Effect of nebivolol on liver regeneration in an experimental 70% partial hepatectomy model

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liver regeneration; nebivolol; partial hepatectomy

Summary Background: Factors affecting liver regeneration are still relevant. The purpose of this study is to investigate the effect of nebivolol treatment on liver regeneration in rats in which 70% partial hepatectomy was performed.
Methods: Three groups were created: the control group, the low dose group, and the high dose group, with 20 rats in each group and 70% hepatectomy was performed in all rats. Immediately after partial liver resection, 2 mL physiological saline solution was administered to the control group via oral gavage, 0.5 mg/kg nebivolol was administered via oral gavage to the low dose group and 2 mg/kg nebivolol was administered via oral gavage to the high dose group. On the 1st and 5th days after liver resection, 10 subjects were sacrificed from each group, and liver weights and the mitotic count and Ki-67 were measured.
Results: Regenerating liver weight on the 1st and 5th days after partial hepatectomy was statistically different in the low dose and high dose nebivolol groups compared to the control group. Mitotic count on the 1st day after partial hepatectomy was significantly higher in the low dose and high dose nebivolol groups than the control group. There was no statistically significant difference detected between the three groups for the 5th day. On the 1st day, Ki-67 rates were significantly higher in both groups given nebivolol than the control group. However, 5th day results were not statistically significant.
Conclusion: Nebivolol increases regeneration after partial hepatectomy in rats.

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1. Introduction

The liver is an organ that has a known regeneration capability. Developments in surgical techniques have led to rapid advances in liver surgery. Nowadays, the most important limiting factor in liver surgery is liver failure after major liver resections. The remaining liver tissue after liver resection is capable of regeneration. This process is a complex condition in which many different mechanisms play a role. Many cytokines have a promoting effect on regeneration, but besides this, some show inhibitory effects. There are many studies related to exposing liver regeneration steps and this topic still remains up to date.

Nebivolol is a pharmacologic agent that is clinically used as an antihypertensive. It is a third generation beta-1 adrenoreceptor antagonist. Unlike other drugs in the same type, it blocks beta-1 adrenoreceptors selectively. It is used a single daily dose. Nebivolol is metabolized in the liver and excreted through the kidneys. It is clinically shown that nebivolol is well tolerated and has fewer side effects than the other beta-blockers. It does not increase peripheral vascular resistance like other beta adrenoreceptor antagonists. Conversely, it creates vasodilation by increasing vascular endothelial nitric oxide (NO) levels. The increasing vascular endothelial level of NO which is produced in the liver has been shown to enhance the regeneration of the liver. To our knowledge, there is no other study that investigates the effects of nebivolol that causes an increase in NO levels in vascular endothelium, on liver regeneration. In this study, our aim is to investigate the effects of different doses of nebivolol on liver regeneration in rats in which we performed 70% partial hepatectomy.

2. Methods

After review of the literature, the study was approved by the Ankara University Experimental Animal Ethics Committee on June 3, 2013 with 2013-6-46 decision number. The experimental study was performed at the Experimental Animal Research Laboratory of Ankara University Faculty of Medicine in April 2013. In the study, 60 female Wistar Albino rats, 8–10 weeks old, ranging in weight from 200 g to 250 g, were fed with standard laboratory chow. Seventy percent hepatectomy was performed in all rats. Physiological saline solution (2 mL), 0.5 mg/kg nebivolol, and 2 mg/kg nebivolol were administered via oral gavage to the control, low dose, and high dose groups, respectively. Rats were divided into three groups: Group 1 (control group, 2 mL oral gavage of saline), Group 2 (low dose nebivolol group, 0.5 mg/kg oral gavage), and Group 3 (high dose nebivolol group, 2 mg/kg oral gavage).

Ketamine HCl (80 mg/kg) and 12 mg/kg xylazine hydrochloride were administered intramuscularly for anesthesia and muscle relaxation. Laparotomy with a 2.5-cm midline incision was performed in all rats. The pedicles of the left lateral and median lobes of the liver were ligated with 4/0 silk and 70% heptectomy was performed as described by Higgins and Anderson. The removed livers were weighed and the weight recorded. After controlling hemostasis, the abdomen was closed with single layer sutures. The above-mentioned doses of nebivolol and saline were given up to 24 hours prior to sacrifice to the groups that will be sacrificed on the 5th day after liver resection. On the 1st and 5th day after liver resection, 10 subjects from each group were sacrificed by creating hypovolemia with drawing an average of 6 mL blood from the inferior vena cava. Then, the remaining liver tissue was removed, weighed and placed in formol for pathological examination.

2.1. The weight of regenerating liver

Wet weights of removed left and median lobes were weighed after partial resection (RW). Total liver weight before resection (TW) was calculated by assuming 70% of liver weight was resected (TW = RW/0.7). The amount of regeneration was calculated by dividing total liver weight on the day of sacrifice (SW) to the calculated weight before resection (SW/TW × 100). This formula is known as Kwon Formula, which derives from the name of the originator.

2.2. Histological and immunohistochemical evaluation

Histological and immunohistochemical evaluation was performed in the Pathology Department of Türkiye Yüksek İhtisas Training and Research Hospital. Tissue samples were fixed in 10% buffer formalin and mitosis were counted from hematoxylin–eosin-stained slides in 10 high power fields (Olympus, CX31, Tokyo, Japan). Ratios are given as percent. To determine Ki-67 expression, sections were taken to the slides with polyclonal antibody and nuclear staining were counted in 1000 cells. Positively stained nuclei were expressed as percent.

2.3. Statistical analysis

Data analysis was performed using SPSS for Windows 11.5 program (SPSS Inc., Chicago, Illinois, USA). Distribution of continuous variables close to normal was investigated with Shapiro Wilk test and homogeneity of variances was investigated by the Levene test. Descriptive statistics are shown as median (minimum–maximum). To control the Type I error in all possible multiple comparisons, Bonferroni adjustment was made. According to the Bonferroni adjustment, p < 0.025 was considered statistically significant for the results.

3. Results

3.1. Regeneration

Median (minimum–maximum) values of regeneration rates of subjects for groups are shown in Table 1. At the end of the 1st day, there was a statistically significant difference.
between the control group and the low dose and high dose groups ($p = 0.007$). At the end of the 5th day, there was a statistically significant difference between the control group and the low dose and high dose groups ($p < 0.001$).

3.2. Mitosis

When the mitotic count calculated under 10 high-power field of microscope was examined at 1 and 5 days after partial hepatectomy of the subjects, we determined that mitotic count in low dose and high dose nebivolol groups was significantly higher when compared to the control group ($p < 0.001$). There was no significant difference between the low dose and high dose neblivolol groups on the 1st day. Statistical analysis of mitosis between the groups on the 5th day showed no significant results ($p = 0.036$; Table 2). Histopathologic appearance is shown in Figure 1.

3.3. Ki-67 proliferation index

On the 1st day, there was a statistically significant difference between the control group and both the low dose and high dose groups and also between the low dose and the high dose groups ($p < 0.001$). However, on the 5th day, there was no statistically significant difference when the groups were compared with each other ($p = 0.343$; Table 3).

4. Discussion

Weight measurement, proliferating cell nuclear antigen (PCNA), and the mitotic index are the most commonly used parameters for the evaluation of liver regeneration. Also, DNA synthesis, cell proliferation, mitochondrial activity, DNA thymidine content, micro RNA, 5-bromo-2-deoxyuridine, plasma fibronectin level, p53, and a stimulatory substance can be used for evaluating regeneration. Currently, mitotic count is the most widely used proliferation index. In our study, we selected regeneration weight ratio (according to Kwon formula), mitotic count, and Ki-67 proliferation index for evaluating regeneration, as they are also the most widely used in the literature. Nebivolol is a third generation beta-adrenoreceptor antagonist which has a double mechanism effect as an antihypertensive, acting as a selective beta-1 blocker, and increasing the level of vascular endothelial NO. In 2003, Cominacini et al. showed the increased levels of NO on

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Regeneration rates of groups at 1 and 5 days after partial hepatectomy.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>1 Day</td>
</tr>
<tr>
<td>Control</td>
<td>42.2 (37.4–55.2)$^{b,c}$</td>
</tr>
<tr>
<td>Low dose nebulol</td>
<td>49.9 (44.3–60.8)$^{b}$</td>
</tr>
<tr>
<td>High dose nebulol</td>
<td>53.6 (42.8–65.5)$^{c}$</td>
</tr>
<tr>
<td>$p$</td>
<td>0.007</td>
</tr>
</tbody>
</table>

$^a$ According to the Bonferroni adjustment, $p < 0.025$ was considered statistically significant for the results.

$^b$ Difference between control group and low dose group was statistically significant ($p < 0.025$).

$^c$ Difference between control group and high dose group was statistically significant ($p < 0.025$).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Mitotic count of the groups on 1 and 5 days after partial hepatectomy.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>1 Day</td>
</tr>
<tr>
<td>Control</td>
<td>1 (0–2)$^{b,c}$</td>
</tr>
<tr>
<td>Low dose nebulol</td>
<td>3 (2–4)$^{b}$</td>
</tr>
<tr>
<td>High dose nebulol</td>
<td>3 (1–6)$^{c}$</td>
</tr>
<tr>
<td>$p$-value$^{a}$</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^a$ Kruskal–Wallis test, comparisons between groups on 1st and 5th days. According to the Bonferroni adjustment, $p < 0.025$ was considered statistically significant for the results.

$^b$ Difference between control group and low dose group was statistically significant ($p < 0.001$).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Ki-67 ratios of groups on 1st and 5th days after partial hepatectomy.</th>
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</thead>
<tbody>
<tr>
<td>Groups</td>
<td>1st Day</td>
</tr>
<tr>
<td>Control</td>
<td>0 (0–1)$^{b,c}$</td>
</tr>
<tr>
<td>Low dose</td>
<td>6 (2–41)$^{b,d}$</td>
</tr>
<tr>
<td>High dose</td>
<td>2 (1–11)$^{c,d}$</td>
</tr>
<tr>
<td>$p$</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^a$ Kruskal Wallis test, comparisons between groups on 1st and 5th days. According to the Bonferroni adjustment, $p < 0.025$ was considered statistically significant for the results.

$^b$ Difference between control group and low dose group was statistically significant ($p < 0.001$).

$^c$ Difference between control group and high dose group was statistically significant ($p < 0.001$).

$^d$ Difference between low dose group and high dose group was statistically significant ($p < 0.011$).
vascular endothelium with the use of nebivolol. There are studies suggesting that nebivolol increases NO production in vascular endothelial cells by calcium independent pathways. Nebivolol is metabolized in the liver and its metabolites increase production of NO in vascular endothelium and induce vasodilatation. Beta-2 adrenergic receptors promote the release of NO from the vascular endothelium. Nebivolol has no inhibitory effect on the release of NO from vascular endothelium, because it selectively blocks beta-1 receptors. Therefore, its effect is stronger. NO increases hepatic microvascular blood flow without raising portal pressure by inducing smooth muscle relaxation in the terminal arterioles and portal vein, as well as relaxation of presinusoidal stellate cells. Vasodilator effects of nitric oxide are based on increase of the cGMP content in vascular smooth muscle and the ability to sustain this effect. This creates an antiapoptotic effect in the liver. There are no studies investigating the effect of nebivolol on liver regeneration. However, Teixeira et al. investigated the impact of nebivolol in rats with a bile duct ligation-induced liver fibrosis model. In this study, they showed less liver damage and increased survival rates in rats administered nebivolol. Reiberg and colleagues suggested in a cirrhotic rat model that treatment with nebivolol increases portal pressure by increasing splanchnic blood flow. Considering the mechanism described above, it can be expected that the use nebivolol has a positive effect on liver regeneration by increasing NO release and Cyclic 3′, 5′-Guanosine Monophosphate (cGMP) production. We observed in the literature that 0.5 mg/kg low dose and 2 mg/kg high dose nebivolol administered via oral gavage can be used safely in experimental studies evaluating nebivolol’s antihypertensive efficacy and postmyocardial infarction remodeling we also used low dose 0.5 mg/kg and high dose of 2 mg/kg of nebivolol same as the literature.

An article Kwon et al. published in 1989 revealed that liver regeneration velocity in 1 day is 44.7%, 3 days is 60.9%, 5 days is 75.9%, and 7 days is 86.2% in rats with 70% hepatectomy, and administration of fibronectin and aprotinin increases the rate of liver regeneration. This study showed that significant increase in the weight measurements occurs on the 5th day. Our study showed, in accordance with this, ratios and regenerating liver weight on the 1st and 5th days after partial hepatectomy were higher than in the control group. Regeneration ratios were found to be significantly higher than the control group in each drug group both on the 1st and 5th days. These findings show that nebivolol treatment increases liver regeneration at both doses. Also, it was observed that the regeneration rate on the 5th day was significantly higher than the 1st day in the groups administered drug.

In an experimental model of partial hepatectomy, Yardmcı and colleagues evaluated liver regeneration by using sildenafil in their study and they showed that sildenafil increases liver regeneration on the 1st and 5th days by the NO pathway. Also Kurokawa et al. created an experimental model; they administered L-arginine as an NO donor to rats with partial hepatectomy and suggested that L-arginine increases liver regeneration. In our study, we similarly suggest that nebivolol increases liver regeneration by the NO pathway.

Mitotic activity is not observed in normal liver but it increases in the liver mass loss or developing tissue damage for any reason. This increase is more evident in rats in the first 24–48 hours, then decreases after 72 hours. Lacom et al. demonstrated that numbers of mitotic cells are much higher 24 and 48 hours after hepatectomy than 72 and 144 hours, in rats with 70% liver resection. Rai et al. investigated the regeneration of liver in rats with partial hepatectomy by using gadolinium chloride, and suggested that mitotic index is significantly high corresponding to regeneration.

In our study, when we evaluated the groups on the 1st day according to mitotic count, we observed that the mitosis rate was statistically higher in the low dose and high dose drug groups compared to the control group, and the difference was significant. This situation suggests that mitosis starts quickly at the end of 24 hours due to use of nebivolol. On the 5th day after partial hepatectomy, there was no statistically significant difference between the groups according to mitotic count. Also, it was observed that higher mitotic count on the 1st day appears to decrease on the 5th day, similar to the results of the hepatectomy model of Kwon et al. Mitotic count of the control group was higher than that of the drug groups on the 5th day, but this was not statistically significant. The decrease of mitotic count in drug groups on the 5th day after partial hepatectomy suggests that regeneration comes to an end and mitotic count decreases.

Ki-67 is a nuclear protein that shows correlation with cell proliferation, and is thus used to measure hepatocyte proliferation after partial hepatectomy. It is associated with ribosomal RNA transcription in a cell cycle. Although it is seen mostly in interphase, it can be seen in most of the active phases of the cell cycle. There is no proliferation in resting (G0) cells. In our study, there was a significant difference between the control group and both the low dose and high dose groups, and also between the low dose and the high dose group, on the 1st day. This result shows that regeneration increases in the early phase both in the low dose and high dose groups, because Ki-67 reflects proliferating cells that are out of G0 phase and in any phase of the mitosis. Also, it is seen that proliferation is higher in the low dose nebivolol group compared to the high dose nebivolol group on the 1st day. This result suggests that...
nebivolol increases regeneration in the early phase both in the low and high dose groups, because Ki-67 reflects cell count in any phase of the mitosis. The high level of Ki-67 in drug groups compared to the control group on Day 1 and similar levels on Day 5 was parallel to the mitotic count. These findings seem to support each other. On the 1st day after partial hepatectomy, the presence of high levels of Ki-67 in the low dose group compared to the high dose group can be considered as an unexpected finding. However, high levels of Ki-67 in both groups is important to show the effect of the drug.

In conclusion, it was found that administration of both low dose and high dose of nebivolol increases liver regeneration on the 1st and 5th days in a rat model of partial hepatectomy. However, more clinical and experimental studies are needed for routine use in humans.

References