Splenectomy changes the biodistribution of pertechnetate ($^{99m}TcO_4^-$) in rats

A esplenectoma altera a biodistribuição do pertecnetato ($Tc^{99m}o_4^-$) em ratos

Ítalo Medeiros Azevedo, Ana Paula Fernandes Neves, Danilo Veras Lobo de Paiva, Jurema Samara Fonsêca Véras, Larissa de Castro Tomasi, Amália Cínthia Meneses Rêgo, PhD, Marília Daniela Ferreira Carvalho, Vítor Brasil Medeiros, Irami Araújo-Filho, MD, PhD, Aldo Cunha Medeiros, MD, PhD.

From the Department of Surgery and Postgraduate Program in Health Sciences, Federal University of Rio Grande do Norte, Brazil
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Correspondence address: Ítalo Medeiros Azevedo, Department of Surgery, Federal University of Rio Grande do Norte, at Ave Nilo Peçanha 620, Natal, RN, Brazil. Email: italo_med@yahoo.com.br
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ABSTRACT

Purpose: To assess if splenectomy alters the biodistribution of sodium pertechnetate in organs and tissues of rats**. Methods**: Twelve Wistar rats were randomly allocated into two groups, A (splenectomized) and B (control), anesthetized with ketamine (50 mg / kg, IM, and sodium thiopental (20 mg / kg-IP) and operated under aseptic conditions. Group A (n = 6) rats underwent laparotomy for splenectomy and group B rats (n = 6) were only anesthetized. Both remained under postoperative observation and after 10 days they were injected with 0.1 mL of sodium pertechnetate (0.66MBq) via orbital plexus. After 30 min, the rats were killed by an overdose of anesthetic and samples of stomach, liver, heart, lung, thyroid, bladder, kidney, brain and femur were harvested. Detection of radioactivity was determined by an automated gamma counter, Wizard Gamma Counter ® Perkin-Elmer. Data were expressed as mean±standard deviation and Student t test was used for independent samples, considering $p<0.05$ as significant. **Results:** There was a lower uptake of pertechnetate in group A than group B in kidney, heart, lung, bladder and femur ($p<0.05$), when compared with controls. The liver of

the splenectomized animals showed radioactive uptake significantly higher than in controls. **Conclusion**: According to the experimental model, we conclude that totalsplenectomy in rats resulted in alteration in the biodistribution of pertechnetate in vital organs.

Key words: Bioavailability. Tc 99m Pertechnetate. Splenectomy. Rats.

Resumo

Objetivo: Avaliar se a esplenectomia altera a biodistribuição do radiofármaco pertecnetato de sódio nos órgãos e tecidos de ratos. **Métodos**: Doze ratos Wistar foram distribuídos aleatoriamente em 2 grupos, A (esplenectomizados) e B (controle), anestesiados com ketamina (50 mg/Kg-IM, e tiopental sódico (20 mg/Kg-IP), operados sob condições asséptica s. Os animais do grupo A (n=6) foram submetidos a laparotomia mediana e esplenectomia total e grupo B (n=6) apenas foram anestesiados. Ambos permaneceram sob observação pós-operatória e após 10 dias foram submetidos à administração de 0,1 ml de pertecnetato de sódio (0,66MBq) via plexo orbital. Após 30 min, foram mortos com superdose de anestésico e submetidos à retirada de segmentos do estômago, fígado, coração, pulmão, tireóide, bexiga, rim, fêmur e cérebro. A detecção da radioatividade foi determinada através de contador gama automático, Wizard Gama Counter Perkin-Elmer®. Dados foram expressos em média±desvio padrão e o teste *t* de *Student* para amostras independentes foi usado, considerando as diferenças significantes com $p<0,05$. **Resultados**: Observou-se menor captação do pertecnetato nos animais do grupo A em relação ao grupo B, no rim, coração, pulmão, bexiga e fêmur com $p<0,05$. O fígado dos animais esplenectomizados apresentou captação radioativa significativamente maior do que nos controles. **Conclusão**: De acordo com o modelo experimental utilizado, pode-se concluir que a esplenectomia total em ratos resultou em alteração na biodistribuição do pertecnetato de sódio em órgãos vitais.

Descritores: Biodisponibilidade. Pertecnetato. Esplenectomia. Ratos.

Introduction

The use of radionuclides has contributed to important advances in health sciences. Diagnostic evaluations are possible by using radiopharmaceuticals labeled with ^{99m} technetium (^{99m}Tc)[1-6]. The widespread use of ^{99m}Tc is due to a number of chemical, physical, economic and ambiental characteristics [l7]. ^{99m}Tc is an artificial radionuclide originating from the disintegration of ^{99}molibdenium, an isotope from the nuclear fission of uranium. Its main features are: half-life of six hours, emission of gamma (γ) rays and radiation energy of 140 Kev. By its high availability, easy for connecting to the red blood cells, biological species, cellular structures, molecular, low cost and negligible environmental impact, it has become the most used radionuclide in nuclear medicine under sodium pertechnetate (Na^{99m}Tc04)[8]. It is known that some drugs can interfere with the biodistribution of radiofarmaceuticals[9-12]. However, little is known about the impact of organ resection on the biodistribution of Na^{99m}Tc04 and publications are unavailable in the literature regarding the effects of splenectomy on biodistribution[13].

If splenectomy interferes with the biodistribution of radiopharmaceuticals, scintigraphic exams may result in images of dubious accuracy, culminating in repeated examinations and unnecessary exposure of patients to radiation. In recent decades, traditional indications for splenectomy have been discarded due to high incidence of deaths due to postoperative sepsis[14]. The emergence of alternative management replaced splenectomy. However, serious cases such as pancytopenia, hematological diseases with high risk of systemic or intracranial bleeding, hypersplenism, gastric carcinoma, portal hypertension and trauma are still indications for esplenectomy[14]. The aim of this study was to evaluate if the excision of the spleen alters the biodistribution of Na^{99m}Tc04 in organs and tissues of operated rats.

Methods

In this experimental study we used 12 Wistar rats weighing 295±23g. The rats were from Vivarium of Center of Health Sciences, Federal University of Rio Grande do Norte, Brazil. They were randomly allocated into 2 groups; A (Esplenectomized) and B (Control), of 6 rats each. The investigational protocol was approved by the Institutional Animal Care Committee, and the research was performed in accordance with the guidelines of the Brazilian College of Animal Experimentation.

The rats were observed in individual cages with food and water ad libitum; They were anesthetized with ketamine (50 mg/Kg) i.m. and sodium thiopental (20

mg/Kg) i.p, operated under aseptic conditions. Splenectomy group animals underwent midline laparotomy and subsequent splenectomy. The laparotomy was closed in layers with mononylon 4-0 suture. Hydration was done with normal saline (10 mL/100g weight) injected subcutaneously in the rats for the first 2 postoperative days. Postoperative pain was treated with tenoxicam (Roche Pharm., Brazil); 0.5 mg/kg was injected i.m. once a day for 3 days. In the control group the rats were only anesthetized. Both groups remained under observation for 10 postoperative days, during which the rats were weighed dayly. The animals were again anesthetized with the above-mentioned anesthetic association and injected with 0.1 ml of $Na^{99m}Tc04$ via the orbital plexus, producing a radioactivity of 0.66 MBq. After 30 minutes, the rats were killed by an overdose of anesthetic (thiopental 100mg/Kg) and samples of stomach, liver, heart, lung, thyroid, bladder, right kidney, right femur and brain were harvested. The samples were washed with 0.9% saline solution, and weighed on a precision scale. The detection of radioactivity in each sample was determined using an automatic gamma counter, Wizard Gamma Counter ® Perkin-Elmer, Finland. The percentage of radioactivity of each organ (%ATI/g) was calculated by dividing the radioactivity of each organ by the total activity administered to each animal. Data were expressed as mean$\pm$SD. The comparison between groups was performed by Student t test for independent samples, using a 0.05 significance.

Results

After analysis of the percentages of radioactive uptake (%ATI/g) from the organs samples, comparing the control and splenectomized groups, it was observed that there was lower uptake of pertechnetate in group A rats than group B in the kidney, heart, lung, bladder and femur. These differences were statistically significant ($p<0.05$). The liver of the splenectomized animals showed significantly higher radioactive uptake than in controls ($p=0.0239$). The other organs showed no significant change in the uptake of $Na^{99m}Tc04$ compared splenectomy and control groups (Table 1).

Table 1 - Values of % radioactivity per gram of tissue (%ATI/g) from rats organs.

Organs	%ATI/g		p-valor(1)
	Splenectomy	Control	
Liver	0.41 ± 0.138	0.22 ± 0.050	0.0239*
Stomach	2.28 ± 0.880	3.33 ± 1.593	0.2305
Kidney	0.25 ± 0.044	0.42 ± 0.074	0.0022 *
Heart	0.10 ± 0.024	0.29 ± 0.061	0.0003*
Lung	0.20 ± 0.077	0.39 ± 0.141	0.0293*
Tyroid	2.91 ± 1.594	3.89 ± 1.447	0.3397
Bladder	0.15 ± 0.086	0.34 ± 0.094	0.0092*
Femur	0.08 ± 0.021	0.14 ± 0.032	0.0119*
Brain	0.01 ± 0.001	0.01 ± 0.003	0.1980

Mean±SD
* p≤0.05, comparing splenectomy group and control.
1. P-valor after analysis by t test for independent samples.

Discussion

^{99m}Technetium (^{99m}Tc) is of great diagnostic utility. The labeling of tissues is based on the reduction capacity of stannous chloride (SnCl2), which acts on the ^{99m}Tc in the form of Na^{99m}Tc04. Several factors influence the biodistribution of different radiopharmaceuticals, among them are surgical procedures[13,32-34]. Changing in biodistribution can lead to repeat examinations for patients undergoing surgery, resulting in unnecessary irradiation. The human spleen is located in the left hypochondrium and has the dimensions 12 cm long by 8 cm wide and 3 cm thick. His weight without blood varies from 75 to 90g; in vivo, the spleen weight varies between 150 and 250g[15]. Despite its size, it is irrigated with 350 liters of blood per day, at a speed of 200 ml/min, accounting for 40% of portal vein flow[16]. Through its great amount of macrophages, spleen has one quarter of the lymphoid tissue of the body, and is able to remove foreign particles from the blood and abnormal cells[17]. This function is very important because these macrophages can phagocyte even without the presence of opsonins. This peculiarity gives great power to the spleen as a defense organ in cases of acute infection. However, this paper only begins to develop over the years, since in childhood the spleen is an organ histologically and physiologically imature[18].

Splenectomy is indicated in several situations, among them, traumatic causes[14]. It is also held in non-traumatic cases, such as the surgical treatment of gastric carcinoma, portal hypertension, idiopathic thrombocytopenic purpura and

splenic cysts[19-21]. As there are no published reports of research involving the study of the biodistribution of $Na^{99m}Tc04$ after splenectomy, this study has sought to fill this gap and contribute to clarify this issue. The distribution, fixation and disposal of radiopharmaceuticals in the organs and tissues depend on the flow circulation, metabolism and binding to tissue and blood elements[22]. However, it is known that red blood cells are remodeled in the spleen, passing from spherical to biconcave discs; it clears substances deposited on their surface, such as proteins, Howell-Jolly bodies (nuclear remnants), Heinz bodies (denatured hemoglobin) and Pappenheimer corpuscles (siderotic granules).

The spleen is also active on the destruction of aging red blood cell, poorly formed or coated with antibodies[14]. Therefore, the spleen is of fundamental importance in the formation of functional red blood cells and destruction of aged red blood cells. Splenectomy can interfere with labeling with radiopharmaceuticals, thereby altering the biodistribution of $Na^{99m}Tc04$. Some studies have related splenectomy as a source of repercussions on the monocytic fagocitosis[23] lipidic profile[24] limph nodes[25], showing that the asplenic state carries systemic consequences. In our study there was a change in the $Na^{99m}Tc04$ biodistribution in various organs. There was lower uptake in kidney, heart, lung, bladder, femur and brain of splenectomized animals than in controls. This finding confirms the hypothesis that removal of the spleen significantly alters the biodistribution of $Na^{99m}Tc04$ in rats, which may be related to the effects of asplenia in the formation of red blood cells or other systems. It has been reported that spleen interferes with hepatic function[26]. In fact, in our study certainly the most relevant finding was the observation that the radioactive uptake in the liver was found to be higher than in controls, indicating that the absence of the spleen increased the uptake of $Na^{99m}Tc04$ in liver. If this is true, we can infer that there are clinical implications after splenectomy, i.e., the liver can undergo changes in their physiology, affecting the uptake of radiopharmaceuticals for diagnostic or therapeutic use.

Splenectomy might somewhat promote hepatic regeneration[26,27], prevent liver fibrosis to a certain degree[28], reduce serum bilirubin concentration and improve liver function[29,30]. In a clinical study, serum total bilirubin concentration was promptly decreased to normal range or pre-operative level on the 7th day in splenectomy group, though it was raised for a transient time. Serum total bilirubin concentration increased on 10 to 14 days in non-splenetomy group. The total bilirubin concentration was significantly lower in the former than in the latter on the 7th day after operation, obviously reflecting the effect of splenectomy on reducing the burden of hepatocyte bilirubin[31].

Conclusion

According to the experimental model used in this study, we can conclude that total splenectomy in rats resulted in alteration in the biodistribution of $Na^{99m}Tc04$ in vital organs.

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