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Evaluation of the effect of anti-toxoplasmic drugs on the biodistribution of the radiopharmaceutical sodium pertechnetate

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ABSTRACT

The human toxoplasmosis is a disease caused by the parasite *Toxoplasma gondii* that can affect the growing baby in pregnancy and it is the most common Central Nervous System infection in patients with AIDS. Its treatment is made by the synergistic association of the drugs pyrimethamine (PM) and sulfadiazine (SDZ).

Objective: The aim of this study was to evaluate whether the combined use of PM and SDZ changes the sodium pertechnetate ($Na^{99m}TcO_4^-$) biodistribution in non-infected animals by the *T. gondii*. It was used on 24 male *Swiss* mice equally divided into four groups: one control group and three treated groups.

Methods: The control group received 0.5 ml of distilled water; the treated group 1 received 0.5 ml of a PM solution; the treated group 2 received 0.5 ml of a SDZ solution and the treated group 3 received 0.5 ml of the PM + SDZ, all by the gavage method for 10 days. On the 10th day and 1 hour after the last dose, all groups received 0.1 ml of $Na^{99m}TcO_4^-$ (0.66 MBq) via the femoral vein. After 40 minutes, the animals were euthanized, and blood and brain samples were isolated. The percentage of total activity injected per gram of organ (%ATI/g) was calculated on the gamma counter. Statistical analysis was performed using the *T*-test, considering a level of significance of $p < 0.05$.

Results: There was a statistically significant increase in %ATI/g in the blood, from the control group to the treated group with PM + SDZ (from 2.53 ± 0.17 to 4.53 ± 0.31) and also in the brain (from 0.09 ± 0.01 to 0.19 ± 0.04). There was no statistically significant difference between the control group and those treated alone with PM and SDZ.

Conclusions: It can be concluded that both the drugs used alone and in combination do not interfere in the biodistribution of the radiopharmaceutical in nuclear medicine exams.

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Introduction

Toxoplasmosis is a cosmopolitan infection caused by the protozoan *Toxoplasma gondii*, which is an obligate intracellular parasite of warm-blooded animals [1–3]. This opportunistic human pathogen induces a devastating disease in immunocompromised individuals, especially HIV/AIDS patients, and congenitally infected neonates [4,5], which requires strong medical care [6,7]. *Toxoplasma gondii* infection causes substantial morbidity and mortality in the United States and infects approximately one-third of persons globally [8]. *Toxoplasma gondii* exhibits a large amount of diversity in Brazil and other South American countries

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[9], probably due to the sexual reproduction of the parasite in several distinct feline species [6,10]. The main routes of transmission of toxoplasmosis in humans are: ingestion of water and food contaminated with oocysts, ingestion of cysts containing bradyzoites in undercooked meat, and the transplacental route by tachyzoites when the mother is infected during pregnancy [5]. In general, toxoplasmosis presents asymptomatically, but it is severe in immunocompromised individuals and in congenital infection [11–13]. The severity of clinical manifestations arising from a congenital infection depends on several factors, such as the type of *T. gondii* strain, which may be a virulent type such as RH (type I), or a cystogenic type such as ME-49 (type II). It also depends on the geographic location and host-related factors such as immunological resistance and gestational period [4,5], since the most severe complications occur in the first gestational trimester [14].

The treatment of toxoplasmosis recommended by the Ministry of Health of Brazil is the association of sulfadiazine (SDZ) and pyrimethamine (PM) combined with folinic acid. SDZ and PM act synergistically in blocking the folate synthesis pathway by inhibiting the enzymes dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR), which are essential for the survival and replication of the parasite [15]. The use of PM can suppress the bone marrow activity and, for this reason, the association with folinic acid is recommended [4]. These drugs bind to the 50S subunit of the pathogen's ribosome preventing RNA-dependent protein synthesis [16]. In addition to the drugs recommended in the treatment of toxoplasmosis, other compounds are being investigated as an alternative treatment for this infection, among which artemisinin, which is a derivative of the plant *Artemisia annua*, a compound commonly used in the treatment of malaria [17–19], and azithromycin which, despite having satisfactory results for the treatment of toxoplasmosis in mice [20–22], is not yet used in humans.

Nuclear medicine is an area of medicine that uses radiopharmaceuticals as a diagnostic tool in 90% of human diseases [23–25]. Scintigraphy is often used for analysis of anatomofunctional organs and systems in patients with tropical diseases, and it is usually made with the administration of technetium-99m sodium pertechnetate ($Na^{99m}TcO_4^-$), a radiopharmaceutical capable of binding to a variety of molecules and cells [24–27]. Over 80% of the commonly used radiopharmaceuticals for human scintigraphy are labeled with technetium-99m (^{99m}Tc) because of its nuclear properties, such as short half-life of only 6 hours, emission range of 140 keV photons suitable for imaging with high detection efficiency and low radiological risk, negligible environmental impact, and easy obtainment from portable generators [24,25].

In the recent years, the interference of the natural or synthetic drugs on the biodistribution of the radiopharmaceuticals used in the nuclear medicine examinations has been discussed by researchers around the world [23–30]. Scientific studies about the interference of anti-toxoplasmic drugs on the biodistribution of radiopharmaceuticals are almost non-existent, allowing the questioning of the existence or not of alterations in the normal biodistribution of radiopharmaceuticals under the use of anti-toxoplasmic drugs to include a clear aim.

Materials and Methods

A controlled study was performed with experimental animals, totalizing 24 male albino Swiss (*Mus musculus*) mice with a body mass of 40 g. The animals were raised with free access to water and food (Purina™, Labina) and maintained under constant environmental conditions (23°C ± 2°C; 12/12 hours of light/dark cycle), according to local regulations for experimentation with laboratory animals. They were obtained from the Bioterium of the Center of Health Sciences of the Federal University of Rio Grande do Norte (UFRN). The protocol conducted in this study was approved by the local Ethical Committee on the Use of Animals of the UFRN, under the number 049.058/2017. The protocol of the biodistribution of the radiopharmaceutical sodium pertechnetate ($Na^{99m}TcO_4^-$) was made following the standards of radiological protection of the National Commission of Nuclear Energy.

The mice were randomly divided into four groups of six animals each, one of which was the control group and the other three were the treated groups. The treated group 1 received 0.5 ml of a solution of PM (0.1 mg/kg/day; Daraprim—Glaxo Wellcome S/A) via gavage for 10 days; the treated group 2 received 0.5 ml of a solution of SDZ (0.075 mg/kg/day; Suladrin—Laboratório Catarinense S/A) via gavage for 10 days; and the treated group 3 received 0.5 ml of association SDZ + PM by the same via and period. The control group received distilled water by the same route, volume, and period. All the animals received an anesthetic combination of xylazine (20 mg/kg) and ketamine

(50 mg/kg) in the same syringe, intraperitoneally, prior to the administration of $Na^{99m}TcO_4^-$ (0.66 MBq) via the femoral vein. The $Na^{99m}TcO_4^-$ was eluted from a $^{99}Mo/^{99m}Tc$ generator produced by the Institute of Energy and Nuclear Research, São Paulo/Brazil, and kindly supplied by the Norteriograndense League against Cancer, Natal/RN, Brazil. Forty minutes after administration of the $Na^{99m}TcO_4^-$, all the animals were anesthetized again to collect a blood sample (0.1 ml) by cardiac puncture and then euthanized by cardiac puncture under general anesthesia. The brain was removed from each animal and washed in 0.9% saline. Brain and blood samples from each animal were weighed on a precision scale (Mark 160®, Bel equipment, Italy) and taken to the Experimental Surgery Laboratory of the Health Sciences Center (UFRN) to count the percentage of radioactivity per gram of organ (%ATI/g) in an automatic gamma counter (Wizard 1470, Perkin-Elmer, Finland). The efficiency of the gamma counter is 86%, as specified by the manufacturer. The percentage of radioactivity per gram (%ATI/g) was calculated by dividing the percentage of total radioactivity of each organ by its weight in grams [23,27–30]. Statistical analyses were performed using the paired *T*-test in the SigmaStat Statistical Program. Statistical difference was considered significant when $p < 0.05$.

Results

Table 1 shows the relationship between the control group and the group treated with the combination of SDZ and PM. There was a statistically significant ($p < 0.05$) increase in the biodistribution of the $Na^{99m}TcO_4^-$ (ATI%/g) in the brain and blood of the treated group when compared with the control group.

Table 2 shows the relationship between the control group and the group treated with PM. There was not any statistically significant difference ($p > 0.05$) in the biodistribution of the $Na^{99m}TcO_4^-$ (ATI%/g) in the brain and blood of the treated group when compared with the control group.

Table 3 shows the relationship between the control group and the group treated with SDZ. There was not any statistically significant difference ($p > 0.05$) in the biodistribution of the $Na^{99m}TcO_4^-$ (ATI%/g) in the brain and blood of the treated group when compared with the control group.

Table 1. Biodistribution of %ATI/g of $Na^{99m}TcO_4^-$ in the blood and brain of the mice treated with the combination of SDZ and PM (PM + SDZ).

Groups organs	Control (%ATI/g)	Treated (PM + SDZ) (%ATI/g)
Blood	2.53 ± 0.17	4.53 ± 0.31**
Brain	0.09 ± 0.01	0.19 ± 0.04*

The values are mean ± standard deviation; *$p = 0.038$; **$p = 0.001$.

Table 2. Biodistribution of %ATI/g of $Na^{99m}TcO_4^-$ in the blood and brain of the mice treated with PM.

Groups organs	Control (%ATI/g)	Treated (PM) (%ATI/g)
Blood	2.53 ± 0.17	2.98 ± 0.68*
Brain	0.09 ± 0.01	0.18 ± 0.03*

The values are mean ± standard deviation; *$p > 0.05$.

Table 3. Biodistribution of %ATI/g of $Na^{99m}TcO_4^-$ in the blood and brain of the mice treated with SDZ.

Groups organs	Control (%ATI/g)	Treated (SDZ) (%ATI/g)
Blood	2.53 ± 0.17	2.26 ± 0.62*
Brain	0.09 ± 0.01	0.11 ± 0.02*

The values are mean ± standard deviation; *$p > 0.05$.

Discussion

The treatment of toxoplasmosis recommended by the Ministry of Health of Brazil is the combination of SDZ and PM combined with folinic acid. PM and SDZ act synergistically against *T. gondii* with a combined activity that is eight times greater than if used alone [12,13].

The enzyme DHFR is a classic target in antiparasitic chemotherapy, widely used in the fight against protozoa of blood and tissue with *Plasmodium* sp. and *Toxoplasma gondii.* SDZ and PM act against *T. gondii* by inhibiting the enzymes DHPS and DHFR, which are essential for the survival and replication of the parasite [15,16].

SDZ is a derivative of sulfonamides and, once absorbed, it is distributed throughout the tissues and liquids of the organism. It can be found in various concentrations in bile, digestive juices, and pleural fluid. It easily crosses the placenta, reaching the fetal circulation and in smaller amounts the breast milk. The pharmacodynamic activity of this medicinal product is due to the fact that it is a structural analog of p-aminobenzoic acid (PABA). Through competitive inhibition, the sulfonamides prevent the incorporation of PABA during the biosynthesis of dihydropteroic acid, functioning in this way as an antimetabolite, which is more toxic to the parasite than to man [17].

PM, *in vitro*, inhibits the growth of tachyzoites at concentrations ≥0.05mg/l and it is parasiticidal.

In murine models of acute toxoplasmosis, it has shown protective activity but its accumulation in the tissues is delayed and should be administered in combination with sulfonamides or other drugs [18,19]. It is a substitute for phenylpyrimidine, the antimalarial drug, of which plasma's half-life in the newborns and children under 18 months of age is approximately 60 hours. In the cerebrospinal fluid, it reaches 10%–20% of serum levels [17].

PM can lead to reversible and usually gradual depression of the bone marrow, causing neutropenia, thrombocytopenia, and anemia. Neutropenia (reversible) is the most important toxic action. Accidental overdose of PM in children may result in vomiting, tremors, convulsions, and bone marrow depression. Therefore, the patient should receive folinic acid (never folic acid, which nullifies the therapeutic action of PM) to prevent changes such as neutropenia, thrombocytopenia, and anemia [19]. Although the treatment controls the forms of rapid proliferation, there is no drug capable of eliminating animal and human tissue cysts, which remain viable for a long time and the infection can be reactivated in cases of immunocompromising [20]. Therefore, the prevention of congenital toxoplasmosis is of fundamental importance for a better control of the infection avoiding the severe sequelae that can occur in the fetus and the newborns [21].

Radiopharmaceuticals are radioactive compounds widely used for diagnosis and treatment of various diseases. Technetium-99m (^{99m}Tc) in the form of sodium pertechnetate ($Na^{99m}TcO_4^-$) is a radionuclide that connects to a wide variety of molecules and cells and is a broadly used radionuclide scintigraphy in the stomach, salivary gland, thyroid and parathyroid glands, the choroid plexus, esophageal reflux, and blood flow [23–25], in addition to its use in experimental research.

The evidence that natural and/or synthetic drugs can affect the biodistribution of radiopharmaceuticals in the setting of nuclear medicine clinic is already known [23,24,26,27]. Several drugs can change the biological effect of the radiopharmaceutical and their interaction can lead to hypo or hyper uptake of radiopharmaceuticals in a particular organ, causing incorrect diagnosis or misinterpretation of results. Repeated scintigraphy may result in unnecessary radiation for patients [23,25,27–29].

The pattern of a radiopharmaceutical biological behavior can be altered by the interaction with natural or synthetic drugs, food, cigarettes, surgical procedures, and parasitic infections which may result in an unexpected result [23,25–27,30]. Knowledge of this interaction is important to avoid the misinterpretation of scintigraphic images [27,31,32]. The alteration of the biodistribution of a radiopharmaceutical due to the effect of a drug in a specific tissue could aid in identifying the toxicologic effect of a substance in an organ [23,26,27].

Several research studies on the influence of antiparasitic drugs and radiopharmaceuticals are described in the literature. Xavier-Holanda et al. [26] showed that glucantime, a leishmanicidal drug, increased the uptake of the radiopharmaceutical methylene diphosphonic acid, labeled with technetium-99m (^{99m}Tc-MDP) in the spleen, kidney, testicles, heart, and liver of rats. Antimalarial drugs also have shown to alter the biodistribution of radiopharmaceuticals. Holanda et al. [23] evaluated the influence of natural and synthetic antimalarial drugs (artemisinin and mefloquine, respectively) on the biodistribution of the radiopharmaceutical methylene diphosphonic acid labeled with technetium-99m (^{99m}Tc-MDP) in W*istar* rats. A significant increase in %ATI occurred in spleen, liver, and blood in rats treated with mefloquine. The %ATI increased in the femur, liver, lungs, spleen, and blood of rats treated with artemisinin. A significant decrease of %ATI occurred in the mefloquine group in the bladder, stout bowel, pancreas, kidneys, brain, and also in the artemisinin group in the bladder, stout bowel, muscle, pancreas, and kidneys [23].

In the current study, our data showed a statistically significant increase of the %ATI/g in the blood and brain of mice treated with the combination of SDZ and PM. SDZ and PM are anti-*Toxoplasma* drugs that act synergistically against *T. gondii* tachyzoites. These drugs bind to plasma proteins, which could affect the biodistribution of $Na^{99m}TcO_4^-$, since 80% of the radiopharmaceuticals are distributed in the body by binding to the same proteins. Although drugs and radiopharmaceuticals are bound to plasma proteins, they probably do not occur through the same binding site. Thus, the uptake of the radiopharmaceutical is not interrupted and therefore, does not interfere with diagnostic imaging by nuclear medicine.

Finally, this study is of great importance to public health, since it is the first to show the interference of anti-toxoplasmic drugs in the biodistribution of radiopharmaceuticals used in nuclear medicine exams. However, increasing the number of experimental animals, as well as performing tests with other anesthetics, would be necessary to give the

scientific community greater knowledge about the subject.

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Conflict of Interests

The authors declare that they have no conflict of interests.

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