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# Consequences of the Magnetic Field, Sonic and Radiofrequency waves and Intense Pulsed Light on the Labeling of Blood Constituents with Technetium-99m

**Patricia Froes Meyer[1], Sebastião David Santos-Filho[1,2], Oscar Ariel Ronzio[3], Ludmila Bonelli[4], Adenilson de Souza da Fonseca[2]*, Iris do Ceu Clara Costa[1], José Brandão Neto[1], Aldo da Cunha Medeiros[1] and Mario Bernardo-Filho[2,5]**

*[1]Programa de Pós-Graduação em Ciências da Saúde; Centro de Ciências da Saúde; Universidade Federal do Rio Grande do Norte; Av. General Gustavo Cordeiro de Farias, s/n; 59010-180; Natal - RN - Brasil. [2]Laboratório de Radiofarmácia Experimental; Departamento de Biofísica e Biometria; Instituto de Biologia Roberto Alcantara Gomes; Universidade do Estado do Rio de Janeiro; Av. 28 de setembro, 87; 20551-030; adenilso@uerj.br; Rio de Janeiro - RJ - Brasil. [3]Universidad de Buenos Aires; Ciudad Autónoma de Buenos Aires - Argentina. [4]Universidade Salgado de Oliveira; Belo Horizonte - MG - Brasil. [5]Coordenadoria de Pesquisa; Instituto Nacional do Câncer; Praça Cruz Vermelha, 23; 20230-130; Rio de Janeiro - RJ -Brasil*

## ABSTRACT

*Sources of magnetic field, radiofrequency and audible sonic waves and pulsed light have been used in physiotherapy to treat different disorders. In nuclear medicine, blood constituents(Bl-Co) are labeled with technetium-99m ($^{99m}$Tc) are used. This study evaluated the consequences of magnetic field, radiofrequency and audible sonic waves and intense pulsed light sources on the labeling of Bl-Co with $^{99m}$Tc. Blood from Wistar rats was exposed to the cited sources. The labeling of Bl-Co with $^{99m}$Tc was performed. Blood not exposed to the physical agents was used(controls). Data showed that the exposure to the different studied sources did not alter significantly ($p>0.05$) the labeling of Bl-Co. Although the results were obtained with animals, the data suggest that no alteration on examinations performed with Bl-Co labeled with $^{99m}$Tc after exposition to the cited agents. The biological consequences associated with these agents would be not capable to interfere with some properties of the Bl-Co.*

**Key words:** Blood constituents; magnetic field, sonic and radiofrequency waves, technetium-99m

## INTRODUCTION

In physiotherapy some devices have been used to treat different disorders or to esthetical propose (Chang et al., 2007, Heinrich, 2007). These devices emit sonic and radiofrequency waves while others are capable to generate magnetic fileds (Johns et al., 2002; Heinrich, 2007). It has described positive effects of sonic waves (bioressonance) on cicatrization process in human beings increasing the collagen synthesis (Capponi and Ronzio, 2006). The use of radiofrequency waves is based on heating of tissue irradiated beyond to 50 $^{o}$C where cell death is induced by protein coagulation and they could be used to treat tumors (Pearce and Thomsen, 1995). Intense pulsed light sources have been used to treat abnormal cicatrices (Perez Rivera et al., 2002). In some reports beneficial effects of magnetic fields on bone metabolism and accelerate hydroxiapatite

* Author for correspondence

osteointegration suggesting osteogenesis stimulation have been described (Giordano et al., 2001).

Radionuclides have been used in investigations (clinical and basic sicences) (Saha, 2004, Joseph et al., 2006). Technetium-99m ($^{99m}$Tc) has been the most utilized radionuclide to label cells or molecules used as radiobiocomplexes (Bernardo-Filho et al., 2005) in the single photon emission computed tomography (SPECT) (Saha, 2004). This radionuclide has also been used in basic research (Pettersson et al., 2005; Fonseca et al., 2007).

Blood constituents labeled with $^{99m}$Tc are used in nuclear medicine (Wong et al., 2004; Harel et al., 2005; Olds et al., 2005) for measurement of red cell volume detection, recognition of gastrointestinal bleeding, identification of hemangiomas, gated blood pool study and other purposes (Saha, 2004). This labeled process depends on an optimal stannous chloride concentration and can be performed using either *in vivo* or *in vitro* methods, or by a combination of both (Saha, 2004). In the red blood cells, the transport of the $^{99m}$Tc-pertechnetate ion by the band-3 system (Callahan and Rabito, 1990) and the stannous ion by the calcium channels (Gutfilen et al., 1992) to the interior of the cells have been suggested.

An experimental model based on the labeling of blood constituents with $^{99m}$Tc has been used to assess some properties of synthetic and natural (Abreu et al., 2006; Fonseca et al., 2007). Moreover, no report has described the effects of physical agents used in physiotherapy on the radiolabeling of blood constituents. Thus, the aim of this work was to evaluate the effect of magnetic field, sonic and radiofrequency waves and intense pulsed light on the labeling of blood constituents with $^{99m}$Tc.

## MATERIALS AND METHODS

### Animals

Adult male *Wistar* rats (3-4 months, 250-300g) were maintained in a controlled environment. The animals had free access to water and food and ambient temperature was kept at 25 ± 2ºC. Experiments were conducted in accordance with the Institutional Committee of Animal Care (*Comissão de Ética para o Cuidado e Uso de Animais Experimentais, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro*) with the protocol number CEA/134/2006.

### Exposition of blood samples to physical agents

Heparinized blood (500µl, n=8 for each agent) was withdrawn from *Wistar* rats (n=8) and exposed to magnetic field (50 gauss, 30 minutes to both poles), sonic waves (3 kHz, 20 minutes), radiofrequency waves (550 kHz, 5 minutes, *Vip Eletrônica*, Brazil) and intense pulsed light (2 pulses, pulse time 0.01 s, 3-7 J/cm2 to each pulse, wavelength 400-1200 nm, Radiance®, Israel).As control, blood samples no exposed to the physical agents.

### Radiolabeling of blood constituents

The experiments were carried following the protocol published elsewhere (Bernardo-Filho et al., 1983). Briefly, after exposition to physical agents, 500µl of freshly prepared solution of stannous chloride (1.2 µg/ml) was added and the incubation continued for further 1 hour. After this period of time, 100µl $^{99m}$Tc (3.7MBq) as sodium pertechnetate ($Na^{99m}TcO_4$), recently milked from a $^{99}$Mo/$^{99m}$Tc generator (*Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo*, Brazil) were added and the incubation continued for another 10 minutes. These samples were centrifuged in a clinical centrifuge (1500rpm, 5 minutes) and aliquots (20µl) of plasma (P) and blood cells (BC) were isolated. Aliquots of 20µl of P and BC were also separated, precipitated with 1.0ml of 5% trichloroacetic acid and centrifuged (1500rpm, 5 minutes) to isolate soluble (SF) and insoluble fractions (IF). The radioactivity in P, BC, SF-P, IF-P, SF-BC and IF-BC were determined in a well counter (Packard, model C5002, Illinois, USA) and the percentage of radioactivity incorporated (%ATI) was calculated (Bernardo-Filho et al., 1983).

### Statistical analysis

Data are reported as (means ± SD) of percentual of radioactivity (%ATI). The One way analysis of variance – ANOVA test was performed to verify possible statistical differences. After that, a rigorous statistical post test (Bonferroni) was chosen to identify the *p* value ($p$<0.05 as lesser significant level) and to compare each experimental group with the control group. InStat Graphpad software was used to perform statistical analysis (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California, USA).

## RESULTS

The Fig. 1 shows the ATI% in blood cells and plasma compartments from whole blood exposed to physical agents. The data indicate that, at conditions used, the magnetic field (South and North poles), sonic and radiofrequency waves and intense pulsed light did not alter significantly ($p$>0.05) the ATI% on the blood compartments.

Fig. 2 shows the ATI% in insoluble and soluble fractions isolated from plasma separated from blood samples exposed to physical agents. These data indicate that magnetic field (South and North poles), sonic and radiofrequency waves and intense pulsed light have not significantly ($p$>0.05) modify the ATI% of fractions of plasma.
The Fig. 3 shows the ATI% in insoluble and soluble fractions isolated from blood cells separated from blood samples exposed to physical agents. Similarly to the results obtained with plasma proteins, magnetic field (South and North poles), sonic and radiofrequency waves and intense pulsed light have not significantly ($p$>0.05) modified the ATI% of fractions of blood cells.

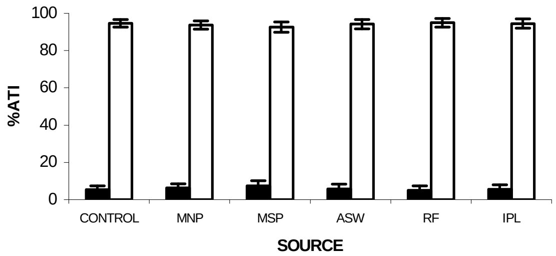


**Figure 1 -** Effect of exposition to physical agents on the distribution of radioactivity between plasma and cellular compartments. Blood from *Wistar* rats was exposed to magnetic north (MNP) and south (MSP) poles, audible sonic (ASW) and radiofrequency (RF) waves and intense pulsed light (IPL). The radiolabeling procedure was performed, plasma and blood cells separated by centrifugation, the radioactivity counted and the %ATI to each fraction calculated. (□) Blood cells and (■) plasma

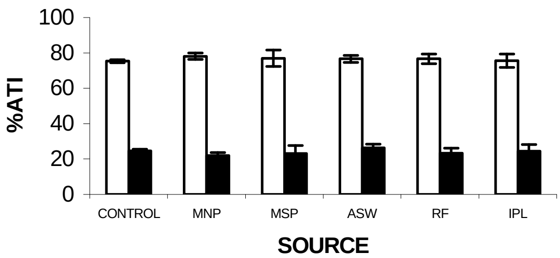


**Figure 2** - Effect of exposition to physical agents on the fixation of radioactivity on soluble and insoluble fractions of plasma. Blood from *Wistar* rats was exposed to the magnetic North (MNP) and South (MSP) poles, audible sonic (ASW) and radiofrequency (RF) waves and intense pulsed light (IPL). After that, the radiolabeling procedure was performed and plasma and blood cells separated by centrifugation. Insoluble and soluble fractions of plasma were obtained by precipitation and the radioactivity counted and the %ATI to each fraction calculated. (■) soluble fraction of plasma and (□) insoluble fraction of plasma

## DISCUSSION

Low frequencies pulsed electromagnetic fields are one of the most athermal common therapies used in the elderly patients by physicians (Heinrich, 2007). It has suggested that the exposition to magnetic field at 15Hz is effective to increases the bone mass (Mc Leod and Rubin, 1997) increasing the local levels of $PGE_2$ and TGF-b1 which decrease osteoclastic bone reabsortion (Lohmann et al., 2003). Other data have suggested no effect of these electromagnetic fields on collagen synthesis (Ahmadian et al., 2006). Although reports suggest an effect of electromagnetic fields on cell function, no modifications on the distribution of radioactivity in the cellular and plasma compartments was found (Fig. 1).

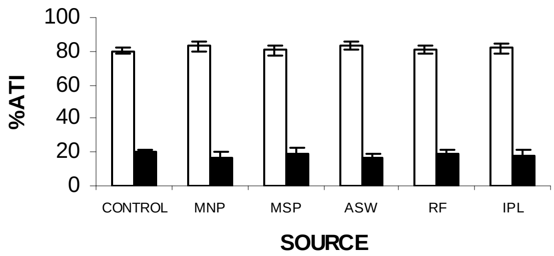


**Figure 3 -** Effect of exposition to physical agents on the fixation of radioactivity on soluble and insoluble fractions of blood cells. Blood from *Wistar* rats was exposed to the magnetic North (MNP) and South (MSP) poles, audible sonic (ASW) and radiofrequency (RF) waves and intense pulsed light (IPL). The radiolabeling procedure was performed; plasma and blood cells separated by centrifugation. Insoluble and soluble fractions of blood cells were obtained by precipitation and centrifugation, the radioactivity counted and the %ATI to each fraction calculated. (■) soluble fraction of blood cells and (□) insoluble fraction of blood cells

Authors have suggested that audible sonic waves could interact with proteins moving them to lymphatic system (Capponi and Ronzio, 2006). No modification on the radiolabeling of plasma and cellular proteins was induced by the source of audible sonic waves used in our experiments (Figures 2 and 3) indicating that the phenomenon reported by Capponi and Ronzio (2006) is not relevant to the studied labeled process. Thus, more studies are necessary to understand the potential applications of these mechanical waves in biomedical sciences as well their adverse effects.

Radiofrequency thermal therapy of tumors is based on heating of target which induces changes in dielectric properties and protein coagulation and fat melting (Pop et al., 2003). The energy absorbed from a radiofrequency source depends strongly on the tissue dielectric properties (Strohbehn, 1983, Van de Kamer et al., 2001). As results, changes in dielectric properties during heating the tissue temperature distribution is affected and resulting thermal damage. Several numerical models for predicting the radiofrequency thermal damage in heart muscle and liver have been proposed, but they either incorporated only temperature-dependent changes in electrical conductivity (Labont´e, 1994) or consider the conductivity to be constant (Haemmerich et al., 2001). However, no alterations on labeling of blood constituents with $^{99m}$Tc were verified when blood samples were exposed to radiofrequency waves in the conditions used in this study. In consequence, the findings described by Strohbehn, 1983, Labont´e, 1994, Van de Kamer et al., 2001, Haemmerich et al., 2001 could be not relevant to the studied labeled process with $^{99m}$Tc.

Intense pulsed light systems are high-intensity light sources, which emit polychromatic and noncoherent light in a broad wavelength spectrum (515-1200 nm) allowing a great variability in selecting individual esthetical treatment of skin (Raulin et al., 2003) as rejuvenation of the aging face (Mezzana and Valeriani, 2007) or skin diseases as erythrosis (Madonna Terracina et al., 2007). No alterations on the labeling of blood constituents with $^{99m}$Tc after exposition to intense pulsed light could suggest a safety to this physical agent used to esthetical propose.

In conclusion, although our data have been obtained with blood from *Wistar* rats, the exposition to magnetic field, sonic and radiofrequency waves and intense pulsed light used in clinical physiotherapy could not alter the examinations performed in nuclear medicine based on blood constituents labeled with $^{99m}$Tc. Furthermore, the biological/physical consequences associated with these physical agents would be not capable to interfere with some properties of the blood constituents.

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## RESUMO

Fontes de campo magnético, ondas sonoras audíveis e de radiofreqüência e luz intensa pulsada são usadas para o tratamento de doenças. Constituintes sangüíneos(CS) marcados com tecnécio-99m($^{99m}$Tc) são utilizados na medicina nuclear. Esse trabalho avaliou as consequências de fontes de campo magnético, ondas sonoras audíveis e de radiofreqüência e luz intensa pulsada na marcação de CS com $^{99m}$Tc. Sangue de ratos Wistar foi exposto às fontes citadas. A marcação de CS com $^{99m}$Tc foi realizada. Sangue não exposto foram utilizadas(controle). Resultados mostraram que os agentes físicos estudados não alteraram significativamente ($p>0.05$) a radiomarcação de CS. Apesar terem sido obtidos com sangue de animais, os resultados sugerem que nenhuma alteração nos exames realizados com constituintes sangüíneos com $^{99m}$Tc em medicina nuclear ocorreria após a exposição às fontes avaliadas. As consequências biológicas associadas a esses agentes não seriam capazes de interferir com algumas propriedades dos CS.

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