The malarial infection can alter the normal biodistribution of a radiopharmaceutical?

Uma infecção por malária pode alterar a biodistribuição normal de um radiofármaco?

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ABSTRACT

Objective: Infection with *Plasmodium berghei* is a model of murine malaria widely used in experimental studies, similar to *Plasmodium falciparum* in humans. The aim of this study was to investigate *in vivo* the influence of *P. berghei* infection on the biodistribution of sodium pertechnetate ($Na^{99m}TcO_4$) in mice. Methods: 14 Swiss mice were divided into two groups: control (n=7) and treated (n=7). Treated group were inoculated with $1x10^5$ *P. berghei* infected red blood cells each. On the 15^{th} day, the infected group and the control group received 0.1 mL (0.66 MBq) of $Na^{99m}TcO_4$. After 40 minutes, all animals were killed and kidney, liver, stomach and blood samples were isolated and the percentage of radioactivity per gram of tissue (%ATI/g) was determined. Parts of tissue samples were used for histological analysis. Data were compared by t-Student test and Mann-Whitney test, considering $p<0.05$ statistically significant. Results: No statistically significant difference in uptake of ATI%/g was observed in any of the organs. Statistically significant histological changes were seen in the liver of the infected group (cell vacuolization and necrosis) and in the kidney (alterations in the parietal cells and mesangial cells). Conclusions: Although the histopathological finding of necrosis has been a significant result, this change did not alter the uptake of technetium-99m at the site of infection. The parasitic infection by acute malaria or the degree of liver injury probably will not affect the performance of nuclear medicine examinations.

Key words: *Plasmodium berghei.* Malaria. Bioavailability. Radiopharmaceutical. 99mTc. Pertechnetate.

RESUMO

Objetivo: A infecção por *Plasmodium berghei* é um modelo murino de malária amplamente utilizado em estudos experimentais, semelhante ao de *Plasmodium falciparum* nos seres humanos. Este estudo objetivou investigar *in vivo* a influência da infecção pelo *P. berghei* na biodistribuição do pertecnetato de sódio ($NaTc^{99m}$ O_4) em camundongos. **Métodos:** 14 camundongos suiços foram divididos em dois grupos: controle (n=7) e tratado (n=7). O grupo tratado foi inoculado com $1x10^5$ hemácias saguineas infectadas por *P. Berghei.* No 15º dia, o grupo infectado e o grupo controle receberam 0,1 mL (0,66 MBq) de $NaTc^{99m}$ O_4. Após 40 minutos, os animais foram sacrificados e amostras de rim, fígado, estômago e sangue foram isolados e a percentagem de radioatividade por grama de tecido (% ATI/g) foi determinada. Amostras dos tecidos foram também utilizadas para análise histológica. Os dados foram comparados pelo teste t de *Student* e *Mann-Whitney*, considerando-se $p<0,05$ estatisticamente significativo. **Resultados:** não houve diferença estatisticamente significativa na captação do %ATI/g em nenhum dos órgãos estudados. Alterações histológicas estatisticamente significativas foram observadas no fígado do grupo infectado (vacuolização celular e necrose) e no rim (alterações nas células parietais e células mesangiais). **Conclusões:** Embora o achado histopatológico de necrose tenha apresentado um resultado significativo, esta mudança não alterou a captação de Tecnécio^{99m} no sítio da infecção. A infecção aguda pelo parasita ou o grau da lesão hepática devido à malária, provavelmente, não influenciará a realização de exames de medicina nuclear**.**

Descritores: *Plasmodium berghei.* Malaria. Biodisponibilidade. Radiofármacos. Tc^{99m}. Pertecnetato.

INTRODUCTION

Currently, malaria is an infectious disease caused by five species of *Plasmodium* in humans*: Plasmodium falciparum (P. falciparum*), *Plasmodium vivax* (*P. vivax*), *Plasmodium malariae* (*P. malariae*), *Plasmodium ovale* (*P. ovale*) and *Plasmodium knowlesi* (*P. knowlesi*)[1,2].

Malaria is one of the most prevalent human parasitic infections and found in the tropics and subtropics around the world. Probably, it is one of the oldest diseases known to man for millennia[1,2]. *P. falciparum* is responsible for nearly all deaths from malaria and is the only species that seems to directly affect the central nervous system causing cognitive sequelae and neurological deficits[1,3,4]. Epidemiologically, 75% of global *falciparum* malaria cases and more than 90% of malaria deaths occur in Africa (south of Sahara)[5]. In 2010, this disease killed about 660,000 people, mostly children under 5 years old. About 40% of the world population live in areas at risk of malaria transmission and *P. falciparum* is the ethiologic agent of malaria that causes more than 1 million deaths each year. According to the WHO[5], there are 99 countries with active

transmission of malaria. Malaria is transmitted by the bite of the *Anopheles* mosquito and it is an entirely preventable and treatable infectious disease[5]. In Brazil, 99.7% of malaria cases are concentrated in the Amazon Region, mainly in the states of Amazonas, Pará and Rondônia, which together account for 85% of cases in this country[5].

The use of radiopharmaceuticals in nuclear medicine is a powerful diagnostic tool capable of detecting inflammatory and infectious human diseases. The clinical images obtained from the use of these radiopharmaceuticals are valuable, because the analysis and correct interpretation help physicians in making decisions and subsequent therapy and/or rehabilitation[6]. Scintigraphy is often used for analysis of anatomofunctional organs and systems in patients with tropical diseases, using Technetium-99m sodium pertechnetate ($Na^{99m}TcO_4$), a radiopharmaceutical capable of binding to a variety of molecules and cells[7,8]. Over 80% of commonly used radiopharmaceuticals for human scintigraphic 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 to obtain from portable generators[8,9].

When injected intravenously, the radiopharmaceutical is distributed through the vascular and interstitial spaces, and is used to obtain diagnostic images of the stomach, salivary glands, thyroid and parathyroid glands, choroid plexus, brain, studies of esophageal reflux and blood flow, beyond its use in experimental research[8,10,11]. The distribution, elimination and fixation of radiopharmaceuticals in the body depend on several factors, such as blood flow, tissue metabolism and their binding to the blood elements. Biodistribution, defined as the concentration and distribution of radioactive elements in organs and tissues, follows a standard uptake that can mean normality or disease[8].

Scintigraphic using ^{99m}Tc-complexed biomolecules is used by malaria patients to observe the bioavailability of organs. The use of radiopharmaceutical hepatobiliary iminodiacetic acid (^{99m}Tc-HIDA) is effective for diagnosis of acute cholecystitis acalculous exclusively in patients affected by *Plasmodium vivax*[12]. To assess the degree of liver damage and functional status of hepatocytes in patients with malaria can be used the glycated human serum albumin labeled with ^{99m}Tc[13]. On the other hand, ^{99m}Tc-labeled sulfur colloid is commonly used to observe the gastric motility in patients with cerebral malaria[11].

Experimental studies in animal models infected with blood or tissue containing parasites such as *Toxoplasma gondii*, *Plasmodium sp., Leishmania sp*. and receiving

sodium pertechnetate, are scarce. Information about changes in the biodistribution of sodium pertechnetate in an organism infected by *Plasmodium* in the acute phase of infection is necessary, since it can directly influence the pathogenesis and clinical diagnosis of malaria or for use in diagnosis by nuclear medicine.

To evaluate the biodistribution of a tracer in a murine model of *P. berghei* is important to verify if the presence of the parasite inside the red blood cells can influence the uptake of the radiotracer and hence its the normal distribution. It is also important to check the possible anatomical and functional changes in a murine model of malaria and thus contribute to the understanding of disease pathogenesis. Therefore, the aim of this study was to investigate *in vivo* the influence of infection by *Plasmodium berghei* on the biodistribution of sodium pertechnetate in mice.

METHODS

Swiss albino mice (2 months old, 25±2 g weight) were used for experimental assays and received water and food *ad libitum* (Purina/Labina®). The animals were maintained under constant environmental conditions (23±2ºC; 12h/12h of light/dark cycle). The experimental protocols were performed using Guidelines for Ethical Conduct in the Care and Use of Animals and approved by the Research Ethics Committee of Bioscience Center of the Federal University of Rio Grande do Norte (UFRN) with the number (CEUA/058/2011). The protocol of the biodistribution of the radiopharmaceutical was made following the standards of radiological protection of the National Commission of Nuclear Energy (CNEN). *Plasmodium berghei* was obtained from the Laboratory of Biology of Malaria and Toxoplasmosis (LABMAT) of Bioscience Center – UFRN.

Randomly, the animals were divided into two groups: control (n=7) and infected (n=7). Briefly, seven male mice were inoculated with $1x10^{5}$ *P. berghei* (NK65 strain) infected red blood cells each, by intraperitoneal route. The mice were kept together overnight, randomly divided in two groups of 3 and 4 per cage. At 3, 5, 7, 9, 11, 13 and 15 days after parasite inoculation, blood smears were prepared from each mouse, fixed with methanol, stained with Giemsa and then microscopically examined (1,000 X magnification). Parasitaemia was determined in coded blood smears by randomly counting up to 1000-3000 erythrocytes. On the 15^{th} day, the infected group (with an average of about 30% of parasitized red blood cells) and the control group (7 animals) received 0.1 mL (0.66 MBq) of $Na^{99m}TcO_4$ via orbital plexus under anesthesia. 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. After 40 minutes, all animals were quickly killed under anesthesia with xylazine (20 mg/kg) and ketamine (50 mg/kg), by intraperitoneal via. Kidney, liver, stomach and blood samples were isolated, washed in 0.9% saline and weighed on a precision scale (Mark 160®, Bel equipment, Italy)**.** The percentage of radioactivity per gram of tissue (%ATI/g) was determined 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[14]. Parts of tissue samples taken were fixed in 10% formaline, cut as 5 µm tissue sections and stained with hematoxylin**-**eosin and dehydrated in ethanol and xylene for histological analysis. The smears were assessed and quantified according to the following histological features: vascular congestion (sinusoidal, centrilobular and portal space), necrosis and fatty liver[15]. The severity of histopathology was expressed in crosses (0 to 3+), obtained from the average of three randomly chosen microscopic fields, considering the following grading: 0+: no change**;** 1+: mild changes (less than 25% of the field examined; 2+: moderate intensity changes (25 to 50% of the field examined); 3+: severe intensity changes (more than 50% of the field analyzed). All data were presented as mean ± standard deviation. The %ATI/g was compared by t-Student test and the histological alterations were compared by Mann-Whitney test, considering $p<0.05$ statistically significant. Statistica 6.0 software was used to analyze the data.

RESULTS

Figure 1 shows the increase in the level of parasitemia of the infected group between the first and fifteenth day of infection. The infected mice with 30% parasitized erythrocytes with *P. berghei* developed a lethal infection after 15th day of infection.

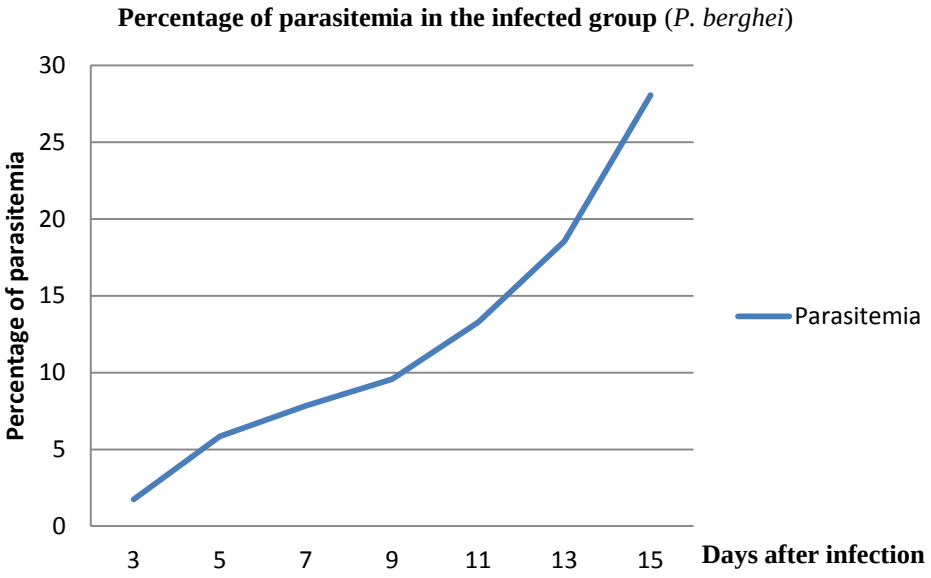

Figure 1 - Percentage of parasitemia in the infected group.

Table 1 shows the relationship between the control group and the infected group. No statistically significant difference (P>0.05) in biodistribution of sodium pertechnetate (ATI%/g) was observed in any of the organs.

Table 1 – Uptake of $Na^{99m}TcO_4$ (%ATI/g) in control group and infected group.

Organs	ATI%/g		*p*-value
	Control group	Infected group	
Blood	6.502 ± 0.6605	7.422 ± 0.3096	0.2693
Liver	2.845 ± 0.3439	3.760 ± 0.3687	0.1036
Kidney	1.217 ± 0.1538	2.202 ± 0.4737	0.0609
Stomach	4.357 ± 0.5784	2.918 ± 0.2394	0.0619

Mean ± SD; No difference was observed between the two groups (p>0.05).

Table 2 shows histological parameters of control group and infected group. Statistically significant histological changes were seen in the liver of the infected group as cell vacuolization and necrosis. Intense inflammatory infiltrate, loss of architecture of the liver parenchyma and malarial pigment were also found.

Table 2 – Histological liver parameters in the control group and infected group.

Histological parameters	Liver		*p*-value
	Control group	Infected group	
Vascular congestion	1.143 ± 0.1429	0.833 ± 0.4014	0.5338
Necrosis	1.285 ± 0.1844	2.667 ± 0.2108	0.0012*
Cell vacuolization	1.071 ± 0.2974	1.833 ± 0.1667	0.0140*
Steatosis	1.142 ± 0.1429	0.900 ± 0.2449	0.5303

Mean ± SD; *p<0.05

DISCUSSION

Plasmodium berghei in the murine model is widely used in studies related to malaria pathogenesis, biological studies and pharmacological tests due to its easy handling and characteristics similar to *Plasmodium falciparum* in humans[16,17].

Plasmodium falciparum is responsible for almost all the mortality from malaria and is the only species that appear to directly affect the central nervous system causing neurological deficits and cognitive sequelae[1,3,4]. It suggests that a toxic substance released by the parasites increases the permeability of the blood brain barrier resulting in cerebral edema, coma, and death[18]. Apparent obstruction to the blood flow in the brain caused by parasitized erythrocytes might be the cause of coma and death in cerebral malaria[19].

In our study, the sacrifice of the infected mice with 30% parasitized erythrocytes was due to the fact that *P. berghei* develop a lethal infection in mice (Figure 1).

The biological behavior of radiopharmaceuticals used for diagnosis in nuclear medicine is well established in the scientific literature. There is evidence that radiopharmaceutical biodistribution may be altered by infectious diseases, procedures, cigarette smoking, surgery, food or natural and synthetic drugs[20-24].

Holanda et al. (2006)[21] evaluated the influence of natural and synthetic antimalarial drugs (artemisinin and mefloquine, respectively) on the biodistribution of radiopharmaceutical methylenediphosphonic acid labeled with technetium-99m (^{99m}Tc-MDP) in rats. In this study, it was observed a significant increase of percentage of total radioactivity injected into each rat (%ATI) in spleen, liver and blood of rats treated with mefloquine. The %ATI increased significantly in femur, liver, lungs, spleen and blood of rats treated with artemisinin. A significant decrease of %ATI occurred in the mefloquine group in bladder, stout bowel, pancreas, kidneys and brain. A significant decrease of %ATI also occurred in the artemisinin group in bladder, stout bowel, muscle, pancreas and kidneys.

Barbosa et al. (2009)[24] showed alterations in the biodistribution of sodium pertechnetate ($Na^{99m}TcO_4$) in rats infected with *Trypanosoma cruzi*, the protozoan that causes Chagas's disease. The authors demonstrated a decreased uptake of the $Na^{99m}TcO_4$ in the colon of chagasic rats, probably due to the inflammatory process and destruction of some histological structures of the organ. However, Rebello et al. (1994)[25] showed no evidence of binding of $Na^{99m}TcO_4$ to the parasite *Schistosoma mansoni* that causes schistosomiasis in Brazil.

The development of *Plasmodium* in the erythrocytes occurs due to the transport of encoded proteins of the parasite to multiple locations, including the cytoplasm and the membrane of the infected erythrocyte. These proteins confer changes in infected erythrocytes, including rigidity and adhesion to the vascular endothelium and an increase in the permeability to various substances[16]. The preferred site of the ^{99m}Tc binding to the erythrocytes is the β chain of hemoglobin. The process of pertechnetate binding to the erythrocytes involves passive diffusion into the cell[26].

The present study demonstrated that the uptake of $Na^{99m}TcO_4$ was not altered in the blood of the animals infected with *P. berghei* despite intense destruction of erythrocytes. This fact probably occurred due to the bind of the $Na^{99m}TcO_4$ (mostly 80%) to plasma proteins[8]. Although no changes have occurred *in vivo, in vitro* studies with red blood cells labeled with technetium-99m are needed.

Although there was no change in the biodistribution of the technetium-99m in the blood, liver, kidney and stomach (Table 1), it is important to demonstrate the potential of ^{99m}Tc as a radiotracer to be used in the diagnosis of acute malaria as the conditions for the coexistence of infected patients and *Plasmodium*.

The histopathological changes observed in the liver of the infected group (Table 2) have been previously observed in others parasitic studies[27]. *Plasmodium berghei* induced liver injury, characterized by the apoptotic and necrotic hepatocytes and dense infiltration of lymphocytes. These pathological changes can be caused by cytokines secreted by *P*. *berghei* that directly damage epithelial cells during parasitic infection. *P. berghei* infection induces activation to produce IL-12, leading to liver injury in a perforin/granzyme-dependent manner[28]. This infection also stimulates lymphocytes and macrophages as a part of the host defense mechanism against the parasites[29]. Necrosis and separation from the lamina propria of most of the epithelial cells and edema occurred due to parasite inducing an atrophy of the epithelial cells[30].

In our study, although the histopathological finding of necrosis has been a significant result, this change did not alter the uptake of technetium-99m at the site of infection. This fact leads us to believe that the parasitic infection by acute malaria, or

the degree of liver injury, probably will not affect the performance of nuclear medicine examinations.

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