

## Metabolic and hematologic consequences of colectomy associated to hepatectomy in rats<sup>1</sup>

### Consequências metabólicas e hematológicas da colectomia associada à hepatectomia em ratos

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#### ABSTRACT

**PURPOSE:** To investigate the influence of partial colectomy associated with hepatectomy on the biodistribution of the <sup>99m</sup>Tc-phytate, on metabolic parameters, as well as labeling and morphology of red blood cells.

**METHODS:** Wistar rats were distributed into three groups (each with six), nominated as colectomy, colectomy+hepatectomy and sham. In the 30<sup>th</sup> postoperative day all rats were injected with <sup>99m</sup>Tc-phytate 0.1mL i.v. (radioactivity 0.66 MBq). After 15 minutes, liver sample was harvested and weighed. Percentage radioactivity per gram of tissue (%ATI/g) was determined using an automatic gamma-counter. Serum AST, ALT, alkaline phosphatase and red blood cells labeling were determined.

**RESULTS:** The liver %ATI/g and red blood cells labeling were lower in colectomy and colectomy+hepatectomy rats than in sham rats ( $p < 0.05$ ), and no difference was detected comparing the colectomy and colectomy+hepatectomy groups. Red blood cells morphology did not differ among groups. Serum levels of AST, ALT and alkaline fosfatase were significantly higher in colectomy+hepatectomy than in colectomy rats ( $p < 0.001$ ).

**CONCLUSION:** Hepatectomy associated with colectomy lowered the uptake of radiopharmaceutical in liver and in red blood cells in rats, coinciding with changes in liver enzymatic activity.

**Keywords:** Colectomy. Hepatectomy. Metabolism. Biological Availability. Technetium. Rats.

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#### RESUMO

**OBJETIVO:** Investigar a influência da colectomia associada à hepatectomia parcial, na biodistribuição do fitato-<sup>99m</sup>TcO<sub>4</sub>, na marcação e morfologia de hemácias e em parâmetros metabólicos.

**MÉTODOS:** Ratos Wistar foram distribuídos em três grupos (seis animais cada), denominados: colectomia, colectomia+hepatectomia e *sham*. No 30<sup>o</sup> dia pós-operatório, em todos eles foi feita injeção de 0,1 mL i.v. de fitato-<sup>99m</sup>TcO<sub>4</sub> (radioatividade 0,66 MBq). Após 15 minutos, uma amostra de fígado foi colhida e pesada. O percentual de radioatividade por grama de tecido (%ATI/g) foi determinado no fígado e hemácias usando-se um contador gama automático. Dosagem sérica de AST, ALT, fosfatase alcalina, morfologia e marcação de hemácias com pertecnato foram determinadas.

**RESULTADOS:** O %ATI/g no fígado e nas hemácias foi menor nos animais dos grupos colectomia e colectomia+hepatectomia do que no grupo sham ( $p < 0,05$ ; teste de Tukey). Nenhuma diferença foi detectada comparando os grupos colectomia e colectomia+hepatectomia. A morfologia das hemácias não diferiu entre os três grupos. Os níveis séricos de AST, ALT e fosfatase alcalina foram significativamente maiores no grupo colectomia+hepatectomia do que no grupo colectomia ( $p < 0,001$ ).

**CONCLUSÃO:** A colectomia associada a hepatectomia contribuiu para reduzir a captação de radiofármaco no fígado e hemácias de ratos, coincidindo com alterações na atividade enzimática do fígado.

**Descritores:** Colectomia. Hepatectomia. Metabolismo. Disponibilidade Biológica. Tecnécio. Ratos.

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## Introduction

The liver is affected by metastases in 50% of patients with colorectal cancer and metastatic disease remains the leading cause of cancer-related death. The metastases may be single or multiple, affecting one or both hepatic lobes and are synchronous with the primary tumor in 16 to 25% of cases, several months or years after diagnosis<sup>1</sup>. The surgical resection of liver metastases is possible in 10 to 15% of patients and it is considered the treatment of choice with potential for cure and five-year survival in 22 to 65% of cases<sup>2</sup>.

One of the most widely used diagnostic tools in identifying diseases and metabolic changes, uses radiopharmaceuticals. On nuclear medicine, pertechnetate ( $^{99m}\text{TcO}_4^-$ ) is used in the diagnosis of gastric, kidney, liver, lung, intestinal, skeletal, endocrine, heart, blood flow and postoperative disorders<sup>3</sup>. Used intravenously, this radiopharmaceutical remains in the circulation long enough to analyse various organic and functional parameters for the diagnosis of diseases. Gradually it comes into equilibrium with the extracellular space, is taken up by tissues, and a small percentage is eliminated by urinary excretion. The  $^{99m}\text{TcO}_4^-$  is used in approximately 85% of diagnosis procedures, has low cost and small environmental impact<sup>3</sup>. Some recent studies have demonstrated significant changes in biodistribution of  $^{99m}\text{TcO}_4^-$  on the postoperative of major surgery<sup>4-8</sup>.

In nuclear medicine, red blood cells can be labeled by  $^{99m}\text{TcO}_4^-$ , and used to detect certain clinical disorders, digestive bleeding, blood flow, heart perfusion, etc<sup>9</sup>. The labeling of blood constituents with  $^{99m}\text{TcO}_4^-$  has been used as a tool in experimental studies *in vitro* and *in vivo*, in order to demonstrate the interaction between drugs and radiopharmaceuticals<sup>18</sup>. The labeling of red blood cells involves intracellular reaction between  $^{99m}\text{TcO}_4^-$  and hemoglobin. It is often used to evaluate pulmonar perfusion<sup>10</sup>, digestive bleeding, liver tumors and hemodynamic disorders<sup>11</sup>. It has been shown that changes on the morphology of red blood cells may arise in the first hours after trauma, persists for several days and may interfere with the their labeling. These changes are more severe in patients with postoperative septic complications<sup>12</sup>.

Any deviation in the uptake of radiopharmaceuticals, on vital organs and red blood cells after surgery, can result undesirable interpretations in the results of scintigraphic examinations, leading to mistaken diagnoses, with serious consequences to the patients. Repetition of the exams can determine an increase of radiation dose for patients and risk for the involved professionals.

This work is part of a research field that has been studying the consequences of major surgery performed in organs of the

digestive system. We have demonstrated relevant postoperative metabolic disorders after massive intestinal resection<sup>4</sup>, total gastrectomy<sup>5,6</sup>, total colectomy<sup>7</sup> and Roux-en-Y gastric bypass<sup>8</sup>, by using this experimental model.

Based on the above concepts, this study aimed to examine in an animal model if colectomy associated with hepatectomy modifies liver biodistribution of  $^{99m}\text{Tc}$ -phytate, metabolic parameters and labeling of red blood cells.

## Methods

Eighteen Wistar rats weighing  $285 \pm 23$ g, were housed in polypropylene cages and kept under controlled conditions of temperature in a clear-dark cycle of 12 hours and allowed *ad libitum* access to food (Labina, Purina®) and water.

All experimental procedures in animals were conducted according to the code of ethics for animal experimentation of the Council for International Organization of Medical Sciences and the Brazilian Law on the Scientific use of Animals (Law No. 11794). The protocol was approved by the Institutional Research Ethics Committee. The anesthesia was accomplished with intraperitoneal injection of 0,2ml/100g of a solution containing 1ml of ketamine (50mg) and 1 ml of xilazine (20 mg) and operated under aseptic conditions. In addition, analgesia (tramadol 20 mg/kg body weight) was applied subcutaneously immediately after surgery and every 12 hours for three days.

The animals from group colectomy underwent laparotomy and a subtotal colectomy was performed, proceeding ileocolic anastomosis with 6-0 polypropilene suture. Cecum, 5 cm of colon and 10 cm of ileum were resected. The laparotomy was closed in layers with 4-0 mononylon. In the group colectomy+hepatectomy the rats were submitted to partial colectomy associated to resection of the left lobe of the liver. In the group sham, we performed medium laparotomy and soft manipulation of the intestine.

The animals were weighed weekly and remained under observation. After 30 days they were anesthetized, the femoral vein was dissected and 0.1 mL of  $^{99m}\text{Tc}$ -phytate was injected i.v. (radioactivity 0.66 MBq). After 15 minutes, 5 mL of blood were harvested by cardiac puncture and the rats were killed with an overdose of anesthetic (sodium thiopental 100mg/Kg). A sample of liver was harvested to examine the biodistribution of  $^{99m}\text{Tc}$ -phytate.

After centrifugation of 2 mL of blood, red blood cells were separated for examination of *in vivo* labeling with pertechnetate and morphology. The liver samples were isolated, washed with 0.9% saline solution and weighed with a precision

scale (Bel-Mark 160-II-Italy). The detection of liver radioactive uptake was determined using an automatic gamma counter, Wizard 1470 (PerkinElmer, Finland). The percentage of radioactivity per gram of tissue (%ATI/g) was calculated dividing the activity of the liver sample by the total activity administered to each animal. The experiment with radiopharmaceutical was carried out in compliance with radiation protection standards recommended by the National Commission of Nuclear Energy. The radiopharmaceutical was provided by Department of Nuclear Medicine of Liga Norte-Riograndense Contra o Câncer (LIGA) and the radioactive waste returned to the LIGA for proper treatment.

*Assay for biochemical levels*

Samples of blood (3 mL) were inserted into test tubes without anticoagulant, centrifuged to 3000 rpm by 10 min and the serum was stocked to -40°C until analysis. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase were measured using a commercial kit on the autoanalyzer (Konelab, Software Version, 60i, Finland).

*Red blood cells morphology*

Blood strains were prepared on slides, them stained with Leishman. The images of red blood cells were captured by digital camera using optical microscopy (Olympus microscope, model BX50, Japan, 1000x,) for analysis. To take the shape of red blood cells and the morphometric measurements we used the ImagePro Plus Software, version 6.0.

*Estatistics*

Data were expressed as mean±sd. The comparison between groups was performed by analysis of variance (ANOVA) and by the multiple comparison test of Tukey, using a 0.05 significance.

**Results**

The animals were randomly divided into three groups of six each. We observed 8% weight loss in group colectomy+hepatectomy at the end of first week. A progressive recovery of weight ocured until day 30. In the other groups there was no weight loss after surgery. At the end of the experiment there was no significant difference in mean weights among groups.

Table 1 shows the results of the percentage of radioactivity per gram of tissue (%ATI/g), found in red blood cells and liver, as well as the tests to investigate the statistical differences among the groups colectomy, colectomy+hepatectomy and sham.

**TABLE 1** - <sup>99m</sup>Tc-phytate biodistribution studies for each group.

Groups	Organ (%ATI/g) <sup>1</sup>	
	Red blood cells <sup>2</sup>	Liver <sup>2</sup>
Colectomy	0.21 ± 0.04	4.35 ± 0.71 <sup>b</sup>
Colectomy + hepatectomy	0.16 ± 0.02 <sup>a</sup>	4.41 ± 0.56 <sup>a</sup>
Sham	0.22 ± 0.05 <sup>a</sup>	5.70 ± 0.87 <sup>ab</sup>
p-value	0.042	0.024

MeanStandard deviation

1. Percent of radioactivity per gram of tissue

2. Dosages in each column, followed by the same letter differ significantly (Tukey test).

Rats from colectomy and colectomy+hepatectomy groups had significantly lower <sup>99m</sup>Tc-phytate uptake in the liver than in the sham group rats (p=0.024). The <sup>99m</sup>Tc-phytate uptake in the liver of colectomy rats was higher than in the colectomy+hepatectomy group rats, but the difference was not significant (p>0.05). Concerning the erythrocytes labeling, we observed a significant difference in %ATI/g when the colectomy+hepatectomy group rats were compared with sham rats (p=0.042). These data are summarized in Table 2.

**TABLE 2** - Serum levels of liver enzymes and alkaline phosphatase in colectomy, colectomy+hepatectomy and sham rats.

Groups	AST (u/L) <sup>1</sup>	ALT (u/L) <sup>1</sup>	Alkaline phosphatase (u/L) <sup>1</sup>
Colectomy	37.67 ± 1.37 <sup>a</sup>	47.33 2.50 <sup>a</sup>	170.6± 22.91 <sup>b</sup>
Colectomy + hepatectomy	155.00 ± 13.67 <sup>ab</sup>	76.33 ± 6.89 <sup>a</sup>	232.3 ± 28.93 <sup>ab</sup>
Sham	44.33 4.72 <sup>b</sup>	51.17 ± 3.19	180.7 ± 20.35 <sup>a</sup>
p-value	<0.001	<0.001	<0.001

Mean ± Standard deviation

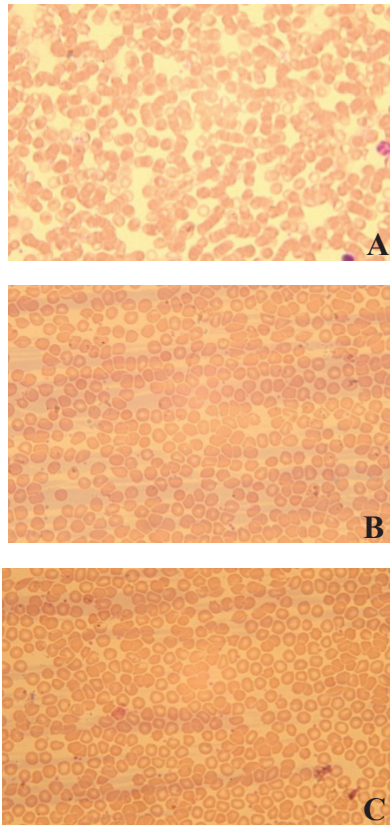
1. Dosages in each column, followed by the same letter, differ significantly (Tukey test).

AST, aspartate animotransferase; ALT, alanine aminotraferase

Serum levels of AST, ALT and alkaline phosphatase were significantly higher in colectomy+hepatectomy rats than in colectomy group rats (p<0.001), signaling that hepatectomy contributed to these results. Comparing the serum levels of AST, ALT and alkaline phosphatase from colectomy group with sham rats, the results were similar (p>0.05) (Table 2).

The qualitative and quantitative evaluation of the shape of red blood cells under optical microscopy was performed and alterations on the shape and morphometric measures were not

found, when compared the three groups (Figure 1).



**FIGURE 1** – Photomicrography of red blood cells from (A) colectomy, (B) colectomy +hepatectomy and (C) sham rats. 1000x.

### Discussion

It is well established in the area of digestive surgery that surgical resection is the most effective treatment for isolated liver metastasis in patients with colorectal cancer<sup>13</sup>. Perioperative morbidity and mortality does not differ between simultaneous resections and staged procedures for selected patients with liver metastases. Meanwhile, patients undergoing simultaneous resections could expect a short duration of surgery and postoperative hospitalization as well as less blood loss during surgery<sup>13</sup>. Although the treatment for patients with synchronous colorectal cancer liver metastases remains controversial, surgical resection of both the primary tumor and liver metastases is the only option offering a potential cure<sup>13</sup>.

In the experimental model used in this work we did not include induction of colon cancer, because our objective was to examine the repercussion of colectomy associated partial hepatectomy without carcinogenesis. After the standardization of the technique in the pilot study, the morbidity was low and there was not mortality during the 30 days of observation of all rats. Despite the great surgical trauma, the animals progressed well

until the end of the experiments. Weight loss in the first week was observed in the animals from colectomy+hepatectomy group, but they had a satisfactory recovery in the three subsequent weeks. These findings corroborate the statement that hepatectomy increased morbidity, but over time the animals had satisfactory evolution.

However, at the end of the observation and collection of biological samples, we detected significant changes in some parameters, when the groups were compared. There was a significant reduction in <sup>99m</sup>Tc-phytate biodistribution in the liver of colectomy+hepatectomy animals, compared with the sham group, but no difference was observed comparing with colectomy group. This finding may be interpreted based on the hypothesis that after 30 days liver regeneration was sufficient to compensate any disfunction, normalizing the <sup>99m</sup>Tc-phytate uptake.

The distribution of radiocolloids in the liver has been shown to correlate well with the severity of chronic liver diseases, the severity of histologic fibrosis, prognosis, and hepatic function<sup>14</sup>. Thus, liver uptake of <sup>99m</sup>Tc-phytate colloid provides a practical index of hepatic function and image, by using planar scintigraphy techniques<sup>15</sup>. In a study from our laboratory it was demonstrated that in splenectomized rats the biodistribution of <sup>99m</sup>Tc-phytate to the liver was higher than in controls, suggesting that the operation favored the hepatic uptake of the radiopharmaceutical. This result coincided with the improvement in liver function, confirmed by the better alanine aminotransferase, aspartate aminotransferase and lactic dehydrogenase activities in splenectomized rats, compared with controls<sup>16</sup>. Otherwise, in the present study the lower liver <sup>99m</sup>Tc-phytate uptake coincided with a lower alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activities in colectomy+hepatectomy rats, than in sham rats.

Nuclear medicine procedures have proven increasingly effective imaging modalities in the study of several disorders<sup>17</sup>. Besides the disease, these procedures could be altered by medications, surgery and natural products that could change the biodistribution of radiopharmaceuticals in a specific target or the fixation of <sup>99m</sup>Tc to blood constituents<sup>16,18</sup>. In general, the labeling of blood constituents could decrease by the action of drugs and trauma:(a) changing of the cellular membrane structure or modifying the transport systems of pertechnetate ions into cells, (b) by direct oxidation or generation of free radicals, (c) by direct inhibition (chelating action) of pertechnetate ions, or (d) binding at the same sites on the blood constituents<sup>3</sup>. In this study we observed that pertechnetate labeling of red blood cells of the colectomy+hepatectomy rats was lower than in sham rats. This

data is relevant, because red blood cells labeling is an important method for diagnosis of digestive and renal bleeding<sup>19</sup>. Interactions of some factors resulting from hepatic resection may be involved with the ion transport systems of pertechnetate ions, decreasing the labeling of red blood cells with pertechnetate. This could in part, explain the data obtained.

### Conclusion

Hepatectomy associated with colectomy lowered the liver and erythrocytes uptake of pertechnetate in rats, coinciding with changes in hepatic enzymatic activity.

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