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Effect of *Thuya occidentalis* on the Labeling of Red Blood Cells and Plasma Proteins with Technetium-99m

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Thuya occidentalis is used in popular medicine in the treatment of condyloma and has antibacterial action. Red blood cells (RBC) labeled with technetium-99m (99mTc) are used for several evaluations in nuclear medicine. This labeling depends on a reducing agent, usually 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 *T. occidentalis* extract on the labeling of RBC and plasma proteins with 99mTc. Blood was withdrawn and incubated with *T. occidentalis* (0.25; 2.5; 20.5; and 34.1 percent v/v). Stannous chloride (1.2 μg/ml) was added and then 99mTc was added. Plasma (P) and blood cells (BC) 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 radioactivity (from 97.64 to 75.89 percent) in BC with 34.1 percent of the drug. In the labeling process of RBC with 99mTc, the stannous and pertechnetate ions pass through the membrane, so we suggest that the *T. occidentalis* effect can be explained (i) by an inhibition of the transport of these ions, (ii) by damage in membrane, (iii) by competition with the cited ions for the same binding sites, or (iv) by possible generation of reactive oxygen species that could oxidize the stannous ion.

INTRODUCTION

Thuya occidentalis (American arbor vitae) extract is used in popular medicine as expectorant, diuretic, antihelmintic, stimulant, and in rheumatism treatment. It acts on the renal epithelium and has a toxic action in bladder muscles. It is used in the treatment of cystitis and prostatic hypertrophy in senile men and in urinary incontinence in women. As a tincture, it is popularly used as an abortifacient, as an agent for cauterization of papillomas and condylomatous warts [1, 2, 3]. The biological effects of *T. occidentalis* have been studied previously [1, 2, 3, 4].

Technetium-99m (99mTc) has been the most utilized radionuclide in clinical nuclear medicine procedures [5, 6, 7], and it has also been used in basic research [8, 9, 10, 11, 12]. Its wide use in nuclear medicine is due to its optimal physical characteristics (half-life of 6h, gamma ray energy of 140 keV and resulting minimal dose to the patient), convenient availability from a 99Mo/99mTc generator, and negligible environmental impact. Almost

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[e] *Abbreviations:* RBC, red blood cells; P, plasma; PP, plasma proteins; BC, blood cells; TCA, trichloroacetic acid; SF, TCA-soluble fracton; IF, TCA-insoluble fraction.

all scanning devices currently in use are optimized for detecting the readioactive emission from this radionuclide [5, 6].

Of the many applications of 99mTc-labeled red blood cells (RBC)[e] most important is in cardiovascular nuclear medicine, where one tries to image the heart to determine its functional status as a pump, to calculate the left ventricular function by measuring the ejection fractions, and to evaluate wall motion abnormalities. Some other applications are in the detection of gastrointestinal bleeding and in the determination of the RBC mass in patients [5, 6, 13]. If one damages the RBC, one can do selective spleen imaging (since damaged cells are rapidly sequestered by the spleen) [14]. RBC have been labeled with 99mTc for *in vitro* [6, 14], *in vivo* [5, 6, 13] or *in vivo/in vitro* [5, 6, 15] techniques. The labeling process with 99mTc depends on a reducing agent and stannous ion (Sn) is usually used for this purpose. When whole blood is employed in the labeling of RBC with 99mTc, radioactivity is found in blood cells, however it is also bound to plasma proteins. This labeling process depends on optimal stannous chloride concentration [16, 17, 18, 19, 20]. Stannous and pertechnetate ions cross the RBC membrane, probably by active transport [21]. The radionuclide is mainly bound to the hemoglobin molecule [22, 23, 24]. Several of the cellular labeling steps have been well-characterized. The band-3 anion transport system [17] and calcium channels [16] may be the ways that 99mTc [17] and Sn [16], respectively, reach the interior of the RBC.

Unexpected patterns of radiopharmaceutical biodistribution can be associated with a disease. However many factors, including drug therapy, radiation therapy, dietary conditions, besides pathological process could affect the biodistibution of the radiopharmaceutical. Any chemical, physical or biological agent which alters (i) the chemical identity of the tracer, (ii) the physiological status of the organ of interest, or (iii) its binding capability to plasma proteins or other blood element, could be expected to modify the radiopharmacokinetics and the disposition of the radiopharmaceutical in the specific target. If unknown, such factors may lead to poor visualization, requiring the repetition of the examination procedure resulting in unnecessary irradiation to the patient or even misdiagnosis. Many drugs have been reported to affect the biodistribution of different radiopharmaceuticals [5, 25, 26]. The labeling of red blood cells with 99mTc has been influenced by patient medications [5, 25] or the labeling conditions [15, 20, 27, 28]. Therapy with β-adrenergic blockers, calcium channel blockers or nitrate may result in normal exercise radionuclide ventriculograms even in the presence of significant coronary artery disease. Thus, the presence of the disease may be missed and/or underestimated [25].

In this way, an unusual behavior of the radiopharmaceutical could, on occasion, be due to a drug. Nevertheless, there is not a well established *in vitro* model to study the interaction of therapeutic drugs with radiopharmaceuticals. Thus, we have evaluated the influence of a *T. occidentalis* extract on the labeling of RBC and plasma proteins with Tc-99m using an *in vitro* technique.

MATERIAL AND METHODS

These experiments were performed without sacrificing the animals. Heparinized whole blood was withdrawn from Wistar rats. Samples of 0.5 ml were incubated with 100 µl of different concentrations of a commercial (Laboratório Simões LTDA, Rio de Janeiro, Brazil) *T. occidentalis* extract (0.25; 2.5; 20.5; and 34.1 percent v/v) for 1 hr at room temperature. A sample of heparinized whole blood was incubated with saline solution (NaCl, 0.9 percent) as control. Then, 0.5 ml of stannous chloride (1.2 µg/ml), as $SnCl_2.2H_2O$ (Merck, Brazil) was added and the incubation continued for another 1 hr. After this period of time, 99mTc (0.1 ml), as sodium pertechnetate, recently eluted from a 99Mo/99mTc generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia

Nuclear, Brazil), was added and the incubation continued for another 10 min. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20 µl) of P and BC were precipitated with 1 ml of trichloroacetic acid (TCA) (5 percent) 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 in a well counter (Clinigamma, gamma counter, LKB, Wallac, Finland). After that, the percent of radioactivity (percent rad) was calculated, as previously described [19, 20]. A statistical analysis (ANOVA test) was employed to compare the experimental data.

RESULTS

Table 1 shows the distribution of the radioactivity in plasma and blood cells from whole blood treated with different concentrations of *T. occidentalis* extract. The analysis of the results indicates that there is a significant decrease ($p < .05$) in the uptake of 99mTc by the red blood cells (from 97.64 to 75.89 percent) with the concentration of 34.1 percent (v/v) of the extract.

Table 2 shows the fixation of the radioactivity in the insoluble fraction of plasma obtained from whole blood treated with different concentrations of T. occidentalis extract. The analysis of the results indicates that there is a significant decrease ($p < .05$)

Table 1. Effect of *Thuya occidentallis* on the labeling of blood cells and plasma with 99mTc.

Thuya occidentallis concentrations (v/v)	Blood cells (BC)	Plasma (P)
0.00 (control)	97.64 ± 1.43	2.24 ± 1.43
0.25	95.40 ± 4.38	4.60 ± 4.38
2.52	92.63 ± 5.91	7.27 ± 5.91
20.50	90.88 ± 4.61	9.12 ± 4.61
34.10	75.89 ± 6.29	24.11 ± 6.29

Samples of heparinized blood were incubated with different concentrations of *T. occidentalis* extract (0.25; 2.5; 20.5; and 34.1 percent v/v). A sample of heparinized whole blood was incubated with saline solution (NaCl, 0.9 percent) as control. Then, stannous chloride (1.2 µg/ml) and 99mTc, as sodium pertechnetate were added. The radioactivity in P and BC was determined in a well counter and the percent of radioactivity (percent rad) was calculated. A statistical analysis (ANOVA test, $n = 12$) was employed to compare the results

in the fixation of 99mTc in plasma proteins when the concentrations of 20.50 and 34.1 percent (v/v) of the referred drug are employed.

Table 3 shows the fixation of the radioactivity in the insoluble fraction of blood cells obtained from whole blood treated with different concentrations of *T. occidentalis* extract. The analysis of the results indicates that there is not a significant decrease in the fixation of 99mTc in insoluble fractions of the blood cells at any of the tested concentrations of the drug.

DISCUSSION

Contrary to the numerous theoretical and practical studies of the conventionally used pharmacologically active agents, the data concerning interaction of diagnostic agents,

Table 2. Effect of *Thuya occidentallis* on the labeling of plasma proteins (PP) with 99mTc

Thuya occidentallis concentrations (v/v)	Insoluble fraction (IF-P)	Soluble fraction (SF-P)
0.00 (control)	79.63 ± 3.71	20.27 ± 3.71
0.25	78.00 ± 5.67	22.00 ± 5.67
2.52	73.65 ± 4.78	26.35 ± 4.78
20.50	22.88 ± 5.56	77.12 ± 5.56
34.10	46.91 ± 3.73	53.09 ± 3.73

Samples of heparinized blood were incubated with different concentrations of *T. occidentalis* extract (0.25; 2.5; 20.5; and 34.1 percent v/v). A sample of heparinized whole blood was incubated with saline solution (NaCl, 0.9 percent) as control. Then, stannous chloride (1.2 μg/ml) and 99mTc, as sodium pertechnetate were added. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20 μl) of P were precipitated with trichloroacetic acid (TCA) 5 percent and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity in SF-P and IF-P was determined in a well counter and the percent of radioactivity (percent rad) was calculated. A statistical analysis (ANOVA test, n = 12) was employed to compare the results

Table 3. Effect of *Thuya occidentallis* on the labeling of blood cells proteins with 99mTc

Thuya occidentallis concentrations (v/v)	Insoluble fraction (IF-BC)	Soluble fraction (SF-BC)
0.00 (control)	79.70 ± 2.12	20.30 ± 2.12
0.25	80.43 ± 2.39	19.57 ± 2.39
2.52	79.59 ± 2.62	20.41 ± 2.62
20.50	79.27 ± 2.51	20.63 ± 2.51
34.10	76.19 ± 3.14	23.81 ± 3.14

Samples of heparinized blood were incubated with different concentrations of *T. occidentalis* extract (0.25; 2.5; 20.5; and 34.1 percent v/v). A sample of heparinized whole blood was incubated with saline solution (NaCl, 0.9 percent) as control. Then, stannous chloride (1.2 μg/ml) and 99mTc, as sodium pertechnetate were added. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20 μl) of BC were precipitated with trichloroacetic acid (TCA) 5 percent and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity in SF-BC and IF-BC was determined in a well counter and the percent of radioactivity (percent rad) was calculated. A statistical analysis (ANOVA test, n = 12) was employed to compare the results.

including radiopharmaceuticals, are relatively scarce. A therapeutic drug can also modify the nature/amount of the 99mTc-radiopharmaceutical bound to blood elements and this may result in unexpected behavior of the radiopharmaceutical. There is not a well-established model to study the interaction of therapeutic drugs with radiopharmaceuticals. Care must be taken when attempting to extrapolate experimental data to the clinical situation, since the observed effects may depend on the amounts of the drug [5, 25, 26].

We agree with Hesslewood and Leung [25] that many reports on drug interactions with radiopharmaceuticals are anecdotal and in some instances a direct cause and effect relationship has not been unequivocally established. This situation could be remedied with the development of *in vitro* tests to evaluate the drug/radiopharmaceutical interactions.

We have studied the effect of a commercial extract of *T. occidentalis* on the labeling of red blood cells with 99mTc and the fixation of this radionuclide to insoluble fractions of plasma (plasma proteins) and to blood cells (blood cells proteins). In the labeling

process of RBC with Tc-99m, the stannous and pertechnetate ions pass through the plasma membrane. Although the exact mechanism of the effect of *T. occidentalis* (34.10 percent v/v) on the labeling of red blood cells is not elucidated, we suggest that it might be explained (i) by a direct inhibition (chelating action) of the referred ions, (ii) by damage induced in the plasma membrane, or (iii) by competition of the cited ions for the same binding sites.

Plasma proteins (PP) were also labeled with the referred radionuclide. 99mTc-labeled PP have been used to locate the placenta, to evaluate cardiac function and pulmonary perfusion, to determine blood volume, and to study gastrointestinal protein loss [5, 6]. When a concentration of *T. occidentalis* extract (20.50 and 34.10 percent v/v) is incubated with whole blood there is a decrease in the fixation of 99mTc in the insoluble fraction of plasma (plasma proteins). The chemical agents present in this extract could (i) bind (chelating action) to stannous and pertechnetate ions and/or (ii) bind to the binding sites of 99mTc in plasma proteins.

Red blood cell proteins can be labeled with 99mTc, mainly in the β-chain of the hemoglobin molecule [22, 23]. The fixation of this radioactivity seems not to be modified by *T. occidentalis* extract. Another possibility is that there is a blocking of the labeling inside the cells, but the 99mTc (free) could be precipitated with the red blood cell ghost.

The genotoxic effect of another natural product extract (*Paullinia cupana*) has been associated to the generation of reactive oxygen species [29] that are oxidizing agents. If *T. occidentalis* would be capable of generating reactive oxygen species, these could oxidize the stannous to stannic ion. This action could explain the decrease on the labeling of red blood cells and plasma proteins with 99mTc in the presence of high concentration of *T. occidentalis* extract.

We can conclude that, depending on *T. occidentalis* concentration, the labeling of red blood cells with 99mTc can be decreased, as well as the fixation of radioactivity in plasma proteins, at least, when an *in vitro* technique to label red blood cells is employed.

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