawn from *Wistar* rats and incubated with various concentrations of biflorin, and solutions of stannous chloride and Tc-99m were added. Plasma (P) and red blood cells (RBC) were isolated, precipitated, and centrifuged, and soluble (SF) and insoluble (IF) fractions were isolated. The results show that the highest concentration (100%) of biflorin is able to reduce the uptake of Tc-99m (%ATI) on RBC and the fixation on IF-P. To study the influence of biflorin on 99mTc lymphocyte labeling, human blood was submitted to a technique with Ficoll-Hypac and centrifuged, and white cells were isolated. Lymphocytes (2.5 mL; 1.0 x 10^6 cells/mL) were obtained and a 0.2 mL solution was incubated with biflorin (0.1 mL). Solutions of stannous chloride and 99mTc were added. Lymphocytes were separated and the %ATI bound in these cells was evaluated. A reduction in %ATI (from 97.85 ± 0.99 to 88.86 ± 5) was observed for RBC and for IF-P (73.24 ± 5.51 to 20.72 ± 6.95). In this case the results showed no decrease in %ATI for the lymphocytes with biflorin.

Keywords: *Capraria biflora*, radiopharmaceuticals, sodium pertecnetate.

INTRODUCTION

Capraria biflora L. is a species that belongs to the Schrophulariaceae family, and is originally from the Antilles and South America. In Brazil, it is distributed among the states of Minas Gerais and Goiás, and in the coastal area between the states of Piauí and Espírito Santo (Menezes, 1949). In this region, it is known as "chá-da-terra", "chá-do-méxico", "chá-da-martinica", "chá-do-rio", "chá-da-américa", "chá-das-antilhas", and "chá-de-

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lima" (Comerford, 1996). Biflorin can be isolated from roots of *C. biflora* L., a substance with an *o*-quinone structure, which has antibiotic activity against Gram-positive bacteria, yeasts, and fungi (Figure 1) (Lima et al., 1958, 1962; Lima; D'Albuquerque, 1958).

We have evaluated the influence of biflorin on the *in vitro* Tc-99m labeling of red blood cells, plasma protein, cell protein, and lymphocytes. Radiopharmaceuticals are molecules or cells that contain a radioactive atom in their structure and can be used in human beings for the diagnosis or treatment of some diseases (Saha, 1998). The interactions between radiopharmaceuticals and other drugs is an important factor that has received a lot of attention from researchers - these interactions can change the chemical nature of the radiotracer and the biochemical medium of the target, or can interfere in the radiotracer's binding to plasma proteins and/or blood cells (Hladik III; Saha, 1987; Srivastava; Straub, 1990; Sampson, 1996). This may result in the radiopharmaceutical's incorrect biodistribution, leading to imprecise diagnosis, repetitive exams, and patients' unnecessary exposure to radiation (Hladik III; Saha, 1987; Hicks; Arkles, 1992; Kelly et al., 1992; Bernardo-Filho et al., 1994; Santos et al., 1995; Eising; Reiners, 1998).

MATERIAL AND METHODS

Biflorin obtention

A hydroalcoholic extract of the roots of *Capraria biflora* L. was concentrated at low pressure and submitted to a chromatographic column using SiO_2 (230-400 mesh) as support and a mixture of toluene/AcOEt (8:2); biflorin was yielded in the form of violet crystals.

Preparation of the solutions

A stock solution was prepared dissolving 25 mg of biflorin in 10 mL of a 0.9% NaCl solution; after centrifugation this was considered as 100% (2.5 mg/mL). Subsequent to successive dilutions, the following solutions were prepared: 50% (1.25 mg/mL), 25% (0.625 mg/mL), 12.5% (0.3125 mg/mL) and 6.25% (0.1562 mg/mL).

Animals

Wistar rats (200-250 g) of both sexes were used, obtained from the Universidade do Estado do Rio de Janeiro. The animals were maintained under constant environmental conditions (22 ± 5 ºC, 12 h of light/dark cycle).

Lymphocyte obtention

Samples of human blood were treated with Ficoll-Hypac. A lymphocyte solution was obtained (10^6 cells/mL) after centrifugation and treatment with a 0.9% saline solution.

Study protocol

The Tc-99m used was obtained from a 99Molibdenium/99mTechnetium generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, Brazil). Radioactivity was determined in a gamma well counter (Clinigamma, LKB, Wallac, Finland). The Follow methodology was based in Oliveira et al. (2000) and Santos-Filho; Bernardo-Filho (2005).

Samples (0.5 mL) of heparinized whole blood (n = 4) from *Wistar* rats were incubated at room temperature for 60 min with 100 µL of biflorin (6.25, 12.5, 25, 50, and 100%). After the incubation period, 0.5 mL of $SnCl_2$ (1.2 µg/mL) was added as $SnCl_2.2H_2O$ and the incubation continued for another hour. The next step was to add 0.1 mL of Tc-99m (3.7 MBq) as sodium pertechnetate ($^{99m}NaTcO_4$), and the incubation continued for another 10 min. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Aliquots (20 µL) of P and BC were precipitated with 1 mL of trichloroacetic acid (TCA 5%), and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity in P, BC, IF-P, SF-P, IF-BC, and SF-BC were determined. Subsequently, the percentage of radioactivity (%ATI) was calculated. A saline solution (NaCl 0.9%) was used for the control experiments.

Samples (200 µL) of the lymphocyte solution (n = 3) were incubated with 100 µL of biflorin (100%) during one hour. After the incubation, 0.1 mL of Tc-99m (3.7 MBq) as sodium pertechnetate ($^{99m}NaTcO_4$) was added and the incubation continued for another 10 min. In the control experiment, the incubation was undertaken with a saline solution (NaCl 0.9%). The aqueous solution (S) and the lymphocytes (L) were separated, and the percentage of radioactivity (% ATI) was calculated.

A statistical analysis (ANOVA) was used in order to compare the results obtained ($p < 0.05$).

RESULTS

Table 1 presents the distribution of radioactivity in plasma and red blood cells from whole blood incubated *in vitro* with different concentrations of biflorin. The analysis of the results indicates that there is an extremely significant decrease ($p < 0.001$) in the uptake of 99mTc in the red blood cells, with the 50% concentration going from 92.92 ± 4.50 to 79.51 ± 3.15 and the 100% concentration reaching 64.06 ± 1.95.

Table 2 shows the fixation of radioactivity in soluble and insoluble fractions of the whole blood plasma incubated *in vitro* with different concentrations of biflorin. The results indicates an extremely significant decrease ($p < 0.001$) in the fixation of 99mTc for the

6.25% concentration (from 71.68 ± 1.70 to 6.30 ± 2.09), the 25% concentration (decrease to 46.83 ± 3.01), the 50% concentration (34.52 ± 9.16), and 100% concentration (29.51 ± 7.11). There is a very significant decrease ($p < 0.01$) for the 12.5% concentration, which dropped to 49.42 ± 3.37.

Table 3 shows the distribution of radioactivity in soluble and insoluble fractions of red blood cells incubated *in vitro* with biflorin. The analysis of the results indicates an extreme decrease ($p < 0.001$) in radioactivity fixation in the insoluble fraction of red blood cells only with the 100% concentration (from 80.83 ± 4.82 to 56.45 ± 8.71).

Table 4 shows the percentage of radioactivity in lymphocytes from the aqueous solution incubated with biflorin (100%). The results indicate no decrease on the lymphocyte labeled at the concentrations used.

DISCUSSION

The *in vitro* studies indicated that biflorin behaves similarly to commercial drugs such as hydralazine, prazosin, digoxin, and methyldopa, which decrease the efficiency of red blood cell labeling with Tc-99m. Other drugs - such as propanolol, chlorothiazide, and furosemide - can also significantly reduce labeling

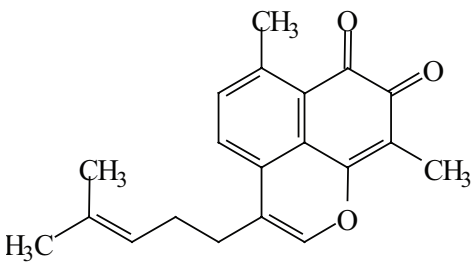

Figure 1. Chemical structure of biflorin, isolated from the roots of *Capraria biflora* L.

Table 1. Influence of biflorin on the labeling of red blood cells and plasma with Tc-99m.

Biflorin concentrations (%)	%ATI	
	BC	P
0.0 (control)	92.92 ± 4.50	7.06 ± 4.50
6.25	97.07 ± 1.16^{ns}	2.92 ± 1.16
12.5	96.7 0± 0.64^{ns}	3.28 ± 0.64
25.0	92.14 ± 1.53^{ns}	7.85 ± 1.53
50.0	79.51 ± 3.15***	20.48 ± 3.15
100.0	64.06 ± 1.95***	35.92 ± 1.95

*** - value of $p < 0.001$ considered extremely significant; ns - not significant; %ATI - percentage of radioactivity; P - plasma; BC - blood cells.

Table 2. Effect of biflorin on the labeling of insoluble fractions of plasma with Tc-99m.

Biflorin concentrations (%)	%ATI	
	IF-P	SF-P
0.0 (control)	71.68 ± 1.70	28.30 ± 1.70
6.25	6.30 ± 2.09***	93.68 ± 2.09
12.5	49.42 ± 3.37**	50.57 ± 3.37
25.0	46.83 ± 3.01***	53.15 ± 3.01
50.0	34.52 ± 9.16***	65.46 ± 9.16
100.0	29.51 ± 7.11***	70.47 ± 7.11

** - value of $p < 0.01$ considered very significant; *** - value of $p < 0.001$ considered extremely significant; %ATI - percentage of radioactivity; SF-P - soluble fraction of plasma; IF-P - insoluble fraction of plasma.

Table 3. Influence of biflorin on the labeling of insoluble fractions of red blood cells with Tc-99m.

Biflorin concentrations (%)	%ATI	
	IF-BC	SF-BC
0.0 (control)	80.83 ± 4.82	19.15 ± 4.82
6.25	88.70 ± 3.76^{ns}	11.28 ± 3.76
12.5	79.38 ± 1.60^{ns}	20.60 ± 1.60
25.0	81.17 ± 2.47^{ns}	18.81 ± 2.47
50.0	77.75 ± 2.68^{ns}	22.23 ± 2.67
100.0	56.45 ± 8.71***	43.53 ± 8.71

Value of $p < 0.001$ considered extremely significant; ns - not significant; %ATI - percentage of the radioactivity; SF-C - soluble fraction of the blood cells; IF-C - insoluble fraction of the blood cells.

Table 4. Percentage of radioactivity (%ATI) of the lymphocytes incubated in vitro with biflorin.

Fraction	%ATI	
	Control	Biflorin (100%)
S	9.54±2.18	10.43±7.47^{ns}
L	90.45±2.18	89.56±7.47^{ns}

%ATI - percentage of radioactivity; ns - not significant; S - aqueous solution; L - lymphocytes.

efficiency in clinical doses (Sampson, 1996). *In vitro* tests have demonstrated that biflorin also decrease the labeling of cellular protein by Tc-99m, which may indicate that it is affecting the permeability of red blood cell membranes.

The processes involved in the passage of $SnCl_2$ and sodium pertechnetate ions into RBCs have not yet been completely revealed, although data suggests that the former passes through the cell membrane by way of selective calcium channels, and the latter by the "band-3 anion" transport system (Sampson, 1996; Callahan; Rabito, 1990; Gutfilen et al., 1992; Smith, 1985; Stryer, 1998).

When a drug or plant extract alters the activity percentage (ATI%) of hemocytes or other blood components, it may be the result of: competition with $SnCl_2$ or sodium pertechnetate for plasma membrane transport mechanisms; morphological modification of the red blood cells at the plasma membrane level, favoring or inhibiting transport through them; competition for other $SnCl_2$, sodium pertechnetate, or radiotracer binding sites; destabilization of the link between Tc-99m and plasma protein, which would alter their distribution within the organism's circulatory system; action as oxidizing or reducing agents and altering the valence of the tin or pertechnetate ions, thus increasing or decreasing the red blood cells' labeling process (Hladik III; Saha, 1987; Santos et al., 1995).

In a general way it was demonstrated that biflorin can alter the binding of radiopharmaceutical Tc-99m to blood components. Thus, the administration of pharmaceutical preparations containing both biflorin and the radiotracer pertechnetate may result in incorrect diagnostic interpretations (Carlsson, 1995).

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