Effect of antimalarial chloroquine on the biodistribution of sodium pertecnetate in swiss mice

Efeito do antimalárico cloroquina na biodistribuição do pertecnetato de sódio em camundongos swiss

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ABSTRACT

Background/Objective: Chloroquine is the prototype anti malarial drug, most widely used to treat all types of malaria except for disease caused by chloroquine resistant *Plasmodium falciparum*. It is also indicated in the treatment of rheumatoid arthritis, lupus erythematosus, giardiasis, amoebic and autoimmune hepatitis. Technetium-99m (^{99m}Tc) in the form of sodium pertechnetate ($Na^{99m}TcO_4$) is a radionuclide that connects to wide variety of molecules and cells. This study aimed to evaluate in vivo the influence of chloroquine on the biodistribution of $Na^{99m}TcO_4^-$ and histological pattern of Swiss mice. **Methods:** We used 14 male Swiss mice: treated group (n = 7) received chloroquine solution and control groups (n=7) received filtered water, for 15 days. Both groups received 0.1 mL (0.66 MBq) of $Na^{99m}TcO_4^-$ and the percentage of radioactivity per gram of tissue (%ATI/g) was determined. Parts of tissue samples were isolated for histologycal analysis. **Results:** Statistically significant difference in biodistribution of sodium pertechnetate (ATI%/g) was observed in blood and liver with increased radiotracer uptake in the treated group. Significant histological changes were seen in mesangial cells in the kidney and significant vascular congestion of the treated group. **Conclusion:** We conclude that the use of chloroquine in swiss mice interferes with the uptake of sodium pertechnetate in some organs and histological changes were visualized probably due to the presence of chloroquine toxicity.

Key-words: Chloroquine. Pertechnetate-Tc99m**.** Radiopharmaceuticals. Bioavailability. Toxicity. Mice.

RESUMO

Introdução/Objetivo: A cloroquina é o protótipo de fármaco antimalárico mais amplamente utilizado para tratar todos os tipos de malária, exceto para a doença causada por *Plasmodium falciparum* resistente à cloroquina. Também é indicado no tratamento de artrite reumatóide, lúpus eritematoso, giardíase, amebas e hepatite auto-imune. Os radiofármacos são compostos radioativos amplamente utilizados para o diagnóstico e tratamento de várias doenças. Tecnécio-99m (^{99m}Tc), sob a forma de pertecnetato de sódio ($Na^{99m}TcO_4^-$) é um radionuclídeo que se liga a uma grande variedade de moléculas e células,sendo utilizado em cintilografias do estômago, glândulas salivares, da tiróide e glândulas paratiróides, o plexo coróide, em análises de refluxo esofágico e de fluxo sanguíneo. Este estudo teve como objetivo avaliar *in vivo* a influência da cloroquina na biodistribuição do $Na^{99m}TcO_4^-$e padrão histológico de camundongos Swiss. **Métodos:** Foram utilizados 14 camundongos Swiss machos: grupo tratado (n = 7) recebeu solução de cloroquina e controle (n = 7) recebeu água filtrada, por 15 dias. Ambos os grupos receberam 0,1 ml (0,66 MBq) de $Na^{99m}TcO_4^-$ eo percentual de radioatividade por grama de tecido (% ATI / g) foi determinada. Amostras de tecidos foram isoladas para análise histológica. **Resultados:** Foi observada diferença estatisticamente significante na biodistribuição do pertecnetato de sódio (ATI% / g) no sangue e no fígado, com aumento da captação do radiofármaco no grupo tratado. Alterações histológicas significativas foram observadas em células mesangiais do rim e congestão vascular do grupo tratado. **Conclusão:** Conclui-se que o uso de cloroquina em camundongos swiss interfere na absorção de pertecnetato de sódio em alguns órgãos e alterações histológicas foram visualizadas provavelmente devido à toxicidade da cloroquina.

Descritores: Cloroquina, Pertecnetato de sódio, Biodisponibilidade, radiofármaco, toxicidade, camundongo.

INTRODUCTION

Chloroquine is a4-amino-quinoline considered the prototype anti malarial drug, most widely used to treat all types of malaria except for disease caused by chloroquine resistant *Plasmodium falciparum*. It is highly effective against erythrocytic forms of *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*, sensitive strains of *Plasmodium falciparum* and gametocytes of *Plasmodium vivax*[1]. Although it has limited action onsome strains of *P.falciparum,* is an important antimalarial been widely used since the 50s due to its efficacy, tolerability, and low cost[2-3]. It is also indicated in the treatment of rheumatoid arthritis, lupus erythematosus, giardiasis and hepatic amoebiasis[4,5,6,7]. This drug presents systemic adverse reactions affecting the gastrointestinal tract, nervous system, skeletal and cardiac muscle, skin andeyes[8,9], accumulates in intracellular acidic organelles, raises the pH, and induces osmotic

swelling and permeabilization of acidic organelles, which account for cloroquine-induced cytotoxicity[10].

After ingestion, chloroquine is rapidly and almost completely absorbed from the gastrointestinal tract(2 –4h)and is deposited in the tissues in considerable quantities in experimental animals, can be found in liver, spleen, kidney and lung from 200to 700 times the plasma concentration[11]. Leukocytes also concentrate the drug. The brain and spinal cord, moreover, contain only 10 to 30 times the amount present in plasma. After administration, is biotransformed via cytochrome P450 enzymes forming the desetilchloroquine which is pharmacologically active and bisdesetilchloroquine, which are cleaned with a half-life of 20 to 60 days. The drug and metabolitec an be detected in urine months after a single dose[12].

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 wide variety of molecules and cells widely used radionuclide scintigraphy in the stomach, salivary gland, thyroid and parathyroid glands, the choroid plexus, esophageal reflux and blood flow[13,14], in addition to its use in experimental research. The evidence that natural and/or synthetic drugs can affect the biodistribution of radiopharmaceuticals (radiobiocomplexes) in setting of nuclear medicine clinic is already known[14,15,16]. 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[16,17].

This study aimed to evaluate in vivo the influence of chloroquine on the biodistribution of $Na^{99m}TcO_4^-$ and histological pattern in organs of Swiss mice.

METHODS

We used 14 male Swiss mice weighing 20±5 g from the vivarium of the Potiguar University of Rio Grande do Norte, Natal, Brazil. The animals had free access to water and standard rodent food (Purina/Labina®) maintained under constant environmental conditions (23±2ºC; 12h/12h of light/dark cycle). The protocol was conducted according to international regulations for animal experimentation and approved by the Research Ethics Committee of Bioscience Center of the Federal University of Rio Grande do Norte (UFRN) with the protocol number CEUA/058/2011. 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. The animals were randomly allocated to two groups of seven animals each one: treated group (n = 7) received orally (gavage) 0.2 mL of chloroquine solution 20 mg/Kg/day and control group (n=7) received filtered water by the same procedure. The animals were treated for 15 days. On the last day of treatment the mice received 0.1 mL (0.66 MBq) of $Na^{99m}TcO_4^-$ via orbital plexus under anesthesia. 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 and stomach samples were washed in 0.9% saline, and with the blood sample weighed on a precision scale (Mark 160®, Bel equipment, Italy). 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 was 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[16]. Parts of tissue samples were fixed in 10% formaline, cut as 5 µm tissue sections, stained with hematoxylin-eosin and dehydrated in ethanol and xylene for histologycal analysis[18]. We evaluated and quantified the changes arising on the following histological features: vascular congestion (sinusoidal, centrilobular and portal space), necrosis and fatty liver. The intensity of histopathological features was expressed in crosses (0-3 +), obtained from the average of three random microscopic fields, considering the following graduation: 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 histhological alterations were compared by Mann-Whitney test, considering $p < 0.05$ statistically significant. Statistica 6.0 software was used to analyze the data.

RESULTS

Table 1 shows the relationship between the controls and the treatedcloroquine group. Statistically significant difference ($p<0.05$) in biodistribution of sodium pertechnetate (ATI%/g) was observed in blood and liverwith increased radiotracer uptake in the treated group.

Table 2 shows histological parameters in control and treated groups. Significant histological changes were seen in mesangial cells in the kidney of the treated group.

Table 3 shows histological parameters in liver in control and treated group with significant vascular congestion.

Table 1 – Biodistribution of $Na^{99m}TcO_4^-$ in control group and in mouse treated with cloroquine.

Organs	ATI%/g		p-value
	Control	Chloroquine	
Blood	6.502 ± 0.6605	15.97 ± 1.336	0.0043*
Liver	2.845 ± 0.3439	4.708 ± 0.8044	0.0491*
Thiroyd	0.3300 ± 0.1663	1.178 ± 0.5653	0.1524
Kidney	1.217 ± 0.1538	1.652 ± 0.1267	0.0626
Stomach	4.357 ± 0.5784	3.990 ± 0.6371	0.6799

Mean ± SD; * $p<0.05$

Table 2 – Histological parameters of kidney in the control group and treated group with cloroquine.

Histological parameters	Kidney		p-value
	Control	Cloroquine	
Mesangial Cells	2.25 ± 0.25	3.20 ± 0.20	0.019*
Lymphocytic infiltrate	2.29 ± 0.18	2.50 ±0.22	0.533
Glomerular capillaries	2.28 ± 0.28	2.40 ± 0.40	0.755

Mean ± SD; * $p<0.05$

Table 3 – Histological parameters of liver in the control group and treated group with cloroquine

Histological parameters	Liver		p-value
	Control	Cloroquine	
Vascular congestion	1.1430 ± 0.1429	1.800 ± 0.2000	0.0202*
Necrosis	0.2857 ± 0.1844	0.8000 ± 0.3742	0.3434
Cell vacuolization	0.5714 ± 0.2974	1.400 ± 0.2449	0.1061
Steatosis	0.1429 ± 0.1429	0.2000 ± 0.2000	0.8763

Mean ± SD; * $p<0.05$

DISCUSSION

The pattern of a radiopharmaceutical biological behavior can be altered by interaction with natural or synthetic drugs, food, cigarettes, surgical procedures and parasitic infections and produce an unexpected result[17,19,20,21, 22]. Knowledge of this interaction is important to avoid the misinterpretation of scintigraphic images[16,23]. The alteration of the bioavailability of a radiopharmaceutical due to the effect of a drug in a specific tissue could aid in identifying the toxicolologic effect of a substance in an organ[24].

Several researches on the influence of antiparasitic drugs and radiopharmaceuticals are described in the literature. Xavier-Holanda et al. showed the Glucantime, an anti-*Leishmania*drug, increased the uptake of the radiopharmaceutical methylene disphosphonic acid, labeled with technetium-99m (^{99m}Tc-MDP) in the spleen, kidney, testicles, heart and liver of rats[15]. Antimalarial drugs also have shown alter the biodistribution of radiopharmaceuticals. Holanda et al.evaluated the influence of natural and synthetic antimalarial drugs (artemisinin and mefloquine, respectively) on the bioavailability of the radiopharmaceutical methylenediphosphonic acid labeled with technetium-99m (^{99m}Tc-MDP) in W*istar* rats. A significant increase of %ATI occurredin spleen, liver and blood in rats treated with mefloquine. The %ATI increased significantly in artemisinin rat treated in femur, liver, lungs, spleen and blood. A significant decrease of %ATI occurred in mefloquine group in bladder, stout bowel, pancreas, kidneys, brain and also in artemisinin group in bladder, stout bowel, muscle, pancreas and kidneys[24].

Chloroquine is 60% bound to plasma proteins and equally cleared by the kidney and liver.In the liver, undergoes biotransformation by cytochrome p450 enzymes complex[12].To an extent of 40% to 70% chloroquine, is eliminated by renal elimination of the unchanged substance and patients with renal insufficiency may be necessary a dose reduction[25]. Chloroquine is a weak base, accumulates in the acidic intracellular organelles, increases pH and induces osmotic swelling and permeation of acidic organelles[10]. However, knowledge of the mechanism of chloroquine cititoxicity is still unknown.It was reported that chloroquine could induce oxidative stress and causes oxidative disorders in cells[26].

The liver is the central organ of the metabolism of drugs as well as detoxification processes becomes a vulnerable organ action of chloroquine[27]. The increased radiopharmaceutical uptake in the liver may have been a result of the metabolism of chloroquine in this organ. Metabolites and oxidative stress generated by chloroquine may have caused morphological changes in both the liver and kidneys and have altered the biodistribution of Tc-99m in the liver and blood.

Proliferation of mesangial cells is an early indicator of nephropathy progression. Diabetic nephropathy is characterized by proliferation of mesangial cells, mesangial expansion, hypertrophy and extracellular matrix accumulation[28]. In our study we observed a significant proliferation of mesangial cells in mice treated with chloroquine. Since chloroquine may change the antioxidant status and cause certain organs become more susceptible to oxidative stress[29,30]. Chloroquine treatment in general resulted in increase in activity of all the lysosomal enzymes in rats[31].

Increased uptake of pertechnetate into the blood may indicate that chloroquine favors the labeling of red blood cells (RBCs) probably facilitating the passage of Tc-99m through the plasma membrane or chloroquine competes with binding sites of TC-99m in red blood cells resulting in greater availability of the radiopharmaceutical in plasma. The increased uptake of Tc-99m in the blood may be due to redox mechanisms. For this, in vitro tagging of red cells should be performed.

Chloroquine has been reported to cause damage to the kidneys, deteriorating renal function in animal models and in humans and exacerbating preexisting renal disease[32,25]. Our results showed mesangial cell proliferation in the group of rats treated with chloroquine.The proliferation of such cells associated or not to the expansion of mesangial matrix are histologic findings frequently observed during the evolution of glomerulopathies[33]. Pari e Murugan showed that treatment with chloroquine cause kidney damage including multiple focuses of hemorrhage, necrosis and swelling tubule also compared to normal kidneys and associated formation of highly reactive radicals as a consequence of oxidative stress caused by chloroquine[30].

Although kidney damage has occurred, no change in radiopharmaceutical uptake was verified in this organ with dose and duration of use, in this study. It was demonstrated an histological liver damage associated with high radiotracer uptake. The results may have clinical implications in patients with chronic use of chloroquine and make use of radiopharmaceuticals for scintigraphic examinations.

CONCLUSION

The use of chloroquine in mice interferes with the uptake of sodium pertechnetate in the liver and blood and causes histological changes in the liver and kidney. Although research is experimental, it is suggested clinical investigations on a possible alteration of the biodistribution of radiopharmaceuticals for scintigraphic examinations in patients using chloroquine.

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