

Article

Forced Swim Alters the Radiolabeling of Blood Constituents from Wistar Rats

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Abstract: The present study investigated the effects of forced swimming on the technetium-99m (^{99m}Tc) labeling of blood constituents (BloCs). Rats (*Wistar*) were submitted to forced swim. In previous experiments, swimming animals would recover for different periods of time. Animals not submitted to swimming were used as control. Blood samples were obtained and the ^{99m}Tc labeling of BloCs was carried out. Blood cells (BCs), plasma (P), insoluble fractions (IF-P and IF-BCs), and soluble fractions (SF-P and SF-BC) were isolated. Radioactivity was determined, and the percentage of ^{99m}Tc incorporated (%ATI) was calculated in each fraction. Results showed that forced swimming decreased the percentage of ^{99m}Tc incorporated (%ATI) in IF-P ($p < 0.05$). It is suggested that the ^{99m}Tc labeling of BloCs could be used to verify the effects of the stress conditions on BloCs and that the radionuclide fixation on plasma proteins might be altered in rats submitted to acute stress induced by forced swimming, returning to control levels after recovery.

Keywords: blood constituents; forced swimming; stress; technetium-99m

1. Introduction

Individuals under biological stress respond negatively to a stress stimulus while they have sufficient reserves. Important alterations to the behavior, metabolic pathways, physiological responses, and the effects of drugs have been described [1–5]. Following the NPR/Robert Wood Johnson Foundation/Harvard School of Public Health Burden of Stress in America Survey (2014), the most common effects on health among those with a great deal of stress are undesirable effects, such as problems with (i) sleep (56%); (ii) emotional well-being (63%); and (iii) difficulty in concentrating, thinking, or making decisions (50%) (<http://www.rwjf.org/en/library/research/2014/07/the-burden-of-stress-in-america.html>). Consequently, it is highly desirable to develop evidence-based methods that minimize the impact of stress [5]. An experimental model such as forced swimming (FS) could be used to better understand the mechanisms that underlie stress.

FS is utilized for inducing stress in rodents [6–8]. Acute stress can be induced by a unique swimming session in a large water tank where the animals are forced to swim wall-to-wall distances, moving the extremities while keeping their nose above the water [9]. Following acute FS stress in C57BL/6J mice, it was reported that a decrease in the circulating and brain concentrations of (3 α ,5 α)-3-hydroxy-pregnan-20-one (3 α ,5 α -THP) could be due to modifications in the biosynthesis/metabolism or changes in the regulation of the hypothalamic–pituitary–adrenal axis [10]. Some behavioral, biochemical, and physiological parameters are modified in animals under this condition, such as social behavior [6,11], learning and memory [12], liver glycogen content [7], and the angiotensin II receptor [13]. Souza et al. [14] showed that the biodistribution of the radiopharmaceutical 99m Tc-methylene diphosphonate was altered due to the FS.

99m Tc is used to label different radiopharmaceuticals, and it is the main radionuclide utilized in nuclear medicine and single-photon emission computed tomography (SPECT) [15]. Functional images of different organs, tissues, and systems are obtained [16–18] and alterations to the 99m Tc radiopharmaceutical can be also associated with a disturbance in the metabolism [19].

99m Tc red blood cells (99m Tc RBCs) can be obtained by in vivo, in vitro, and combined in vitro/in vivo methods. In general, the basic mechanism of this radiolabeling process is almost the same. RBCs are “pre-tinned” (stannous ions) for some minutes before the addition of the 99m Tc as sodium 99m Tc-pertechnetate. Stannous ions (Sn^{++}) enter into the cell and are strongly bound to cellular components. Pertechnetate, as 99m Tc, diffuses freely across the erythrocyte membrane and is reduced in the presence of Sn^{++} inside the cell and binds to the β chain of the hemoglobin [15,20].

99m Tc RBCs have been used for evaluating volemia [21] and cardiac function [22] and detecting gastrointestinal bleeding sites [23]. In one case report, 99m Tc RBCs were used to localize intrathoracic bleeding [24].

Authors reported that synthetic and natural medications might alter the bioavailability of radiopharmaceuticals [25,26], as well as the radiolabeling of blood constituents [25,27–30].

99m Tc RBCs obtained by an in vitro technique can be used to determine the blood and red cell volume [31], to detect gastrointestinal blood loss and hemangioma, for gated blood pool studies [15], and to obtain selective imaging of the splenic tissue (when RBCs are heat-damaged or heat-denatured) in a variety of clinical scenarios [32].

Plasma proteins have been used as Tc-99m-labeled macro-aggregated albumin to measure pulmonary perfusion in mice [33], as 99m Tc-albumin nanocolloid in abdominal and pelvic region scintigraphy [34], and as 99m Tc-human serum albumin to determine the plasma volume [35].

Considering that in vitro blood constituent labeling with 99m Tc can be modified by medications, an experimental assay to try to verify the effects of drugs was developed [27–30].

The cardiac function has been evaluated in stressful situations such as exercise by radiopharmaceutical ventriculography [36,37]. Gwozdzinski et al. [38] reported: (i) a significant decrease in membrane thiols after exercise (60 min), with an increase in plasma thiols immediately after and 60 min after the exercise; (ii) a significant decrease in the level of ascorbic acid in the erythrocytes after exercise; and that (iii) a single-bout of exercise can mobilize defensive antioxidant components

in blood against oxidative stress. Considering these findings and the undesirable effects of stress on health [39], the aim of this work was to evaluate the effects of stress induced by FS on in vitro blood constituent labeling with ^{99m}Tc .

2. Materials and Methods

2.1. Animals

Wistar rats (male, 3–4 months, 250–300 g) were housed (six animals per cage) in controlled conditions with 12 h light/12 h dark cycles, light at 6:00 am, and controlled temperature ($25 \pm 2^\circ\text{C}$). Food and water were freely accessed by all animals. The Committee on animal research of Universidade do Estado do Rio de Janeiro (UERJ) had approved the protocols used in this current study (CEA/122/2006).

2.2. Forced Swimming and Recovery

Rats were removed from their home cages and immediately submitted to FS in an aquarium ($70 \times 50 \times 40$ cm, $25 \pm 2^\circ\text{C}$) with water filled to 30 cm high for different periods of time (2.5, 5, or 20 min). Following Souza et al. [14], with slight modifications, the animals were forced to swim (CS) and not interrupt their swimming or place their feet or tails on the walls or bottom of the aquarium.

A study related to the recovery from FS was also performed, where animals rested for different amounts of time (5, 20, or 60 min) after 2.5 min of swimming before the blood constituent radiolabeling procedure.

Animals not submitted to swimming were used as control (NS).

2.3. In-Vitro Blood Constituent Radiolabeling

The experiments were performed according to a previously published protocol [40].

All the tubes (vials) utilized were closed (rubber cap) and, to try to eliminate the air from the tubes (vacuum), a syringe was used in the various steps of the procedure.

Figure 1 briefly summarize all the steps of the radiolabeling procedure.

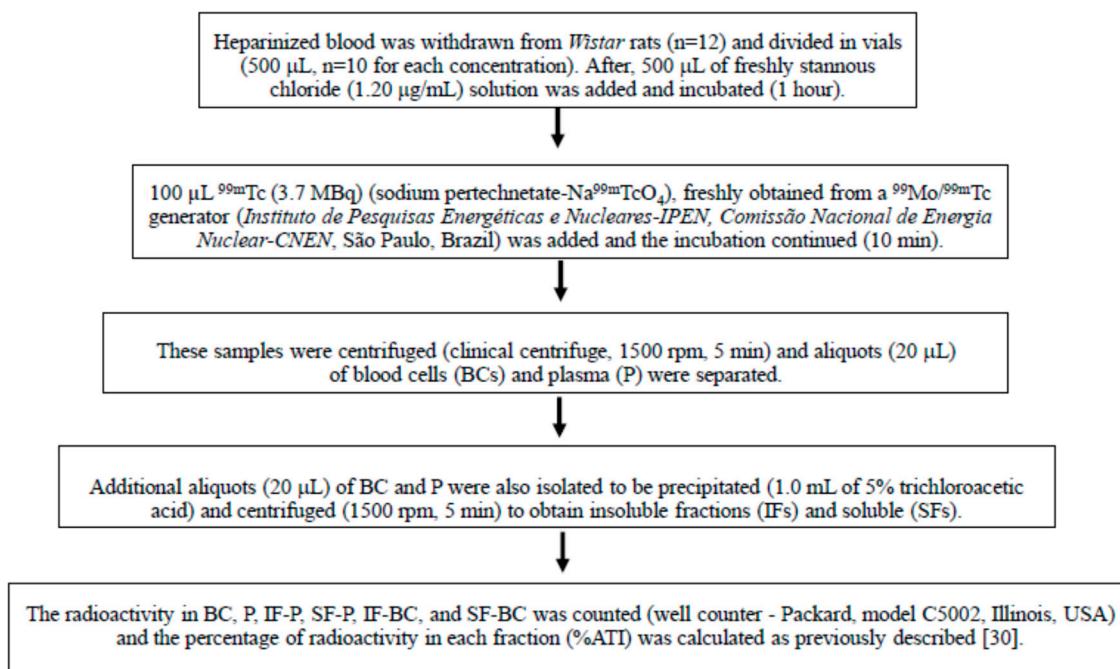


Figure 1. The ^{99m}Tc (99m-technetium) labeling of blood constituents withdrawn from Wistar rats.

2.4. Statistical Analysis

Data are presented as mean \pm SD of the percentage of ^{99m}Tc incorporated (%ATI) from the labeling assay ($n = 10$ for each FS time or recovery). One-way analysis of variance (ANOVA) was conducted to verify statistical significance. Statistical post-test (Bonferroni) was used to verify the p -value ($p < 0.05$) and to compare the control group (treated with 0.9% NaCl) with each treatment. InStat GraphPad software was used to perform the statistical analysis (GraphPad InStat version 3.0 for Windows 95, GraphPad Software, San Diego, CA, USA).

3. Results

3.1. The Distribution (ATI%) of P and BC Compartments from the Blood Isolated from Animals Submitted to FS

Figure 2 shows no significant ($p > 0.5$) alteration to the ATI% in plasma (P) and blood cells (BCs), suggesting that FS might not modify the distribution of the ^{99m}Tc between the plasma and cellular compartments.

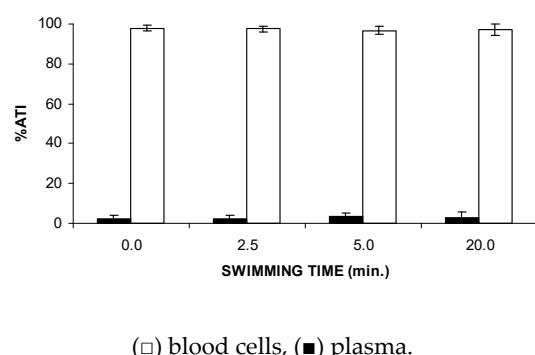


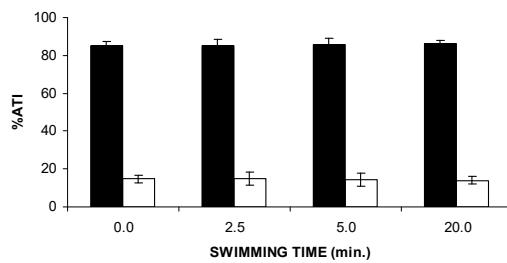
Figure 2. Effect of the forced swimming (FS) on the distribution of ^{99m}Tc between the cellular and plasma compartments. Wistar rats were submitted to FS, blood samples were withdrawn, and the labeling of blood constituents with ^{99m}Tc procedure was carried out. Blood cells and plasma were separated by centrifugation. The radioactivity in blood cells and plasma was measured and the percentage of ^{99m}Tc incorporated (%ATI) was calculated.

3.2. The Fixation (ATI%) on Soluble and Insoluble Fractions Isolated from Blood Cells Obtained from Wistar Rats Submitted to FS

Similarly, based on the results obtained with the blood compartments, Figure 3 shows no significant ($p > 0.05$) alteration to the ATI% of the insoluble fraction isolated from blood cells (IF-BCs) in the blood from the animals submitted to FS, suggesting no modification of the ^{99m}Tc fixation on the cellular proteins in the studied stress condition.

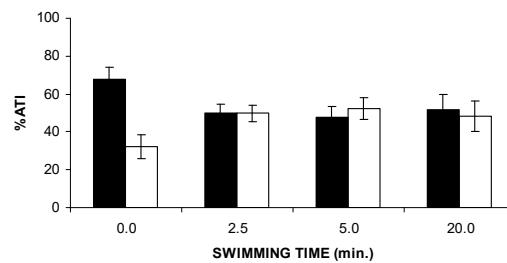
3.3. The Fixation (ATI%) on Soluble and Insoluble Fractions Isolated from the Plasma Obtained from Wistar Rats Submitted to FS

Figure 4 shows that the FS significantly ($p < 0.05$) altered the fixation (ATI%) of the ^{99m}Tc on the soluble and insoluble fractions obtained from plasma (SF-P and IF-P, respectively) at all of the time periods evaluated (2.5 to 20 min), suggesting a reduction in the ^{99m}Tc fixation on the plasma proteins from the animals submitted to the studied stress condition.



(□) blood cells, (■) plasma.

Figure 3. Effect of the forced swimming (FS) on the distribution of ^{99m}Tc between the cellular and plasma compartments. Wistar rats were submitted to FS, blood samples were withdrawn, and the labeling of blood constituents with ^{99m}Tc procedure was carried out. Blood cells and plasma were separated by centrifugation. The radioactivity in blood cells and plasma was measured and the percentage of ^{99m}Tc incorporated (%ATI) was calculated.

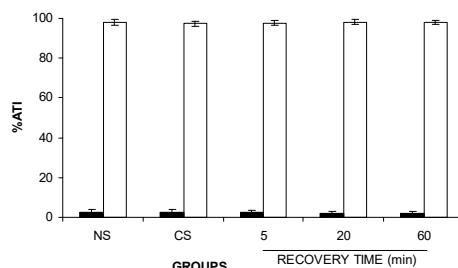


(□) insoluble fraction of blood cells, (■) plasma.

Figure 4. Effect of the forced swimming (FS) on the distribution of ^{99m}Tc between the cellular and plasma compartments. Wistar rats were submitted to FS, blood samples were withdrawn, and the labeling of blood constituents with the ^{99m}Tc procedure was carried out. Blood cells and plasma were separated by centrifugation. The radioactivity in blood cells and plasma was measured and the percentage of ^{99m}Tc incorporated (%ATI) was calculated.

3.4. The Fixation (ATI%) in P and BC Compartments of Wistar Rats after Recovery from FS

Data of the ATI% on P and BCs in Figure 5 suggest no significant alteration ($p > 0.05$) to the distribution of the ^{99m}Tc between the cellular and plasma compartments after recovery from FS.

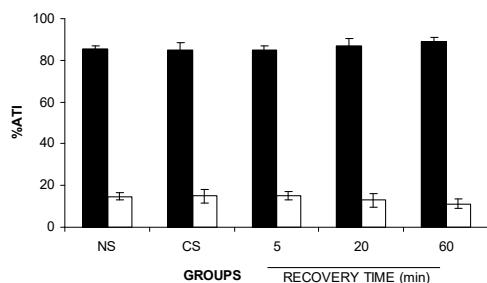


NS—control group, CS—control swim group, (□) blood cells, (■) plasma.

Figure 5. ATI% in the blood cells and plasma from Wistar rats after recovery from the forced swimming. Wistar rats were submitted to forced swimming for 2.5 min, and recovery was followed for different periods of time. After that, blood samples were withdrawn and the labeling of blood constituents with the ^{99m}Tc procedure was carried out. Blood cells and plasma were separated by centrifugation. The radioactivity in blood cells and plasma was measured and the percentage of ^{99m}Tc incorporated (%ATI) was calculated.

3.5. The Fixation (ATI%) in SF-BC and IF-BC Fractions Isolated from Wistar Rats after Recovery from the FS

Similarly, based on the results obtained with BCs and P, data of the %ATI on SF-BCs and IF-BCs in Figure 6 indicate no significant alteration ($p > 0.05$) in the fixation of ^{99m}Tc on cellular proteins after recovery from the FS.

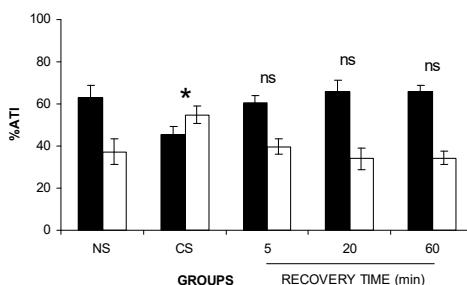


NS—control group, CS—control swim group, (□) insoluble fraction of blood cells, (■) soluble fraction of blood cells.

Figure 6. ATI% in the soluble and insoluble fractions of the blood cells from Wistar rats after recovery from the forced swimming (FS). Wistar rats were submitted to FS for 2.5 min and recovery was followed for different periods of time. After that, blood samples were withdrawn and the labeling of blood constituents with the ^{99m}Tc procedure was carried out. Blood cells were separated by centrifugation and insoluble and soluble fractions of blood cells were isolated by precipitation in trichloroacetic acid centrifugation. The radioactivity in each fraction was measured and the percentage of ^{99m}Tc incorporated (%ATI) was calculated.

3.6. The Fixation (ATI%) in SF-P and IF-P Fractions Isolated from Wistar Rats after Recovery from the FS

Figure 7 indicates that after 5 min of recovery from a 2.5-min FS, the ATI% in SF-P and IF-P presented the same value obtained with the NS group. Similar data were obtained with the highest time of recovery (20 and 60 min).



NS—control group, CS—control swim group,
(□) insoluble fraction of blood cells, (■) soluble fraction of blood cells

Figure 7. ATI% in the soluble and insoluble fractions of the blood cells from Wistar rats after recovery from the forced swimming (FS). Wistar rats were submitted to FS for 2.5 min and recovery was followed for different periods of time. After that, blood samples were withdrawn and the labeling of blood constituents with the ^{99m}Tc procedure was carried out. Plasma was separated by centrifugation and insoluble and soluble fractions of plasma were isolated by precipitation in trichloroacetic acid centrifugation. The radioactivity in each fraction was measured and the percentage of ^{99m}Tc incorporated (%ATI) was calculated.

4. Discussion

Several studies have related metabolic disorders to stress in different organs and body systems. Heart, circulatory, gastrointestinal, immunological, and nervous systems can present altered function

in individuals with stressful lifestyles [5,41–44]. In some conditions, the stress can be desirable, as in the cardiac evaluations with radiopharmaceuticals [36,37].

Changes in blood parameters have been evaluated in physical activities such as cycling [38], whole-body vibration exercise [45], and FS-induced [6–8] stress.

Plasma proteins and RBCs labeled with ^{99m}Tc have been used to evaluate various clinical applications [32,34,35] and in experimental models [25–30,36].

In the current work, the effects of FS on the in-vitro ^{99m}Tc blood constituent labeling were assessed. The data analysis showed that the FS for different amounts of time (2.5, 5, or 20 min) did not alter the allocation of radioactivity between BC and P compartments (Figure 2). The presence of the radionuclide ^{99m}Tc on the cellular proteins (blood cells) was not modified (Figure 3). No alteration was verified after different periods of recovery from FS (Figures 5 and 6). These data agree with other studies that obtained no alterations in RBC radiolabeling in patients after dipyridamole-thallium stress testing [46] without interference in the pathways and chemical actions of the Sn^{++} and pertechnetate ions [31]. Antioxidant enzymes such as superoxide dismutase are highly active and present at high concentrations in RBCs, constituting the most important components of the antioxidant defense system of blood [47,48]. This could be related to the absence of alterations to the RBC radiolabeling from animals submitted to FS. Vierck et al. [49] reported that running and cycling (moderate and high intensity) reduce the carotenoid levels in the skin, whereas sports and both exercise intensities had comparable findings. Moreover, it is suggested that above a determined threshold, physical activity also induces oxidative stress on the surface of the body (skin) associated with a reduction in the antioxidant level.

Authors suggested that medications could interfere with the fixation of ^{99m}Tc in blood on the same sites as blood constituents, inducing alterations in the erythrocyte membrane structure or altering the Sn^{++} and pertechnetate ion transport into cells, inhibiting (via chelating action) the Sn^{++} and pertechnetate ions, and oxidizing or generating free radicals that might oxidize the Sn^{++} [27,40]. The influence of stress conditions on such radiolabeling has yet to be evaluated. Regarding the plasma proteins, the oxidative status of a plasma compartment could be an important factor in altering the fixation of ^{99m}Tc on plasma proteins. Indeed, studies have demonstrated that different stressful situations could induce alterations to the plasma antioxidant systems in animals and human beings [50–54].

Our findings suggest that the binding of ^{99m}Tc to plasma proteins from rats might be altered after FS (Figure 4). Moreover, the data indicate that the swimming-induced alteration to the fixation of ^{99m}Tc on plasma proteins is not time-dependent, returning to control level after 5 or 20 min of recovery. These data suggest that this assay could be used to assess the effects of stress on the blood constituents. Stressful situations such as the hemodialysis procedure have been related to increases of oxidative stress and the alteration/damage of the antioxidant defense [54]. Other authors have demonstrated that the amount of a reduced form of human albumin and glutathione peroxidase are increased after hemodialysis [55,56], while catalase, superoxide dismutase, glutathione-S-transferase, and glutathione peroxidase experimentally induce myocardial infarction (rats) [57]. Finkler et al. [58] reported that the negative consequences of exercise are generally related to the difference between the number of antioxidants (antioxidant enzymes and low-molecular-weight antioxidants) and the free radicals (reactive nitrogen and oxygen species) during physical exercise. In plasma, oxidative stress could lead to the excessive production of free radicals and/or a reduction in the antioxidant defenses, with an uncontrolled redox status of the cell. This imbalance could be by-passed with the production or activation of these antioxidant enzymes, as well as endogenous substances such as glutamine. These defense mechanisms could require some time to appear and block the oxidative stress effects, explaining the data obtained with the fixation of ^{99m}Tc on plasma proteins from rats submitted to swimming after recovery (Figure 7). Moreover, some oxidative stress markers in plasma and RBCs could also sustain and justify this finding.

The limitations of the current research were related to the number of animals utilized in this work and the time used for the recovery from FS. Moreover, the scarcity of publications evaluating the effect of stress on blood constituent labeling limited the discussion of the results. Although there were some limitations in the current investigation, some important findings were observed.

5. Conclusions

The 99m Tc labeling of blood constituents could be used to evaluate the effects of stress conditions on the blood constituents related to FS. Moreover, the fixation of radionuclides on plasma proteins might be altered in rats submitted to acute stress induced by FS, returning to control levels after the recovery time.

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