Clinics in Surgery

Research Article
Published: 20 Jul, 2020

Radiotherapy Enhances Kupffer Cell Function and Modifies the Spleen Macrophage Clearance

Fernando Pereira de Faria[1,2], Andy Petroianu[2], Paula Peixoto Campos[3], Marcela Guimarães Takahashi de Lazari[3], Jony Marques Geraldo[4] and Clara Bicalho Nascimento[4]*

[1]Department of Nuclear Energy, Federal University of Minas Gerais, Brazil

[2]Department of Surgery, Federal University of Minas Gerais, Brazil

[3]Department of General Pathology, Federal University of Minas Gerais, Brazil

[4]Radiotherapy Center of the Luxemburgo Hospital, Brazil

Abstract

Introduction: Although the effects of X-ray irradiation, used in megavoltage radiotherapy are well-known, literature investigating their influence on the cell defense system is still scarce.

Objective: To verify the Kupffer cell and splenic macrophage clearance function after megavoltage X-ray irradiation.

Method: Fourteen adult male Wistar rats were distributed into three groups: group 1 (n=6) - non-irradiated; group 2 (n=4) - irradiated animals, studied after 24 h; group 3 (n=4) - irradiated animals, studied after 48 h. Animals were anesthetized and irradiated with 8 Gy of X-ray in the abdominal region. A colloidal carbon solution was injected in the left internal jugular vein (1 ml/kg); after 40 min, the livers and spleens were removed for histological analysis of the phagocytosis by Kupffer cells and splenic macrophages. Kupffer cells and splenic macrophages containing pigments in consecutive microscopic fields were then counted. Averages obtained for each group were compared among the three groups.

Results: An increase in the number of Kupffer cells containing colloidal carbon pigments in irradiated livers, as well as damage to hepatocytes and bile ducts, was observed. In the spleen, the uniform distribution of the colloidal carbon pigments in red and white pulps shifted to the presence of black pigments inside the macrophages of the marginal zone in the two irradiated groups.

Conclusion: X-ray irradiation is associated with an increase in the function Kupffer cells, and shifts the splenic macrophage clearance without being linked neither to apparent morphological changes nor to the necrosis or apoptosis of splenic cells.

Keywords: Liver; Spleen; Kupffer cell; Macrophage; Clearance function; X-ray

OPEN ACCESS

Correspondence:
Andy Petroianu, Department of Surgery, Federal University of Minas Gerais, Avenida Afonso Pena, 1626 – apto. 1901, Belo Horizonte, MG 30130-005, Brazil, Tel: 55-31- 3274-7744; Fax: 98884-9192; E-mail: petroian@gmail.com

Received Date: *15 May 2020*
Accepted Date: *02 Jul 2020*
Published Date: *20 Jul 2020*

Citation:
de Faria FP, Petroianu A, Campos PP, Takahashi de Lazari MG, Geraldo JM, Nascimento CB. Radiotherapy Enhances Kupffer Cell Function and Modifies the Spleen Macrophage Clearance. Clin Surg. 2020; 5: 2867.

Introduction

The Mononuclear Phagocyte System (MPS) consists of cells originating from bone marrow pluripotent stem cells. These are released in the blood circulation as monocytes and may remain in circulation for a short period of time or may undergo tissue diapedesis, in which they differentiate as macrophages. Macrophage precursors are also found in other tissues, such as the red spleen pulp, liver parenchyma, lung parenchyma, intestinal Peyer's patches, and lymph nodes [1]. However, most of the MPS is found in the liver and spleen; in the main blood clearance organs for microorganisms, including bacteria, fungi, viruses, parasites, foreign bodies, and inhaled particles, as well as in cells with morphological disorders. The MPS also participates in the modulation of endocrine immunity and the regulation of hematopoiesis. Moreover, it works in the production of enzymes, the complementing of system components, the binding of proteins and cytokines, the promotion of clotting and angiogenesis factors, as well as the stimulation of growth factors [1,2].

Macrophages are found in the peritoneal and pleural tissues, alveolar septa, lymph nodes, liver, splenic and sinusoidal vascular beds, etc. [1]. Liver macrophages, described by Kupffer in 1876, are known as Kupffer Cells (KC) and are located on the internal side of the sinusoidal endothelium [3,4]. The KC constitute 35% of the liver's non-parenchymal cells [5] and contain multiple receptors, including complement, toll-like opsonins and immunoglobulins [6], which are responsible for the phagocytosis of bacteria in the blood flow. The KC also operates in immunity surveillance, as

they secrete cytokines and chemokines, which recruit and activate immune cells in response to the presence of pathogens and circulating anomalous structures [7].

In cancer treatment, megavoltage X-ray irradiation, used in Radiotherapy (RT), reaches body structures beyond the radiation field, regardless of what technique is applied, which only ensures reduced doses in tissues outside the target [8]. RT modifies the tumor's microenvironment by damaging blood vessels and peritumoral cells of the immune system. This effect leads to an intense inflammatory response with disorders in cell functions. The irradiation inhibits the access of CD8+ lymphocytes to tumors, and activates immunosuppressive pathways that lead to increasing radio resistant suppressor cells, such as M2 macrophages and regulatory T cells [9].

Although the effects of megavoltage X-ray irradiation used in RT are well-known, medical literature investigating its influence on the mononuclear phagocyte system is still scarce. Considering the lack of knowledge on the effects of megavoltage RT in MPS organs, this work was designed to evaluate the clearance function of Kupffer cells and splenic macrophages after megavoltage X-ray irradiation.

Methods

This work was approved by the Ethics Committee on Animal Use of the Federal University of Minas Gerais (UFMG) (CEUA-Registration number: 115/2018).

A total of 14 adult male Wistar rats were studied, each with an average weight of 265 ± 17 g, allocated at the UFMG School of Medicine's Biotherium. The animals were placed in appropriate cages and were provided with food and water ad libitum. The mice were distributed into three groups:

Table 1: Mean (M) and Standard Error of Mean (SEM) of the number of Kupffer cells and splenic macrophages containing colloidal carbon pigments per animal group.

Groups	N	Liver	Spleen
		M ± SEM	M ± SEM
1	6	277.2 ± 26.0*	429.25 ± 35.9*
2	4	468.0 ± 17.8**	410.25 ± 9.6**
3	4	458.0 ± 17.5***	432.25 ± 7.6***

N: total animals
Group 1: Control - non-irradiated animals, colloidal carbon solution was intravenously injected; 40 min later, liver and spleen were removed for study.
Group 2: Animals irradiated with 8 Gy in the abdomen; after 24 h, colloidal carbon solution was intravenously injected; 40 min later, liver and spleen were removed for study.
Group 3: Animals irradiated with 8 Gy in the abdomen and after 48 h; colloidal carbon solution was intravenously injected; 40 min later, liver and spleen were removed for study.
Liver: Comparison between: * and **, p=0.0001 (one-tailed Student's t-test); * and ***: p=0.0005 (one-tailed Student's t-test); ** and ***, p=0.69 (two-tailed Student's t-test).
Spleen: Comparison (two-tailed Student's t test) between: * and **, p=0.701; * and ***, p=0.951; ** and ***, p=0.123.

Group 1 (n=6) - non-irradiated animals (control group). After four hours of fasting, the animals underwent intraperitoneal anesthesia, using 80 mg/kg of ketamine chloride, together with 7 mg/kg xylazine chloride [10]. Next, a 50% (1 ml/kg) colloidal carbon solution was injected into the left internal jugular vein. After forty minutes, the rats were euthanized, using 240 mg/kg of ketamine chloride, together with 21 mg/kg of xylazine chloride [10]. The liver and spleen were removed and immersed in a 10% buffered formaldehyde solution for future standard histological procedures and histological studies on glass slides, using hematoxylin and eosin stain.

Group 2 (n=4) - the animals were anesthetized using the technique applied to Group 1 and underwent irradiation using the Isocenter

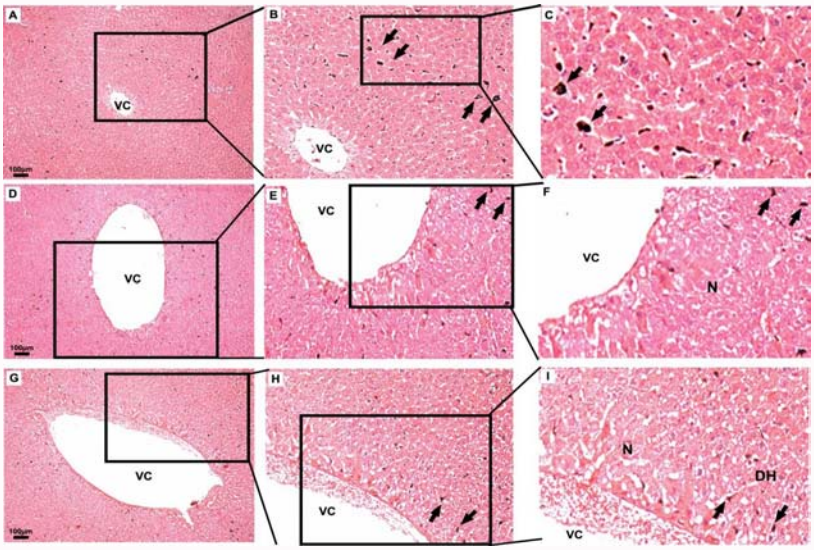

Figure 1: Histological images of 8 Gy irradiated livers and of control, with no irradiation.
A - Liver Centrilobular Vein (CV) of an animal in the control group (non-irradiated) (H&E, 100x).
B and C - Liver parenchyma area showing an area of preserved hepatocytes organized in rows, and the presence of sinusoids. The rows in all frames indicate colloidal carbon pigments in Kupffer cell cytoplasm (H&E, Zoom in the marked area).
D - Liver CV area in an irradiated animal (Group 2), showing its dilation when compared to the image in frame A (H&E, 100x).
E and F - Liver parenchyma area showing necrosis, with necrosis and homogeneous eosinophilia and absence of nucleus, indicated by the letter N (H&E, Zoom in the marked area).
G - Liver CV area in an irradiated animal (Group 3), showing its dilation when compared to the image in frame A (H&E, 100x).
H and I - Liver parenchyma area showing necrosis, with necrosis and homogeneous eosinophilia and absence of nucleus, indicated by the letter N. There are Hydropic Degeneration (HD) areas containing a lace-like pattern and central nucleus (H&E, Zoom in the marked area).

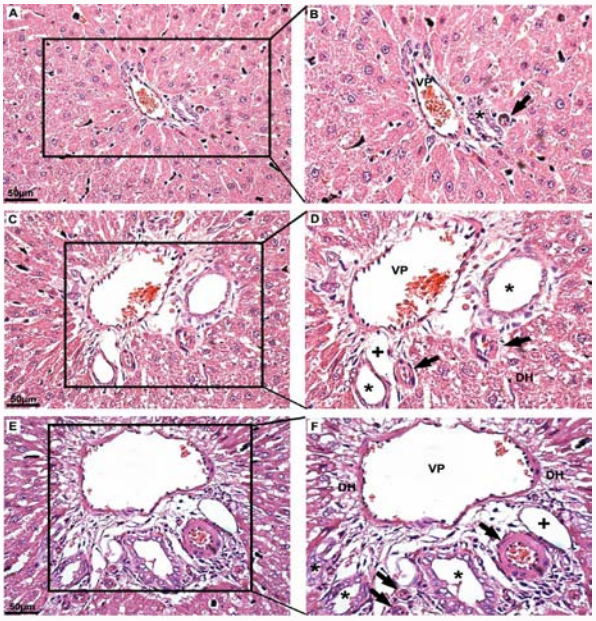

Figure 2: Hepatic portal spaces of control group (non-irradiated rats) and irradiated with 8 Gy X-ray.
A - Hepatic portal space of a non-irradiated animal (Control group) (H&E, 400x).
B - Detail of the area marked in (A) with preserved hepatocytes organized in rows, and the presence of sinusoids, bile duct (*), liver artery branch (arrow), and portal vein (PV) (H&E, 400x, zoom in the marked area).
C - Liver portal space area in an irradiated animal (Group 2) (H&E, 400x).
D - Detail of an area in (C), showing PV dilation and normal hepatic artery branches. Hydropic Degeneration (HD) areas with dilated hepatocytes, as well as lace-like pattern cytoplasm and central nucleus. Increased number of arterioles (arrows), lymphatic vessel (+), and bile duct (*) (H&E, 400x, zoom in the marked area).
E - Liver portal space area in an irradiated animal (Group 3) (H&E, 400x).
F - Detail of an area in (E), showing PV dilation and normal hepatic artery branches. Hydropic Degeneration (HD) areas with dilated hepatocytes, as well as lace-like pattern cytoplasm and central nucleus. Increased number of arterioles (arrows), lymphatic vessel (+), and bile duct (*) (H&E, 400x, zoom in the marked area).

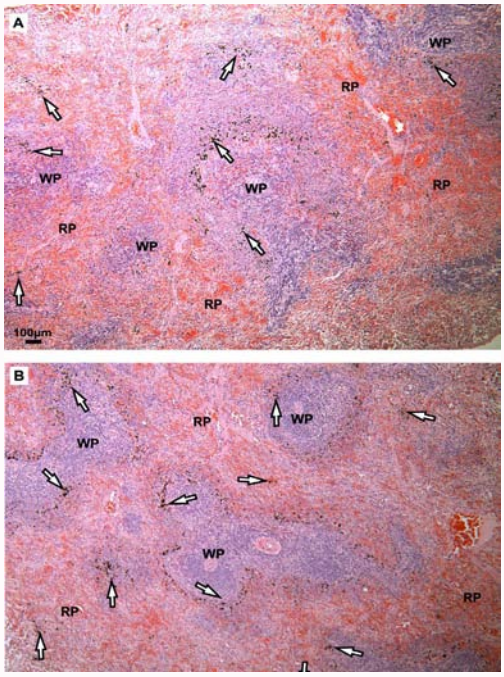

Figure 3: Distribution of colloidal carbon pigments in the splenic parenchyma of non-irradiated and irradiated (8 Gy X-ray) rats.
A) Non-irradiated splenic parenchyma with macrophages containing carbon pigments (arrows) uniformly distributed in the red (RP) and white (WP) pulps (H&E, 100x).
B) Irradiated splenic parenchyma with macrophages containing carbon pigments (arrows) concentrated only in the marginal zone (arrows) surrounding the White Pulp (WP), which contains no black pigment, as well as the Red Pulp (RP) (H&E, 100x).

technique [8] with 8 Gy (4 Gy in anteroposterior incidence and 4 Gy in posteroanterior incidence) in the abdominal region, using a 6-MV linear accelerator. The rats received the colloidal carbon solution, in accordance with the procedure described for Group 1, 24 h after irradiation. After forty minutes, the rats were euthanized, using 240 mg/kg of ketamine chloride, together with 21 mg/kg xylazine chloride [9]. The liver and spleen were removed and immersed in a 10% buffered formaldehyde solution for future standard histological procedures and histological studies on glass slides, using hematoxylin and eosin stain.

Group 3 (n=4) - the animals were anesthetized using the technique applied to Group 1 and underwent irradiation as performed in Group 2. The rats received the colloidal carbon solution, in accordance with the procedure described for Group 1, 48 h after irradiation. After 40 min, the rats were euthanized, using 240 mg/kg of ketamine chloride together with 21 mg/kg of xylazine chloride [10]. The liver and spleen were removed and immersed in a 10% buffered formaldehyde solution for future standard histological procedures and histological studies on glass slides, using hematoxylin and eosin stain.

The KC containing black colloidal carbon pigments in their cytoplasm were counted in 12 consecutive optical microscopic fields per slide, using a 400x magnification. The splenic macrophages containing these pigments in their cytoplasm were counted in 16 consecutive optical microscopic fields, using a 1000x magnification, in immersion oil.

The one-tailed Student's t-test was used to compare, two by two, the average number of KCs containing colloidal carbon pigments in the irradiated and control groups. The same test was used to compare splenic macrophages containing colloidal carbon. To compare averages in the two irradiated groups, the two-tailed Student t-test was used. In the comparisons, differences corresponding to $p<0.05$ were considered to be significant.

Results

The animals remained stable during the experiments. The distribution of phagocytized carbon pigments was uniform in all studied liver sections along the sinusoidal capillaries. The number of KCs that phagocytized colloidal carbon pigments was higher in the irradiated animal groups ($p<0.05$). No difference in this number was detected in the irradiated groups (Table 1). Necrosis and degeneration were detected in hepatocytes, including perivenular types, the dilation of centrilobular veins (Figure 1), as well as the proliferation of bile ducts in portal tracts of the irradiated animals' livers, but not in the control group animals (Figure 2).

The spleen parenchyma presented a uniform distribution of the macrophages containing colloidal carbon inside the red and white pulp in the control group. The macrophages with colloidal carbon were located almost exclusively in the marginal zone around the

white pulp after irradiation (Figure 3), without any morphological damage detected in the spleen parenchyma. The total mean number of macrophages containing carbon colloid per microscopic field was no different from the control group. These histological analyzes identified no disorder in the splenic morphology nor in necrosis or apoptosis [11-14].

Discussion

Since the 1990s, our research team has studied the phagocytic function of MPS organs in the presence of alcoholism, partial splenectomy, autogenous splenic implants, oophorectomy, and pregnancy [15-26]. Quantification of the clearance activity in animal models is performed by means of histological studies after the intravenous infusion of colloidal substances, as well as by means of radioactive counts of the MPS in gamma radiation detectors, after the intravenous infusion of radiolabeled bacteria [27-35].

According to studies conducted with colloidal substances, splenic macrophages and KCs are responsible for 85% to 90% of the intravascular phagocytic activity [36]. Other studies have shown that the liver plays a major role in the blood clearance of radiolabeled bacteria, followed by the spleen and the lungs, which are more effective in clearing colloidal substances and bacteria [28].

In addition to the megavoltage RT, cancer has been treated with the use of nanoparticles associated with radionuclides and antitumor drugs, directed toward the tumor environment [37,38]. These complexes are injected into the bloodstream in order to destroy cancer cells; however, they are also removed from the blood by the MPS and act upon its cells. It is estimated that approximately 99% of the nanoparticles are removed from circulation by the MPS, including the KC and the splenic macrophages. Therefore, the antitumoral effect is caused by only 1% of what is administered to the organism [39,40].

Since 1923, there have been reports on the influence of low energy X-ray irradiation on MPS activity in animal models, by measurements of the blood's clearance of colloidal substances and bacteria [41-62]. These studies were conducted in accordance with full-body irradiation protocols, which are rarely used for RT nowadays. The radiobiological effects depend, among other factors, on the radiation energy and the irradiated body region [8]. Therefore, it is possible for the MPS to be affected by megavoltage RT in a different manner from that observed in low-energy irradiation.

The increase in the number of KC containing colloidal carbon is consistent with experimental results *in vitro* using macrophage derivatives from human monocytes irradiated with a 10 Gy X-ray [63] and macrophage derivatives from rodent monocytes irradiated with 8 Gy [64]. These studies have reported the increase in the studied macrophages' phagocytic capacity, which is most likely associated with the activation of its pro-inflammatory state caused by radiation [63]. Knowledge of the KC behavior after irradiation is of interest for treating cancer with antitumoral drugs carried by nanoparticles and concomitant RT, considering that KC-phagocytized nanoparticles carry these drugs [37-40].

Radiobiological damages to the liver parenchyma in patients subjected to total or partial liver irradiation to control hepatocellular carcinoma are well-known and include the activation of hepatic stellate cells and the increase in hepatic enzymes, such as alkaline phosphatase, aminotransferases, lactate dehydrogenase, hepatomegaly, and ascites, immediately after the RT [65-68]. In animal models, irradiation is associated with KC activation, centrilobular vein dilation, and the destruction of hepatocytes [12], with an increase in oxidative stress and lipid peroxidation [69]. Other alterations observed after RT include steatosis, [70], congestion of vascular sinusoids [65,68], hepatic fibrosis, and hepatocyte apoptosis [30,31].

In this work, the detection of hepatocyte necrosis and dilated centrilobular veins in irradiated animals are in agreement with previous studies involving rats that had been previously irradiated with 8 Gy of X-ray in the abdominal region in a 6-MV linear accelerator [12]. Hepatocyte necrosis is a short-term consequence of this irradiation on hepatocyte DNA, which affects its structure, resulting in its death by necrosis [8]. The dilation of centrilobular veins results from vascular alterations throughout the liver parenchyma and requires additional studies to understand its physiopathology.

The proliferation of bile ducts observed in portal tracts of irradiated animals is an attempt to achieve liver regeneration. Bile duct branches contain stem cells capable of differentiating into several cell types of this parenchyma [71-73]. Previous studies with Wistar rats found the proliferation of bile ducts only after fraction doses of liver focal radiation therapy [68]. However, this work showed that duct proliferation also takes place soon after a single dose of megavoltage X-ray irradiation.

Splenic macrophages are found around the sinusoidal capillaries, forming the so-called Billroth cords, in the white and red pulps, as well as in the marginal zone around the white pulp [15,16]. These cells remove abnormal red blood cells; store metals, especially the Fe ion resulting from hemoglobin metabolism; exhibit antigens to the B and T lymphocytes; secrete inflammatory cytokines; and modulate immune responses [15,16].

The shifts of the splenic macrophage clearance function from the red and white pulps to the marginal zone after irradiation reveals that megavoltage RT with a single 8 Gy dose in the abdominal region of the animals changes the splenic activity. The literature establishes that high fraction RT doses cause splenic hypotrophy through the destruction of white pulp [74]. However, in this work, no microscopic or macroscopic short-term morphological alterations, apoptosis, or necrosis in the spleen were found during the short observation period. Therefore, the absence of colloidal carbon uptake in most of the splenic parenchyma is not due to the absence of macrophages, but rather to the loss of their functional capacity. On the other hand, irradiation did not interfere in the ability of macrophages in the marginal zone to remove colloid from the blood stream.

Conclusion

X-ray irradiation is associated with an increase in the function of Kupffer cells, and shifts the splenic macrophage clearance without being linked neither to apparent morphological changes nor to the necrosis or apoptosis of splenic tissues.

Acknowledgment

The authors thank Dr. Alexandre Pereira de Faria and Dimitri Favalessa for their support in the experimental procedures with animals, Prof. Dr. Alfredo José Afonso Barbosa for his contribution in histological studies, and to the Radiation Therapy Center at Hospital Luxemburgo, Belo Horizonte, Brazil for irradiating the animals.

The authors acknowledge the CAPES (Coordination for the Improvement of Higher Education Personnel), to CNPq (Brazilian

National Council for Scientific and Technological Development), to FAPEMIG (Minas Gerais State Research Foundation), and to the financial support provided by UFMG's Office for Research.

References

1. Hume DA, Irvine KM, Pridans C. The mononuclear phagocyte system: The relationship between monocytes and macrophages. Trends Immunol. 2019;40(2):98-112.
2. Gordon S, Plüddemann A. The mononuclear phagocytic system. Generation of diversity. Front Immunol. 2019;10:1893.
3. Kumar V, Abbas AK, Aster JC. Pathologic basis of disease. 9th ed. Philadelphia, PA: Saunders; 2015. p. 821-3.
4. Kubes P, Jenne C. Immune responses in the liver. Annu Rev Immunol. 2018;36:247-77.
5. Bilzer M, Roggel F, Gerbes AL. Role of Kupffer cells in host defense and liver disease. Liver Int. 2006;26(10):1175-86.
6. Helmy KY, Katschke KJ Jr, Gorgani NN, Kljavin NM, Elliott JM, Diehl L, et al. CRIg: A macrophage complement receptor required for phagocytosis of circulating pathogens. Cell. 2006;124(5):915-27.
7. He JQ, Katschke KJ Jr, Gribling P, Suto E, Lee WP, Diehl L, et al. CRIg mediates early Kupffer cell responses to adenovirus. J Leukoc Biol. 2013;93(2):301-6.
8. Khan FM, Gibbons JP. Physics of radiation therapy. 5th ed. Hong Kong: Lippincott Williams & Wilkins; 2014. p. 182-3.
9. Jarosz-Biej M, Smolarczyk R, Cichoń T, Kułach N. Tumor microenvironment as a "game changer" in cancer radiotherapy. Int J Mol Sci. 2019;20(13):3212.
10. Damy SB, Camargo RS, Chammas R, De Figueiredo LFP. Fundamental aspects of animal experimentation - applications in experimental surgery. Rev Assoc Med Bras. 2010;56:103-11.
11. Scheibe PO. Number of samples-hypothesis testing. Nucl Med Biol. 2008;35(1):3-9.
12. Özyurt H, Özden AS, Çevik Ö, Özgen Z, Cadirci S, Elmas MA, et al. Investigation into the role of the cholinergic system in radiation-induced damage in the rat liver and ileum. J Radiat Res. 2014;55(5):866-75.
13. Wang S, Lee K, Hyun J, Lee Y, Kim Y, Jung Y. Hedgehog signaling influences gender-specific response of liver to radiation in mice. Hepatol Int. 2013;7(4):1065-74.
14. Wang S, Lee Y, Kim J, Hyun J, Lee K, Kim Y, et al. Potential role of Hedgehog pathway in liver response to radiation. PLoS One. 2013;8(9):e74141.
15. Petroianu A. Historical aspects of spleen and splenic surgeries. In: Petroianu A, editor. The spleen. 1st ed. Betham eBooks; 2011. p. 3-19.
16. Kierszenbaum AL, Tres LL. Histology and cell biology: An introduction to pathology. 3rd ed. Philadelphia, PA: Saunders; 2012. p. 328-330.
17. Sabino KR, Petroianu A, Alberti LR. Influence of the acute alcoholism on the phagocytic function of the mononuclear phagocytic system. J Med Life. 2011;4(4):421-3.
18. Petroianu A. Assessment of phagocytic function in remnants of subtotal spleen implantation. Rev bras hematol hemoter. 2003;25(1):25-31.
19. Petroianu A, Barbosa AJA. Quantitative studies on the macrophage phagocytosis in whole spleen and in the remnant of subtotal splenectomy. Med Sci Res. 1991;19:373-5.
20. Petroianu A, Simal CJ, Barbosa AJA. Impairment of phagocytosis by mammalian splenic macrophages by 99m-Tc sulphur colloid. Med Sci Res. 1992;20:847-9.
21. Petroianu A, Barbosa AJA. Splenic macrophage phagocytic function after subtotal splenectomy in the dog. Med Sci Res. 1992;20:127-8.
22. Araújo ID, Simal CJR, Souza IF, Neto JEO, Junior PSB. Phagocyte inhibition in the rat liver spleen and lung. Acta Cir Bras. 1994;9:169-73.
23. Araujo ID, Marques MS, Lage RP, Ferraz de Souza IK, Petroianu A. Assessment of reticuloendothelial system phagocytosis following laparotomy in rats. Austr J Med Sci. 1996;17:79-81.
24. Petroianu A, Marques RG, Coelho JMC. Regenetration of phagocytic function after splenic autotransplantation. Biomed Res. 2002;13:15-8.
25. Marques RG, Petroianu A, Coelho JMC, Portela MC. Morphology and phagocytic function of regenerated autogenous splenic implant in rats. Acta Cir Bras. 2004;19(6):642-8.
26. Vasconcellos LS, Sabino RK, Petroianu A. The influence of oophorectomy and pregnancy on the phagocytic function of the phagocytic mononuclear system in experimental model. J Bras Patol Med Lab. 2005;41(3):153-7.
27. Marques RG, Petroianu Y, Coelho JM. Bacterial phagocytosis by macrophage of autogenous splenic implant. Braz J Biol. 2003;63(3):491-5.
28. Marques RG, Petroianu A, de Oliveira MBN, Bernardo-Filho M, Boasquevisque EM, Portela MC. Bacterial clearance after total splenectomy and splenic autotransplantation in rats. Appl Radiat Isot. 2002;57(6):767-71.
29. Marques RG, Petroianu A. Distribution of *Escherichia coli* in mononuclear phagocytic system organs after total splenectomy isolated or combined with splenic autotransplantation in rat. Rev Col Bras Cir. 2003;30(5):330-6.
30. Petroianu A, da Silva RG, Cardoso VN, Alberti LR, da Silva MG. Effect of spleen surgeries on *Escherichia coli* distribution on the mononuclear phagocytic system. Int J Surg. 2010;8(1):48-51.
31. Petroianu A, Simal CJR, Barbosa AJA. Assessment of phagocytic function in remnants of subtotal splenectomy and in autologous spleen implantation. Med Sci Res. 1993;21:715-7.
32. Marques RG, Lucena SBSG, Caetano CER, de Sousa VO, Portela MC, Petroianu A. Blood clearance of Howell-Jolly bodies in an experimental autogenic splenic implant model. Br J Surg. 2014;101(7):820-7.
33. Petroianu A, Marques RG, Oliveira MBN, Filho MB. Importância da preservação de tecido esplênico para a fagocitose bacteriana. Acta Cir Bras. 2002;17(6):388-93.
34. de Matos Filho AS, Petroianu A. Spleen function after preservation in a physiological solution. J Surg Res. 2015;199(2):586-91.
35. Petroianu A, Silva RG, Silva MG, Diniz SOF, Cardoso VN. Influence of surgical procedures on spleen on distribution of *Escherichia coli* in mononuclear phagocyte system. Rev Col Bras Cir. 2003;30(1):65-71.
36. Saba TM. Physiology and physiopathology of the reticuloendothelial system. Arch Intern Med. 1970;126(6):1031-52.
37. Wang Z, Zhao K, Zhang Y, Duan X, Zhao Y. Anti-GPC3 antibody tagged cationic switchable lipid-based nanoparticles for the co-delivery of anti-miRNA27a and sorafenib in liver cancers. Pharm Res. 2019;36(10):145.
38. Cullis PR, Hope MJ. Lipid nanoparticle systems for enabling gene therapies. Mol Ther. 2017;25(7):1467-75.
39. Tavares AJ, Poon W, Yi-Nan Zhang, Dai Q, Besla R, Ding D, et al. Effect of removing Kupffer cells on nanoparticle tumor delivery. Proc Natl Acad Sci USA. 2017;114(51):E10871-80.
40. Andón FT, Digifico E, Maeda A, Erreni M, Mantovani A, Alonso MJ, et al. Targeting tumor associated macrophages: The new challenge for nanomedicine. Semin Immunol. 2017;34:103-13.
41. Warren SL, Whipple GH. Bacterial invasion of the blood stream as influenced by X-ray destruction of the mucosal epithelium of the small intestine. J Exp Med. 1923;38(6):713-23.
42. Chrom SvA. Studies on the effect of roentgen rays upon intestinal epithelium and upon the reticulo-endothelial cells of the liver and spleen. Acta Radiologica. 1935;16(6):641-60.

43. Miller CP, Hammond CW, Tompkins M. The incidence of bacteremia in mice subjected to total body X-radiation. Science. 1950;111(2890):540-1.

44. Barrow J, Tullis JL, Chambers FW Jr. Effect of X-radiation and antihistamine drugs on the reticulo-endothelial system measured with colloidal radiogold. Am J Physiol. 1951;164(3):822-31.

45. Callaway JL, Kerby GP. Splanchnic removal of bacteria from the circulating blood of irradiated rabbits. AMA Arch Derm Syphilol. 1951;63(2):200-6.

46. Lamson BG, Tullis JL. The progression of morphologic lesions in Swiss mice exposed to 625r, 2000 KVP, total body X-radiation. Mil Surg. 1951;109(4):281-93.

47. Wish L, Furth J, Sheppard CW, Storey RH. Disappearance rate of tagged substances from the circulation of roentgen irradiated animals. Am J Roentgenol Radium Ther Nucl Med. 1952;67(4):628-40.

48. Kaplan HS, Speck RS, Jawetz E. Impairment of antimicrobial defenses following total body irradiation of mice. J Lab Clin Med. 1952;40(5):682-91.

49. Shechmeister IL, Bond VP, Swift MN. The susceptibility of irradiated mice to infection as a function of post-irradiation time. J Immunol. 1952;68(1):87-95.

50. Shechmeister IL, Paulissen LJ, Fishman M. Subtotal body x-radiation and susceptibility of mice to Salmonella enteritidis and *Escherichia coli*. Proc Soc Exp Biol Med. 1953;83:205-9.

51. Fitch FW, Barker P, Soules KH, Wissler RW. A study of antigen localization and degradation and the histologic reaction in the spleen of normal, X-irradiated and spleen shielded rats. J Lab Clin Med. 1953;42(4):598-620.

52. Hammond CW, Tompkins M, Miller CP. Studies on susceptibility to infection following ionizing radiation. I. The time of onset and duration of the endogenous bacteremias in mice. J Exp Med. 1954;99(5):405-10.

53. Shechmeister IL, Fishman M. The effect of ionizing radiation on phagocytosis and the bactericidal power of the blood. I. Effect of radiation on migration of leucocytes. J Exp Med. 1955;101(3):259-74.

54. Fishman M, Schechmeister IL. The effect of ionizing radiation on phagocytosis and the bactericidal power of the blood. II. The effect of radiation on ingestion and digestion of bacteria. J Exp Med. 1955;101(3):275-90.

55. Di Luzio NR. Effects of X-irradiation and choline on the reticulo-endothelial system of the rat. Am J Physiol. 1955;181(3):595-8.

56. Gordon LE, Cooper DB, Miller CP. Clearance of bacteria from the blood of irradiated rabbits. Proc Soc Exp Biol Med. 1955;89(4):577-9.

57. Gyi KK, Marcus S. Effects of acute and chronic exposure to X-radiation on phagocytic activity. J Immunol. 1957;79(4):312-8.

58. Benacerraf B, Kivy-Rosenberg E, Sebestyen MM, Zweifach BW. The effect of high doses of X-irradiation on the phagocytic, proliferative, and metabolic properties of the reticulo-endothelial system. J Exp Med. 1959;110(1):49-64.

59. Benacerraf B, Sebestyen MM, Schlossman S. A quantitative study of the kinetics of blood clearance of p32 labelled *Escherichia coli* and *Staphylococci* by the reticulo-endothelial system. J Exp Med. 1959;110(1):27-48.

60. Nelson EL, Becker JR. The effect of whole body X-irradiation on the bactericidal activity of phagocytic cells. I. Survival of *Pseudomonas aeruginosa* within phagocytes from peritoneal exudates of mice. J Infect Dis. 1959;104(1):13-9.

61. Nelson EL, Becker JR. The effect of whole body X-irradiation on the bactericidal activity of phagocytic cells. II. Survival of *Pseudomonas aeruginosa* within liver and spleen of mice. J Infect Dis. 1959;104(1):20-3.

62. Zweifach BW, Kivy-Rosenberg E, Nagler AL. Resistance to whole body X-irradiation in rats made tolerant to bacterial endotoxins. Am J Physiol. 1959;197:1364-70.

63. Pinto AT, Pinto ML, Cardoso AP, Monteiro C, Pinto MT, Maia AF, et al. Ionizing radiation modulates human macrophages towards a pro-inflammatory phenotype preserving their pro-invasive and pro-angiogenic capacities. Sci Rep. 2016;6:18765.

64. Conrad S, Ritter S, Fournier C, Nixdorff K. Differential effects of irradiation with carbon ions and X-rays on macrophage function. J Radiat Res. 2009;50(3):223-31.

65. Koay EJ, Owen D, Das P. Radiation-induced liver disease and modern radiotherapy. Semin Radiat Oncol. 2018;28(4):321-31.

66. Lawrence TS, Robertson JM, Anscher MS, Jirtle RL, Ensminger WD, Fajardo LF. Hepatic toxicity resulting from cancer treatment. Int J Radiat Oncol Biol Phys. 1995;31(5):1237-48.

67. Sempoux C, Horsmans Y, Geubel A, Fraikin J, Van Beers BE, Gigot JF, et al. Severe radiation-induced liver disease following localized radiation therapy for biliopancreatic carcinoma: Activation of hepatic stellate cells as an early event. Hepatology. 1997;26(1):128-34.

68. Rave-Fränk M, Malik IA, Christiansen H, Naz N, Sultan S, Amanzada A, et al. Rat model of fractionated (2 Gy/day) 60 Gy irradiation of the liver: Long-term effects. Radiat Environ Biophys. 2013;52(3):321-38.

69. DeLeve LD, Shulman HM, McDonald GB. Toxic injury to hepatic sinusoids: Sinusoidal obstruction syndrome (veno-occlusive disease). Semin Liver Dis. 2002;22(1):27-42.

70. Christiansen H, Batusic D, Saile B, Hermann RM, Dudas J, Rave-Frank M, et al. Identification of genes responsive to gamma radiation in rat hepatocytes and rat liver by cDNA array gene expression analysis. Radiat Res. 2006;165(3):318-25.

71. Zhang J, Zhou S, Zhou Y, Feng F, Wang Q, Zhu X, et al. Hepatocyte growth factor gene-modified adipose-derived mesenchymal stem cells ameliorate radiation induced liver damage in a rat model. PLoS One. 2014;9(12):e114670.

72. Carpino G, Renzi A, Franchitto A, Cardinale V, Onori P, Reid L, et al. Stem/progenitor cell niches involved in hepatic and biliary regeneration. Stem Cells Int. 2016;2016:3658013.

73. Overi D, Carpino G, Cardinale V, Franchitto A, Safarikia S, Onori P, et al. Contribution of resident stem cells to liver and biliary tree regeneration in human diseases. Int J Mol Sci. 2018;19(10):2917.

74. Barbosa AJA. Some Aspects of Splenic Pathology. In: Petroianu A, editor. The spleen. 1a ed. Betham eBooks; 2011. p. 75-83.