Effect of cyclosporine on liver regeneration in partial hepatectomized rats[1]

Giorgio Alfredo Pedroso Baretta[I], Ozimo Gama Filho[II], Edimar Leandro Toderke[II], André Ricardo Dall'Oglio Tolazzi[III], Jorge Eduardo Fouto Matias[IV]

DOI: http://dx.doi.org/10.1590/S0102-86502015001000007

[I]Fellow Master degree, Postgraduate Program in Surgical Clinic, Department of Surgery and Liver Transplantation, Federal University of Parana (UFPR), Curitiba-PR, Brazil. Intellectual and scientific content of the study; technical and surgical procedures; acquisition, analysis and interpretation of data; manuscript writing.
[II]Fellow Master degree, Postgraduate Program in Surgical Clinic, Department of Surgery and Liver Transplantation, UFPR, Curitiba-PR, Brazil. Technical and surgical procedures, acquisition and analysis of data.
[III]Fellow Master degree, Postgraduate Program in Surgical Clinic, Department of Surgery and Liver Transplantation, Federal University of Parana (UFPR), Curitiba-PR, Brazil. Technical and surgical procedures, acquisition and analysis of data.
[IV]PhD, Associate Professor of Surgery, Coordinator of the Postgraduate Program in Surgical Clinic, Department of Surgery and Liver Transplantation, UFPR, Curitiba-PR, Brazil. Conception, design, intellectual and scientific content of the study; technical and surgical procedures; analysis and interpretation of data; manuscript writing, critical revision.

ABSTRACT

PURPOSE: To evaluate the influence of the cyclosporine in liver regeneration in rats submitted to an experimental model of 70% hepatectomy.

METHODS: Forty male rats were randomly divided in four subgroups (C.24h, C.7d, E.24h, E.7d), according to the drug used and the day of sacrifice (24 hours and 7 days). Cyclosporine (10mg/Kg/day) was given to the study subgroup and 1 ml of 0.9% sodium chloride was to the control subgroup. Resection of left lateral lobe and median lobe performing 70% of liver mass. During the animals' death, KWON formula was applied. Counting of mitotic figures and percentage of positive nucleus with PCNA and Ki-67 were evaluated.

RESULTS: In the 2nd, 4th PO and death days, E.7d lose more weight than C.7d. Regarding to the KWON formula, the C.7d regenerated more than the C.24h and the same with the E.7d. Comparing between the groups, only E7d subgroup was statistically significant compared with C.7d, showing the stimulating effect of cyclosporine in liver regeneration. Immunohistochemestry had significant results between the study subgroups. The mitotic index revealed statistical differences in the control subgroups.

CONCLUSION: Cyclosporine, in spite of being an immunosuppressive drug, has a positive effect in liver regeneration, although reduce the animal's body weight.

Key words: Liver Regeneration. Cyclosporine. Hepatectomy. Rats.

Introduction

A notable capacity of the liver is the ability to regenerate itself after an injury like hepatectomy. The first report of liver regeneration tells about the punishment of Prometheus in the Greek mythology. He was punished by being chained to a rock after he has revealed to the mortals the secret of fire protect by the gods of Olympus. Every day, an eagle fed of his liver, which regenerated itself during the night[1].

Liver doesn't regenerate in a true sense, a global tissue hyperplasia occurs until that the original liver mass has been restored[2]. Higgins and Anderson published one of the first studies about liver regeneration in 1931[3]. They removed the left lateral and median lobes of rat liver which amounts 70% of liver rat mass.

At any given time, in rats, about one of 20000 hepatocytes is dividing, and each hepatocyte can divide once or twice at most. After large liver resection, all the liver cells simultaneously go into the G1 phase due to the influence of IL-6 and TNF-α. These cytokines stimulate the hepatocytes to answer the growing factor as HGF (hepatocyte growing factor), TGF-α (transforming growth factor alpha) and HB-EGF (heparin binding – epidermal growth factor). 12 to 15 hours after the hepatectomy, the hepatocytes enter in the S phase, and six to eight hours after the DNA synthesis, the liver cells go to the G2 and M phase. The differences between the beginning of proliferation and the peaks of mitosis after partial hepatectomy (24 hours in rats and 42 hours in mice) show the variability of G1 phase in the species[4].

The degree of hepatocyte proliferation is proportional to the extension of injury, with local mitotic action in small resections; but in injuries greater than 10%, all the liver cells can proliferate. In resections over than 50%, a second peak of mitosis occurs after three to five days in rats[1]. The regenerative process finishes within seven to 14 days[5]. Regulatory growth factors has been studied in the last years, and some of them are well-known and can be divided in three categories: (1) mitogenic agents or growth stimulating factors (HGF, EGF, TGF-α). They are capable in inducing DNA synthesis and mitosis in hepatocytes at rest (G0 phase); (2) comitogenic agents. They stimulate proliferation indirectly, potentiating the effect of mitogenic agents and reducing the effect of inhibiting agents; (3) inhibiting factors. Capable of inhibiting induced mitogenesis in primary hepatocyte cultures[6,7].

Some studies have shown the influence of pretherapy with cyclosporine in liver regeneration after a 70% hepatectomy[8-11], although some papers don't confirm the same results[12,13].

Due to the immunosuppressive properties of cyclosporine and its positive effects on liver regeneration demonstrated in some reports, the possibility of augmentation in hepatocyte proliferation after liver resection, living donor liver transplantation and split liver procedure, appears as a hypothesis of being studied. The purpose of this study is to evaluate the effect of pretherapy with cyclosporine on liver regeneration using a well established animal model of 70% hepatectomy.

Methods

This study was carried out in compliance with the guidelines of the Brazilian Society for Animal Experimentation (COBEA) and Federal Law 6638. Forty male adult Wistar rats (*Rattus norvegicus albinus, Rodentia mammalia*) divided into two groups of twenty were used. Each one was divided into subgroups of 10 rats. The control group had two subgroups of 10 rats each (C.24h and C.7d) and the study group as well (E.24h and E.7d) according to the sacrifice day. All animals were 180 days old and had an average weight of 508 ± 37.86 g. They were kept in plastic cages, within a controlled light-dark, temperature and humidity environment and had water and commercial feed *ad libitum*. Cyclosporine 10mg/Kg/day was administered by gavage for three days before the surgical procedure to the study subgroups and the control subgroups received 1 ml of 0.9% sodium chloride solution by gavage as well. After inhalatory anesthesia with isoflurane, the animals from all subgroups were submitted to hair removal at the ventral abdominal wall and antisepsis with polyvinyl pyrrolidone-iodine. A 4 cm longitudinal incision was performed in the abdomen wall and the peritoneal cavity explored. The left lateral and median hepatic lobes were resected and the pedicle stitched with 3-0 monocryl. This kind of hepatectomy add up to 67% to 70% of the parenchyma of the liver rat. After checking hemostasis, abdominal closure was carried out with 3-0 prolene. The resected segments was weighted and the value recorded for posterior analysis of the Kwon's formula[14].

The gavage with cyclosporine or sodium chloride solution was maintained daily until animals' death. The control subgroup (C.24h) and the study subgroup (E.24h) were killed 24 hours after the hepatectomy. The other two subgroups were killed 7 days after the liver resection (C.7d and E.7d). Sacrifice was by inhaled lethal dose of ethylic ether. The regenerated liver was resected and weighted to calculate the percentage of regeneration. A portion of the superior right lobe was sampled, fixed in 10% buffered formalin and sent for histological examination. Slides were prepared with 4 micrometer thick cuts. These were stained with hematoxylin-eosin to evaluate the mitotic figure count while immunohistochemistry was used to evaluate PCNA and Ki67

positive nuclei count. Liver regeneration was assessed using four methods: Kwon's formula, percentage of mitotic figures in 100 hepatic cells, by the average of PCNA and Ki67 positive nuclei in ten fields.

Kwon's formula gives the regeneration rate based on weight.

% = D / E. 100 where G = R / 0.7

Where: D = liver weight per 100 g body weight on the day that the animal was killed.

E = the estimated liver weight per 100 g of body weight before the hepatectomy and is calculated using the weight of the resected liver (R). G = estimated liver weight at the time of the hepatectomy.

The body weight of all animals was evaluated in the first day of pretherapy, before the hepatectomy, during the death procedure in all subgroups and on second and fourth post-operative days in the seven days subgroups.

Results

When evaluated initial body weight (PCI) and death body weight (PCM), all subgroups lose weight and the differences were statistically significant (Figure 1).

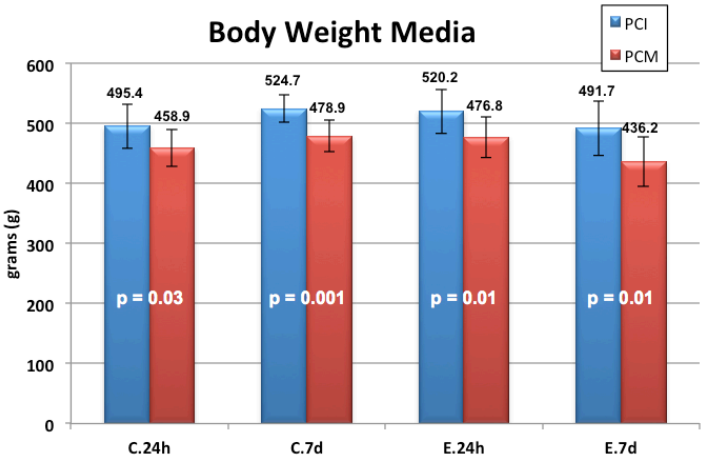

FIGURE 1 – Initial and death body weight media ± standard deviation. (**A**) C.24H: 24 hours control group; (**B**) C.7d: 7 days control group; (**C**) E.24H: 24 hours experimental group; (**D**): E.7d: 7 days experimental group; (**E**) PCI: initial body weight; (**F**) PCM: death body weight. Statistical comparations among groups (p).

When seven days experimental and control groups were compared during the research, the first one lost weight statistically significant on second and fourth postoperative days and on death's moment too (Figure 2).

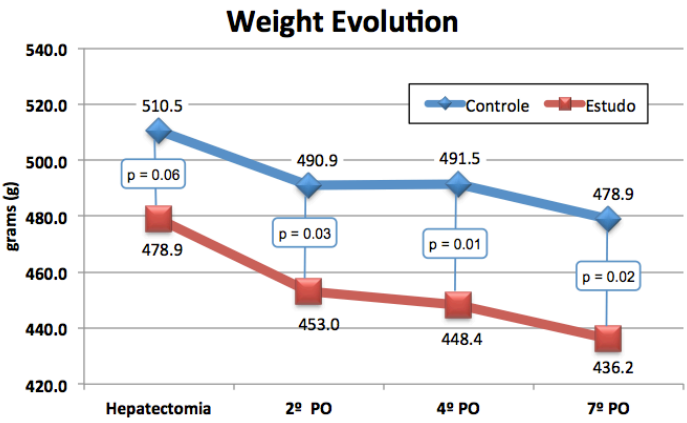

FIGURE 2 – Seven days control and experimental subgroups during the research. Statistical comparations among groups (p).

We found no statistical differences among all the subgroups when compared total liver weight, weight of resected segment during hepatectomy and estimated weight of remaining liver. When evaluated using Kwon' formula, liver regeneration rates in experimental and control groups were found to be different. At 24 hours, the control group had an average liver regeneration rate of 60.72% compared with 62.93% of experimental group (p = 0.49) (Table 1). After one week, the liver regeneration rate in the control group was 83.61% versus 93.05% in the experimental group (p = 0.05) (Table 2).

TABLE 1 – Statistical analysis of liver regeneration by kwon' formula in 24 hours and seven days in the control and experimental groups.

GROUPS	PERCENTAGE OF LIVER REGENERATION BY KWON' FORMULA				p
	min-max	media	±	sd	
Control 24 hours	55.47-63.82	60.72	±	2.27	0.0001
Control 7 days	68.60-99.60	83.61	±	11.46	
Experimental 24 hours	51.80-76.40	62.93	±	9.47	0.0001
Experimental 7 days	82.88-105.10	93.05	±	7.91	

Note: min–max – minimum and maximum values, sd – standard deviation, p – statistical significance value.

TABLE 2 – Statistical analysis of liver regeneration by kwon' formula in 24 hours and seven days in the control and experimental groups.

GROUPS	PERCENTAGE OF LIVER REGENERATION BY KWON' FORMULA				p
	min-max	media	±	sd	
Control 24 hours	55.47-63.82	60.72	±	2.27	0.49
Experimental 24 hours	51.80-76.40	62.93	±	9.47	
Control 7 days	68.60-99.60	83.61	±	11.46	0.05
Experimental 7 days	82.88-105.10	93.05	±	7.91	

Note: min–max – minimum and maximum values, sd – standard deviation, p – statistical significance value.

When regeneration was assessed according to the average number of mitotic figures in a total count of 100 hepatocytes, group C.24h was found to contain 0.3 ± 0.7 mitotic figures and group E.24h 0.5 ± 0.5 mitotic figures in 100 hepatocytes (p = 0.26). After seven days, group C.7d contained 1.9 ± 1.5 mitotic figures and group E.7d 1.6 ± 1.5 mitotic figures in 100 hepatocytes (p = 0.66).

The PCNA-positive nuclei count in ten fields was assessed in all groups and significant statistical difference was found only when compared the experimental groups (p = 0.0001) (Table 3).

TABLE 3 – Statistical analysis of percentage of PCNA-positive nuclei among groups.

GROUPS			PERCENTAGE OF PCNA-POSITIVE NUCLEI		p
			media ± sd	x media ± sd	
Control 24 hours	x	Control 7 days	67.4 ± 17.6	x 57.9 ± 22.2	0.30
Experimental 24 hours	x	Experimental 7 days	74.3 ± 13.4	x 42.3 ± 15.6	0.0001
Control 24 hours	x	Experimental 24 hours	67.4 ± 17.6	x 74.3 ± 13.4	0.34
Control 7 days	x	Experimental 7 days	57.9 ± 22.2	x 42.3 ± 15.6	0.09

Note: sd – standard deviation, p – statistical significance value.

As occurred with PCNA, the Ki67-positive nuclei count in ten fields was assessed in all groups and significant statistical difference was found only when compared experimental groups (p = 0.04) (Table 4).

TABLE 4 – Statistical analysis of percentage of KI67-positive nuclei among groups.

GROUPS			PERCENTAGE OF KI67-POSITIVE NUCLEI		p
			media ± sd	x media ± sd	
Control 24 hours	x	Control 7 days	58.3 ± 19.8	x 44.7 ± 18.0	0.12
Experimental 24 hours	x	Experimental 7 days	65.2 ± 24.2	x 41.5 ± 27.6	0.04
Control 24 hours	x	Experimental 24 hours	58.3 ± 19.8	x 65.2 ± 24.2	0.49
Control 7 days	x	Experimental 7 days	44.7 ± 18.0	x 41.5 ± 27.6	0.70

Note: sd – standard deviation, p – statistical significance value.

Discussion

The influence of cyclosporine in liver regeneration has been a controversial issue. In an experimental point of view, for studying liver regeneration, most of researchers chooses the stimulus like liver resection, where the remaining liver mass is normal, using for this reason the animal model of 70% hepatectomy described by Higgins and Anderson[3]. This is a simple and reproducible model with low mortality, in resistant and low cost animals. The authors used adult Wistar rats, completely developed, weight over 350g, in order to analyze the phenomena of liver regeneration with no humoral, general or specifics mechanisms.

Kim *et al.*[11] and Daoudaki *et al.*[8] demonstrated augmentation in hepatocyte proliferation in hepatectomized rats that were submitted to pretherapy with cyclosporine. However, other authors didn't achieve the same results[12,13]. In this study, the authors used oral cyclosporine in a 10mg/Kg/day with rigid plastic orogastric tube as described by other researchers[9-11,15-18]. All groups lost body weight in the moment of death with statistical significance. When compared 7 days experimental and control groups, the first one lost more weight than the last one on the second, fourth and death day (p = 0.03; p = 0.01; p = 0.02 respectively), proving the influence of the drug on animals weight during the research. Tannuri *et al.*[19] had the same results when resected 70% of newborn liver rats and treated one group with cyclosporine pretherapy (p = 0.01).

Biondo-Simões *et al.*[20] compared 90 days young rats and 560 days adult rats submitted to 70% hepatectomy. These animals were killed 24 hours and seven days after the procedure and liver regeneration was analyzed with Kwon's formula, mitotic index and immunohistochemistry with PCNA. The young animals regenerated more than the older ones in 24 hours and seven days, but in seven days the adults reached the same hepatic mass that the young rats reached in 24 hours. In this present study, using Kwon's formula, seven days control group regenerated more than the 24 hours group (p = 0.0001) and the same occurred in the experimental group (p = 0.0001). That's because the cell cycle of the hepatocytes has a peak in 24 hours[4] and in non-parenchymal cells that peak occurs 24 hours later[21]. In resections over than 50%, like in this research, a second peak of mitosis in rats occurs three days after the first one[1]. When compared 24 hours control/experimental groups and seven days control/experimental groups, only in the last one there was statistical significance (p = 0.05), with higher media in the rats treated with cyclosporine.

Dahmen *et al.*[22] compared the effect of four immunosuppressants on liver regeneration after Higgins and Anderson' experimental model. The calcineurin inhibitors had hepatotrophic effects and a better survival rate than the antiproliferative drugs that diminished the regeneration of the

viscera. Recently, Nagayoshi *et al.*[23] reported the dose-dependent effect of cyclosporine on liver regeneration in rats submitted to a 70% hepatectomy and killed in 24 hours, three, seven and 14 days after the procedure. Control group and experimental with cyclosporine 5mg/Kg/day didn't have statistical significance, but the experimental group with cyclosporine 10mg/Kg/day was significant.

Liver regeneration can also be evaluated by mitotic index and immunohistochemistry with proliferating cellular nuclear antigen (PCNA) and Ki-67. Some authors reported augmentation in the mitotic index after partial hepatectomy in rats that received cyclosporine 24 hours before and after the resection[17]. These authors compared four groups: hepatectomy only, hepatectomy with azatioprine, hepatectomy with methilprednisolone and the last one with cyclosporine. The azatioprine and methilprednisolone groups had significative reducing in mitotic index. In our study, when compared 24 hours and seven days control groups ($p = 0.006$) and experimental groups ($p = 0.007$), an augmentation in mitotic figures was found. It was due to the use of adult rats with no vicious in regenerating capacity. Biondo-Simões *et al.*[20] showed that advanced age is related, in rats, with a late liver regeneration. Other authors demonstrated similar results. Two peaks of cellular proliferation were described. The first one begins in the S phase 18 hours after hepatectomy and finishes around 26 hours after the liver resection. The second begins in the S phase 26 hours after hepatectomy and comes over 34 hours after the procedure[24].

In this study, when PCNA was evaluated, cyclosporine didn't stimulate liver regeneration. Significant difference was seen only when compared the experimental groups ($p = 0.0001$), suggesting that the second peak of hepatocyte proliferation occurs after the first 24 hours[25] and in adult and old rats, the liver takes more time to recover its volume[20]. No significant statistical differences between control and experimental groups were comproved. Assy *et al.*[25] studied the use of PCNA in liver regeneration in rats submitted to a 30% and 70% hepatectomy. Peak of PCNA with statistical difference was observed after 36 to 48 hours after the procedure ($p < 0.01$) when compared with sham group, but no significant difference in the first 24 hours[25]. Ki-67 index (percentage of positive cells / percentage of evaluated cells) has been used to quantify the proliferation of determined tumor or tissue, in our case, liver in regeneration. In this study, we had the same results that with PCNA.

There is controversy in the literature regarding regeneration effect of immunosuppressive therapies. We recently investigated two other immunosuppressive drugs – tacrolimus and sirolimus – using the same regeneration parameters used in the present study with interesting positive results for both drugs concerning liver regeneration[26,27]. The results of the present study show that pretherapy with cyclosporine has stimulatory effect in liver regeneration in the end of seven days after 70% hepatectomy, despite of causing weight loss. Although, low significative results were found with common histology and immunohistochemistry, other researches and studies should be done in order to demonstrate real cyclosporine effects on liver regeneration.

Conclusions

Cyclosporine, although used as immunosupressive therapy in transplant patients, can stimulate liver regeneration in adult rats submitted to 70% hepatectomy. Common histology and immunohistochemistry didn't show the same results as the Kwon formula.

References

1. Koniaris LG, Mckillop I, Schwartz SI, Zimmers TA. Liver regeneration. J Am Coll Surg. 2003 Oct;97(4):634-59. PMID: 14522336.
2. Fausto N, Laird AD, Webber EM. Liver regeneration 2. Role of growth factors and cytokines in hepatic regeneration. FASEB J. 1995 Dec;9(15):1527-36. PMID: 8529831.
3. Higgins GM, Anderson RM. Experimental pathology of the liver; I. Restoration of the liver by the white rat following partial surgical removal. Arch Pathol 1931;12:186-202.
4. Fausto N, Riehle KJ. Mechanisms of liver regeneration and their clinical implications. J Hepatobiliary Pancreat Surg. 2005;12(3):181-9. PMID: 15995805.
5. Michalopoulos GK, Defrances MC. Liver regeneration. Science. 1997 Apr 4;276(5309):60-6. PMID: 9082986.
6. Ramalho FS, Ramalho LNZ, Zucoloto S, Silva Jr. OC. Regeneração Hepática – algumas definições num universo de incertezas. Acta Cir Bras. 1993;8(4):177-89.
7. Ramalho FS, Ramalho LNZ, Zucoloto S, Silva Jr. OC. Regeneração hepática. In: Silva Jr. OC, Zucoloto S, Júnior AB. Modelos experimentais de pesquisa em cirurgia. São Paulo: Robe Editorial; 1998. p.244-58.
8. Daoudaki M, Fouzas I, Stapf V, Ekmekcioglu C, Imvrios G, Andoniadis A, Demetriadou A, Thalhammer T. Cyclosporine A augments P-glycoprotein expression in the regenerating rat liver. Biol Pharm Bull. 2003 Mar;26(3):303-7. PMID: 12612437.
9. Kim YI, Calne RY, Nagasue N. Cyclosporine A stimulates proliferation the liver cells after partial hepatectomy in rats. Surg Gynecol Obstet. 1988 Apr;166(4):317-22. PMID: 3353828.
10. Kim YI, Nakashima K, Kawano K, Kobayashi M. Evidence that cyclosporine is hepatotoxic and hepatotrophic in 70% hepatectomized rats and mice. Eur Surg Res. 1990;22(4):231-7. PMID: 2265657.
11. Kim YI, Kobayashi M, Egashira T, Kawano K, Morimoto A, Kai T, Shimada T. Augmentation of hepatocyte proliferation by immunosuppressant pretherapy is associated with up-regulation of malondialdehyde production. Res Exp Med. 1993;193(5):337-45. PMID: 7506444.
12. Provencher SJ, Demers C, Bastien MC, Villeneuve JC, Gascon-

Barré M. Selective effect of cyclosporine A on the early recovery of drug metabolism in the partially hepatectomized. Hepatology. 1997;26:A382. PMID: 10101139.

13. Provencher SJ, Demers C, Bastien MC, Villeneuve JP, Gascon-Barré M. Effect of cyclosporine A on cytochrome P-450 mediated drug metabolism in the partially hepatectomized rat. Drug Metab Dispos. 1999 Apr;27(4):449-55. PMID: 10101139.
14. Kwon AH, Uetsuji S, Yamamura M, Hioki K, Yamamoto M. Effect of administration of fibronectin or aprotimin on liver regeneration after experimental hepatectomy. Ann Surg. 1990 Mar;211(3):295-300. PMID: 1689991.
15. Kikuchi N, Yamaguchi Y, Mori K, Takata N, Goto M, Makimo Y, Hamaguchi H, Hisama N, Ogawa M. Effect of cyclosporine on liver regeneration after ortothopic reduced-size hepatic transplantation in the rat. Dig Dis Sci. 1993 Aug;38(8):1492-9. PMID: 8344106.
16. Kikuchi N, Yamaguchi Y, Mori K, Takata N, Goto M, Makimo Y, Hamaguchi H, Hisama N, Ogawa M. Liver regeneration after ortothopic reduced-size hepatic transplantation in the rat. Hepatogastroenterology. 1994 Apr;41(2):170-3. PMID: 8056408.
17. Kim YI, Salvini P, Auxilia F, Calne RY. Effect of cyclosporine A on hepatocyte proliferation after partial hepatectomy in rats: comparison with standard immunosuppressive agents. Am J Surg. 1988 Feb;155(2):245-9. PMID: 3341550.
18. Kim YI, Kawano K, Iwao Y, Shimada T, Kobayashi M. Influences of cyclosporine pretherapy on 90% hepatectomizes rats. Eur Surg Res. 1992;24(4):226-33. PMID: 1505599.
19. Tannuri ACA, Tannuri U, Coelho MCM, Mello ES, Santos NASR. Effects of immunosuppressants on hepatocyte cell mitosis during liver regeneration in growing animal models of partial hepatectomy. Transplant Proc. 2008 Jun;40(5):1641-4. doi: 10.1016/j.transproceed.2008.01.077.
20. Biondo-Simões MLP, Matias JEF, Montibeller GR, Siqueira LCD, Nunes ES, Grassi CA. Effect of aging on liver regeneration in rats. Acta Cir Bras. 2006 Jul-Aug;21(4):197-202. PMID: 16862337.
21. Labrecque D. Liver regeneration: a picture emerges from the puzzle. Am J Gastroenterol. 1994 Aug;89(8 Suppl):S86-S96. PMID: 8048418.
22. Dahmen U, Gu YL, Shen K, Dirsch O, Li J, Fan LM, Broelsch CE. Onset of liver regeneration after subtotal resection is inhibited by the use of new immunosuppressive drugs. Transplant Proc. 2002 Sept;34(6):2312-3. PMID: 12270412.
23. Nagayoshi S, Kawashita Y, Eguchi S, Kamohara Y, Takatsuki M, Miyamoto S, Mochizuki S, Akihiko S, Tokai H, Hidaka M, Tajima Y, Kanematsu T. Metabolism for cyclosporine A during liver regeneration after partial hepatectomy in rats. World J Gastroenterol. 2008 Nov 7;14(41):6355-9. PMID: 19009651.
24. Baratta B, Rizzoli R, Galliani I, Vitale M, Rizzi E, Matteucci A, Galanzi A, Zamai L, Mazzotti G. Early events of liver regeneration in rats: a multiparametric analysis. Histochem Cell Biol. 1996 Jan;105(1):61-9. PMID: 8824907.
25. Assy N, Gong Y, Zhang M, Pettigrew NM, Pashniak D, Minuk GY. Use of proliferating cell nuclear antigen as a marker of liver regeneration after partial hepatectomy in rats. J Lab Clin Med. 1998 Mar;131(3):251-6. PMID: 9523849.
26. Gama Filho O, Toderke EL, Baretta GAP, Sakamoto DG, Agulham MA, Tambara EM, Matias JEF. Tacrolimus-based immunossupression favours liver regeneration induced by extent hepatectomy in rats. Rev Col Bras Cir. 2010 Jun;37(3):218-25. PMID: 21079895.
27. Toderke EL, Baretta GAP, Gama Filho O, Matias JEF. Sirolimus influence on hepatectomy-induced liver regeneration in rats. Rev Col Bras Cir. 2014 Mai-Jun;41(3):203-7. doi: 10.1590/S0100-69912014000300012.

Correspondence:
Giorgio Alfredo Pedroso Baretta
Alameda Presidente Taunay, 1375/51
80430-000 Curitiba – PR Brasil
Tel/Fax: (55 41)3259-6655
giorgio.baretta@gmail.com

Received: Sep 29, 2014
Review: Nov 28, 2014
Accepted: Dec 30, 2014
Conflict of interest: none
Financial source: Coordination of Improvement for Higher Academic Staff (CAPES)

[1]Research performed at Laboratory of Experimental Surgery, Department of Surgery and Liver Transplantation, Federal University of Parana (UFPR), Curitiba-PR, Brazil. Part of Master degree thesis, Postgraduate Program in Surgical Clinic. Tutor: Jorge Eduardo Fouto Matias.