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Effects of Growth Hormone on Hepatic Regeneration

Abstract: The aim of this experimental study was to determine the effects of growth hormone on hepatic regeneration after partial hepatectomy.

Thirty pathogen free Sprague-Dawley rats were divided into three groups, each containing 10 rats.

The animals were subjected to a sham operation in Group 1, and to left hepatic lobectomy in Groups 2 and 3. The animals in Groups 1 and 2 received saline solution (0.2 mg/kg/day), while growth hormone (Lilly Humotrope, Lilly France Usine de Fegersheim, France) (0.2 mg/kg/day) was given to the animals in Group 3 for seven days. On the seventh postoperative day, the animals were sacrificed and total hepatectomy was performed. The mitosis rate of the hepatocytes and Ki-67 monoclonal antibody positivity were determined.

The animals in Group 3 exhibited a higher hepatocyte mitosis rate and greater Ki-67 monoclonal antibody positivity than the animals in Groups 1 and 2. It was concluded that the growth hormone had positive effects on hepatic regeneration.

Key Words: Growth hormone, hepatic regeneration.

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Introduction

Regeneration of normal and injured liver is one of the most important issues in hepatology and hepatic surgery (1).

The polygonal cells of the mammalian liver have a high regeneration capacity. After partial hepatectomy, the regeneration lasts until the liver reaches sufficient volume (2).

Because of the vast functional reserve of the liver and its regeneration capacity, an extensive hepatic resection to remove 80% of the liver can be tolerated by most patients with a normal liver (3). Hepatic regeneration or restorative hyperplasia is an interesting subject for research (3,4). Although there have been reports about metabolic changes and hepatotrophic factors, the pathophysiology of regeneration is unclear (3-6). Hepatocytes exhibit mitosis very rarely, but 24 hours after partial resection, cell replication occurs and stops when the liver reaches its initial mass. This process lasts approximately 4-5 weeks. Humoral factors from the injured liver and other organs lead to hepatic regeneration (3-8). These factors are insulin, glucagon, hypophysial hormones, and arginin (2,5,6).

Growth hormone (GH) is one of the hormones of the somatolactogen family and is an anabolic hormone that improves protein metabolism in critical illness. GH is also the major regulator stimulating the synthesis and secretion of insulin like growth factor-1 (IGF-1) from various tissues. The anabolic effects of GH on protein metabolism are mainly mediated by IGF-1 (9-12)

Ki-67 is a human nuclear antigen that is present in proliferating cells, but is absent in resting cells (13)

In order to investigate the effects of growth hormone on hepatic regeneration after partial hepatectomy, an experimental study was performed, and mitosis rate and Ki-67 antibody positivity were investigated

Materials and Methods

Thirty pathogen free Sprague-Dawley rats weighing 250±25gr were fasted for 12 hours before the operation. Under aseptic conditions anesthesia was performed with intramuscular ketamine HCl (30mg/kg). The animals were divided into three groups, each containing 10 animals.

Group 1 (sham operation): Laparotomy was performed and then the liver was delivered from the abdomen by compressing the ribs. It was then replaced in the abdomen and the abdomen was closed with 3/0 silk. The animals received 0.2 mg/kg/day saline solution subcutaneously for seven days.

Group 2: The animals were subjected to left hepatectomy and received saline solution of 0.2mg/kg/day for seven days.

Group 3: The animals were subjected to left hepatectomy and received growth hormone (Lilly Humotrope, Lilly France Usine de Fegersheim, France) of 0.2mg/kg/day for seven days.

On the seventh postoperative day, by the injection of intracardiac 3% gluteraldeyde, the animals were sacrificed and total hepatectomy was performed. The livers were fixed in 10% formaldehyde solution and subjected to the standard procedure for paraffin embedding. In order to determine the mitosis rate, 4-μm thick sections were stained with H&E and examined under light microscopy. The mitosis rate was determined by random evaluation of at least 1000 hepatocytes and was expressed as a percentage (number of cells with mitosis/total number of hepatocyte cells X 100).

In order to determine Ki-67 antibody positivity, 4-μm sections were stained with Ki-67 monoclonal antibody (Biogenex, AM297-5m, USA) and Ki-67 positivity was determined as described previously (14). The results were expressed as a percentage (number of cells with mitosis/total number of hepatocyte cells X 100).

An Arc.Sin transformation was carried out on the results, which were expressed as a percentage, and then a variant analysis and LSD post hoc test were used for statistical analysis, with $p < 0.05$ considered significant.

Results

Two animals in Group 2 and one in Group 3, died in the postoperative period and were excluded. The livers in Group 1 exhibited no macroscopic changes and no mitosis in the hepatocytes on microscopic examination (Figure1). The macroscopic appearance of the livers in Groups 2 and 3 were similar, and there was granulation around the wounds. The mitosis rate was 18% in Group 2 (Figure 2) and 33% in Group 3 (Figure 3). Statistical analysis showed significant differences in mitosis between Group 1 and Group 2, Group 1 and Group 3, and Group 2 and Group 3 ($p < 0.01$) (Table 1).

Table 1.   Mitosis rates

Groups	Mitosis rate (%)
Group 1	0*
Group 2	18±1.53*
Group 3	33±2.52*

*$p < 0.01$ as compared with the other groups

Ki-67 antibody positivity was 3.5% in Group 1, 20.4% in Group 2 (Figure 4) and 27.6% in Group 3 (Figure 5). Statistical analysis showed significant differences in the Ki-67 antibody rate between Group 1 and Group 2, Group 1 and Group 3 ($p < 0.01$), and Group 2 and Group 3 ($p < 0.05$) (Table 2).

Table 2.   Ki-67 positivity

Groups	Ki-67 positivity (%)
Group 1	3.5±0.17*
Group 2	20.4±1.08**
Group 3	27.6±1.96

*$p < 0.01$ as compared with the other groups,
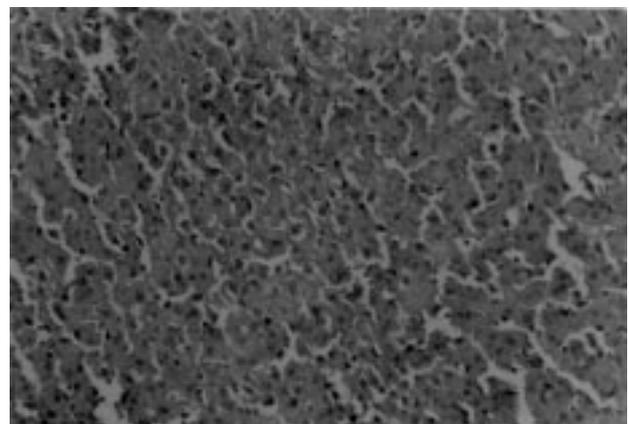**$p < 0.05$ as compared with Group 3

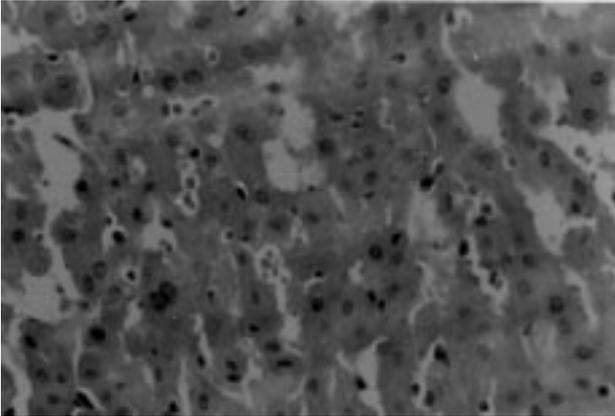Figure 1.   Absence of mitosis in Group 1 (H&EX100)

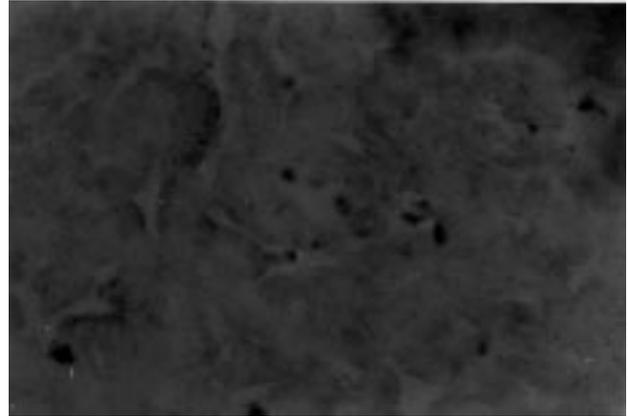Figure 2.   Regenerative changes in Group 2 (H&EX200)

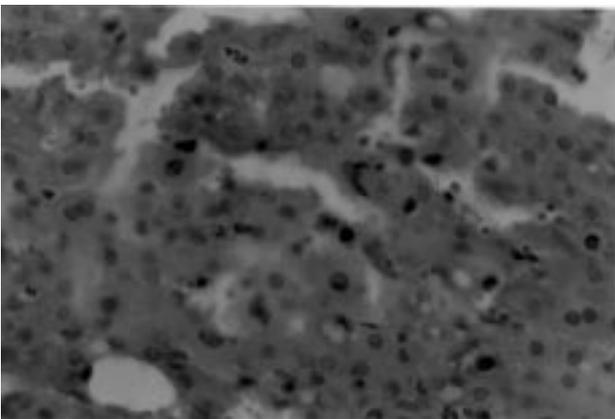Figure 5.   Mitotic activities in Group 3 (Ki-67X400)
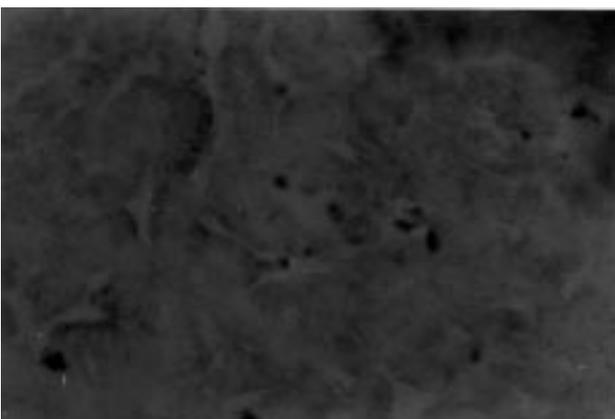

Figure 3.   Regenerative changes in Group 3 (H&EX200)

Figure 4.   Mitotic activities in Group 2 (Ki-67X400)

Discussion

The liver has many important metabolic functions which have an influence on other organs and systems. Today, hepatic surgery is carried out in major medical establishments for reasons of either preoperative preparation or postoperative care and dealing with complications (1).

Mortality in elective hepatic surgery is aproximately 5% and is generally related to postoperative complications (3,15-17). Hepatic resection impairs metabolic, immunologic, coagulative, cardiovascular, respiratory and renal functions (18,19). Multisystem organ failure, which has a 30-100% mortality rate, occurs at a rate of 7 to 22% due to these metabolic changes (20-22).

Regeneration of the mammalian liver has been studied for many years (23). The differences between the regeneration of the normal and injured liver is one of the main issues in hepatology and hepatic surgery. Studies of humans and animals have highlighted many technical difficulties (24). DNA synthesis and enzymatic activities, like ornithine decorboxilase, thymidine kinase, and polyamine oxydase, are used as markers in hepatic regeneration in vitro, but in vivo these markers are not adequate (23). Despite all these diffuculties, it has been observed that regeneration of the liver is common after partial hepatectomy. However, the many factors involved in regeneration are less understood. There have been many reports related to the series of metabolic changes and to hepatotrophic factors (3-6). It has been shown that regeneration in the remaining liver tissue after partial hepatectomy starts on the first day (3,25-28).

In those studies which have aimed to determine the regeneration criteria, many different markers and materials have been used. These consist of: DNA synthesis and mitosis number, liver volume, cell proliferation, mitochondrial activity, DNA thymidine content, 5-bromo,2-dioxyuridine, proliferating cell nuclear antigen (PCNA), plasma fibronectin level, and stimulator substance (10,25,29,30).

In order to evaluate the effects of hepatocyte growth factors on hepatic regeneration, many studies have been performed. It has been shown that hepatocyte growth factor promotes liver regeneration, ameliorates hyperbilirubinemia in hepatectomized cholestatic rats, and prevents postoperative liver failure (31,32). In the present study, we aimed to evaluate the effects of GH on liver regeneration in hepatectomized rats.

Gerders et al. (13), in 1983, found Ki-67 antigen and monoclonal antibody against it in the cell nucleus. In prognostic conditions, this antibody shows a correlation with central nervous system tumors (glioma, olygodendroglioma, pineoblastoma, primary central nervous system lymphoma and neurofibroma), non-Hodgkin lymphoma, soft tissue sarcomas and carcinoma of the breast. Ki-67 differs from other methods and materials in terms of its value in the classification of the whole cycle, rather than the "S phase" only. This classification is used as a sign of cell proliferation (12,13, 33-37).

After the period of fetal life, the liver becomes rich in GH receptors, and contains few IGF-1 receptors. Therefore, most effects of GH are localized in the liver (9, 38). In addition, it has been discovered that there is a high concentration of GH receptors in this tissue (3). It has also been reported that GH accelerates cell protein synthesis by increasing mRNA synthesis in the cell and shows an increasing effect on the target tissue (39).

There is evidence that GH takes on the role of continuity of the structure and function of intestinal mucous membrane and stimulates the proliferation of the mucous membrane epithelium. Gomez de Segura et al. (10) evaluated the effect of GH on the proliferation of the mucous membrane of rat intestine. They reported that GH receptors were found in the intestinal mucous membrane, which demonstrates that GH directly affects the GI tract and causes proliferation. In our study, we did not have the opportunity to determine GH receptor in the liver, but we found that the group which recieved GH after partial hepatectomy had a higher level of Ki-67 antibody positivity, which was used as a marker of mitosis rate and cell proliferation (p<0.01). This situation may be due to the higher regeneration rate in the group which received GH.

Studies have shown that GH increases protein synthesis by increasing mRNA in the nucleus, and promotes cell growth and proliferation (12, 38, 40). In our study, the high level of Ki-67 monoclonal antibody positivity in the study group compared to the controls (p<0.05) may be due to completion of the cell cycle and proliferation.

IGF-I and IGF-II, which are known as somatomedins, mediate GH in anabolic and growth effects. In particular, IGF-I mediates many interactions (38,41)

Huang et al. (41) reported that body weight, intestinal mucous membrane thickness, and mucosal DNA and protein were higher in IGF-treated burned rats than in the controls, and they concluded that GH both directly and, by increasing IGF-I levels, indirectly prevents mucosal atrophy. Although we did not use any parameters to determine the effect of GH on the liver, the higher mitosis rate (p<0.01) in the GH-treated group shows a correlation with those results.

Inoue et al. (12) reported that rats with sepsis showed a decrease in the number of bacteria and greater survival when treated with GH. Edwards et al. (42) reported that GH has positive effects on immunity, protein metabolism and wound healing. In our study, we determined a higher mitosis rate in the study group than in the controls (p<0.01). The higher level of Ki-67 monoclonal antibody positivity (p<0.05) and the results given above are similar to those of other studies.

On the basis of these results, after partial hepatectomy, GH increases hepatocyte regeneration in the early stages. However, the present study must be supported by further experimental and clinical studies.

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