The influence of estrogen on liver regeneration. An experimental study in rats[1]

A influência do estrogênio na regeneração hepática. Estudo experimental em ratos

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ABSTRACT
Purpose: To recognize the regenerative capacity influenced by the administrating of estradiol. **Methods**: 42 female Wistar rats were used, divided into two groups, the control and the experiment group. A resection of approximately 70% of the liver was made in the liver of these animals. The control group received an intramuscular injection of one ml of peanut oil. The experiment group were given estradiol hexahydrobenzoate (50µg) diluted in one ml of peanut oil. Calibrations were done after 36 hours and 7 days, using three methods: the formula of Kwon *et al.*[21], to recognize gain in volume, counting of the mitosis figures in five fields and the percentage of positive PCNA nuclei. **Results**: Gain in volume (mass) was similar in both groups after 36 hours (p=0.1873) and higher in the experiment groups after seven days (p=0.0447). Microscopy showed a similar number of mitosis figures after 36 hours (p=0.3528) and a tendency to be higher in the experiment group after 7 days (p=0.0883). The average of positive PCNA nuclei was higher in the experiment group both after 36 hours (p=0.0009) and 7 days (p=0.0000). **Conclusion**: The estradiol hexahydrobenzoate improved liver regeneration in rats submitted to a 70% hepatectomy.
Key words: Liver Regeneration. Liver. Estradiol. Hepatectomy.

RESUMO
Objetivo: Reconhecer a capacidade regenerativa influenciada pela administração de estradiol. **Métodos**: Utilizaram-se 42 ratos Wistar, fêmeas, divididos em dois grupos controle e experimento. Realizou-se a ressecção de, aproximadamente, 70% do fígado destes animais. Ratos do grupo controle receberam injeção intramuscular de um mililitro de óleo de amendoim, enquanto que os do grupo experimento receberam hexaidrobenzoato de estradiol (50µg) diluídos em um mililitro de óleo de amendoim. Fizeram-se as aferições com 36 horas e 7 dias, com 3 métodos: Fórmula de Kwon *et al.*[21] para reconhecer ganho de volume, contagem das figuras de mitose existentes em 5 campos e percentual dos núcleos PCNA positivos em 5 campos. **Resultados**: O ganho de volume (massa) foi semelhante nos dois grupos com 36 horas (p=0,1873) e maior no grupo experimento com 7 dias (p=0,0447). À microscopia observou-se número de figuras de mitose em número semelhante com 36 horas (p=0,3528) e tendência a ser maior no grupo experimento com 7 dias (p=0,0883). A média de núcleos PCNA positivos foi maior no grupo experimento tanto com 36 horas (p=0,0009) quanto com 7 dias (p=0,0000). **Conclusão**: O hexaidrobenzoato de estradiol favoreceu a regeneração hepática em ratos submetidos à hepatectomia 70%.
Descritores: Regeneração Hepática. Fígado. Estradiol. Hepatectomia.

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Introduction

Liver regeneration capacity has been known for centuries. It is a complex process involving cytokines and hormones.

Although commonly used, the meaning of the term regeneration is only that of restoring the volume of the viscera and not strictly its initial appearance. In truth, what happens is hypergenesis with compensatory hypertrophy of the remaining segments until the primitive liver mass has been reestablished[2,3,4].

The process is an organic protection mechanism against loss of functioning liver tissue be it by chemical, viral or traumatic aggression or surgical resection[5,6,7]. There is highly ordered and organized tissue growth until the liver regains its original weight, with a small variation of 5-10%. However, there is a period of transitory liver insufficiency during the recomposition of the liver mass, which has led to the growing search for factors capable of influencing hepatocellular proliferation and the regenerative process[8,9].

There seems to be a clear distinction between the cells that originate the new hepatocytes and after liver resections and those which originate following cellular necroses with acute liver insufficiency. After hepatectomies, regeneration is done by the replication of the normally quiescent hepatocytes, without activation of the progenitor cells, while regeneration after losses induced by toxins occurs through the replication and differentiation of intra-hepatic progenitor cells (oval cells)[10].

The anxiety of accelerating agents in the regeneration process is justified by the importance of a short period of hepatic insufficiency following a hepatectomy, which would lead to much wider resections.

There is evidence that suggests estradiol plays an important role in liver regeneration. In male rate, the plasma concentration of estradiol is increased in the first six hours following a hepatectomy[11-13]. It has been suggested that this may play an important role in facilitating initial liver regeneration[14]. Fisher *et al.*[15] report that the administration of 17-beta-estradiol induced the rapid beginning of translocation of estrogenic cytoplasm receptors to the nuclei of the parenchymatous cells[15]. The DNA synthesis is increased following the nuclear internalization of the receptors[15]. Francavilla *et al.*[16] and Klatskin *et al.*[17] found partial blockage of regeneration after the administration of tamoxyphen[16-17]. Mizushima *et al.*[18] observed that the administration of estrogens in rats following hemorrhagic trauma improved the functioning of the liver. It is interesting to note that the administration of estradiol reduces the seric level of interleukin-6 and the non-offering of estradiol led to the raising of the levels of interleukin-6[18]. It seems logical to think that there is a feedback between IL-6 and estradiol.

Chiu *et al.*[19] demonstrated that patients with liver function deficiency, following hepatectomies, saw an improvement in their function when treated with estradiol.

Although it is acknowledged that estrogens do indeed stimulate liver regeneration, a search for this information in the literature turns up very few results and thus the interest to know its real importance.

This paper sought to establish a more precise relationship of the effect of estrogen on liver regeneration by seeking to recognize the regenerative capacity of the liver of animals treated with estradiol and compare them to the animals in the control group.

Methods

The project which originated this study was submitted for analysis by the Ethics in Health Sciences Research Committee at the Federal University of Paraná and approved and allocated the number CEP/SD:NA.010.001.08.1

The study was carried out within the guidelines of the Brazilian College of Animal Experiments (COBEA) and Federal Law 6638.

Forty-two female, Wistar rats (*Rattus norvegicus albinus, Rodentia mammalia*), aged 120±10 days and weighing on average 250±72 grams were used. They were kept in an animal house in a light-dark cycle with the natural humidity and room temperature of the environment, with free access to water and normal food.

The sample was divided into two groups, control (C) and experiment (E). There were 18 rats in the control group and 24 in the experiment group. Under an anesthetic of 0.1ml/100g of weight and a mixture of 1ml of ketamine (50mg) and 1ml of xylazine (20 mg), the animals were submitted to trichotomy of the ventral abdominal wall and antisepsis with poly vinyl pyrrolidone-iodine. A median laparotomy was the performed and a partial hepatectomy, with resection of the median and left lateral lobes. This hepatectomy is equivalent to a resection of approximately 67% of the viscera volume[20]. The resected segments were weighed and noted. Following the laparorrhaphy, the rats in the control group were given an intramuscular injection of one ml of peanut oil, while the experiment group were given estradiol hexahydrobenzoate (50µg) diluted in a ml of peanut oil, every 24 hours until calibration. These were carried out after 36 hours and seven days when ten animals from each group were submitted to euthanasia. In this way the subgroups C_1, C_2, E_1 e E_2 were obtained. The rats were then submitted to another laparotomy with total resection of the liver, which was weighed and the measure noted on the chart.

The resected parts were set in covered formalin at 10% and forwarded for histopathological study, with the histological cuts colored by hematoxylin and eosin for the counting of mitosis figures in five fields and by immunohistochemicals through the use of PCNA (cellular proliferation nuclear antigen) for the count of positive and negative nuclei and DNA replication in five fields.

Regeneration was evaluated using three methods: the formula of Kwon *et al.*[21], by the count of mitosis figures in five fields and by the count of positive PCNA nuclei in five fields. The formula of KWON *et al.*[21] provides the rate of regeneration based on weight.

$$\% = D/E \,.100$$

Where E = R/0.7 and D = weight of the liver per 100g of the weight of the animal when sacrificed, with "E" being the estimated weight per 100g of animal weight before the partial hepatectomy, calculated from the weight of the resected liver (R).

Results

One death was registered in the control group after seven days.

Gain of mass was similar for both groups after 36 hours (p=0.1873) and higher in the experiment group after 7 days (p=0.0447) (Table 1).

TABLE 1 – Percentage of liver mass gain at the two times studied in the control and experiment group

	C.36h	E.36h	C.7d	E.7d
Average	58.36	61.00	93.04	101.51
DP	5.68	7.18	13.54	7.64
%DP	9.73	11.77	14.55	7.52
Maximum	65.63	74.27	118.25	116.37
Minimum	46.11	49.02	78.39	88.57
T test for 36 hours		0.187338		
T test for 7 days		0.044713		

The histological cuts revealed the number of mitosis figures in the 36-hour evaluation with average values of 2.78 ± 0.83 for the control group and 2.64 ± 0.81 for the experiment group (p=0.3528). In the evaluation after seven days, the average for the control group was 5.89 ± 0.78 and for the experiment group it was 5.18 ± 1.33 (p=0.0883) (Figure 1).

The average of positive PCNA nuclei was higher in the experiment group both after 36 hours (p=0.0009) and 7 days (p=0.0000). The values of the averages obtained were 10.44 ± 2.96 for the control group and 14.00 ± 1.26 for the experiment group after 36 hours and 6.00 ± 0.87 for the control group and 10.09 ± 2.51 for the experiment group after 7 days (Figure 2).

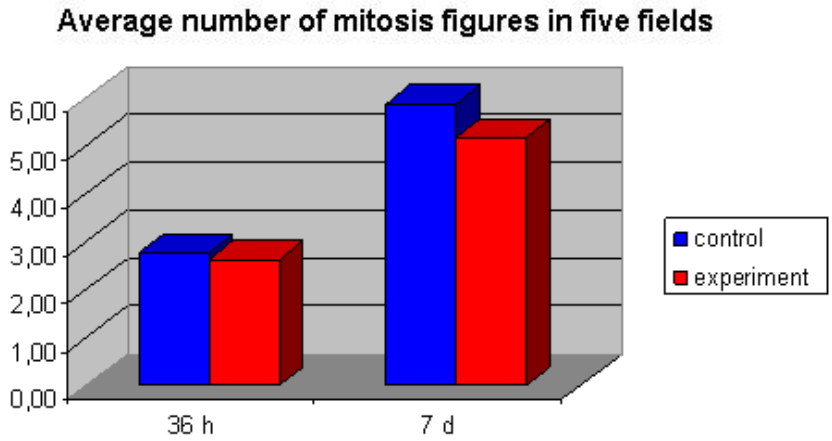

FIGURE 1 – Average number of mitosis figures at both times

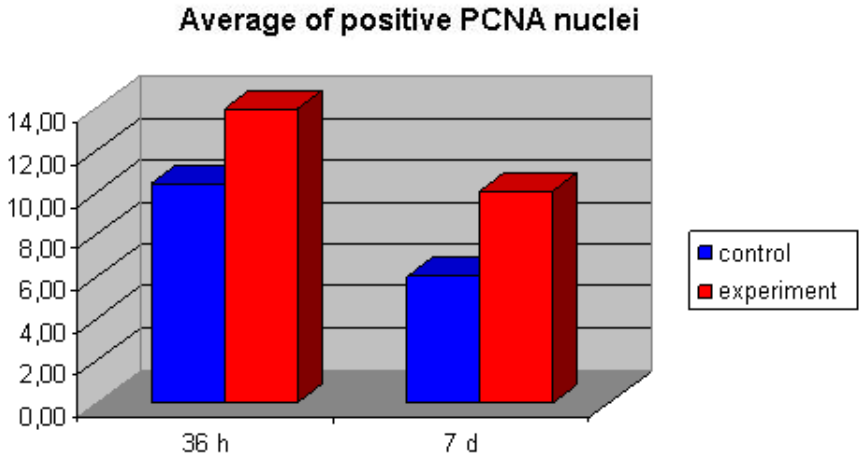

FIGURE 2 – Demonstration of the average of positive PCNA nuclei in the two groups and on both two occasions of evaluation

Discussion

It has been suggested that estrogen may play an important role in facilitating the beginning of liver regeneration[14]. Estrogens have recognized hepatotropic effects[15] like a role in liver carcinogenesis[22].

While a little exposure to 17α-ethinil-estradiol can stimulate liver growth, in rats[23,24] prolonged exposure inhibits the proliferation of hepatocytes[25]. 17α-ethinil-estradiol reduces the number of cells in the S phase and in the mitosis, suggesting previous blocking of the DNA synthesis[26].

With the assumption that exposure to estrogen for short periods might aid liver regeneration, Chiu *et al.*[19] administered estradiol to patients with liver function deficiency after hepatectomies and found that their function improved[10]. Kopplow *et al.*[27] demonstrated that dehydroepiandrosterone is mitogenic, but reduces the regenerative capacity of the liver[27]. The administration of tamoxiphen, however, led to partial blockage of the liver regeneration[12,13,17]. The liver cells have estrogenic receptors. Thus the administration of tamoxiphen, by competing with the estrogens for the receptors, would account for the blockage of the regeneration.

Hepatectomies are accompanied by the elevation of the seric levels of estradiol[11]. The administration of estradiol to rats with abdominal trauma reduces the seric level of interleukin-6 and the non-offering of estradiol led to the elevation of the levels of interleukin-6[18]. Interleukin-6 is a cytokine produced by cells with immunological activity such as macrophages, T cells and the Küpffer cells of the liver. This cytokine promotes liver regeneration during chronic lesions and after hepatectomies. In rats, the level of interleukin-6 increases in the first hours following hepatectomy to 2/3 and reaches its peak after 24 hours. It was reported that the reduction of interleukin-6 lowers liver regeneration after partial hepatectomies[28].

In this study, the administration of estradiol hexahydrobenzoate determined a higher number of positive PCNA hepatocytes at both times studied, although this number was higher after 36 hours. The number of mitosis figures proved to be higher after seven days in both groups, with no significant difference between one group and the other. The regenerated liver mass was similar in both groups after 36 hours (p=0.1873) and higher in the group that was given estradiol hexahydrobenzoate after 7 days (p=0.0447), corroborating the same positive relationship reported by Ramalho *et al.*[5], one of the positive factors described in the literature. It is possible that these results attribute to estradiol a beneficial effect for liver regeneration that is more significant in the early stages, an initial facilitation already suggested by Liddle *et al.*[11,12].

Conclusion

Estradiol hexahydrobenzoate improves the liver regeneration of rats submitted to a 70% hepatectomy.

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