Does estrogen contribute to the hepatic regeneration following portal branch ligation in rats?

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Kawai T, Yokoyama Y, Kawai S, Yokoyama S, Oda K, Nagasaka T, Nagino M, Chaudry IH, Nimura Y. Does estrogen contribute to the hepatic regeneration following portal branch ligation in rats? Am J Physiol Gastrointest Liver Physiol 292: G582–G589, 2007. First published October 19, 2006; doi:10.1152/ajpgi.00374.2006.—The aim of this study was to determine whether estrogen plays any role in the hepatic regeneration of nonligated lobe following portal branch ligation (PBL). Male rats were subjected to PBL on the left and middle lobes. Two and 7 days after PBL, the rats were killed and blood and liver samples were analyzed. Sham animals underwent only laparotomy. The serum estradiol levels were significantly elevated on day 2 following PBL and returned to normal levels on day 7. The expression of estrogen receptors (ER) in the liver evaluated by Western blotting did not show any change in the nonligated lobe compared with sham. Immunohistochemical study for ER showed a predominant ER expression in the hepatocyte nucleus in periportal area (zone 1), although there was no apparent difference in the amount and expression pattern between sham and PBL. However, chronic inhibition of ER by an ER antagonist (ICI 182,780) showed a significantly lower regeneration rate of the nonligated lobe compared with vehicle treatment. Liver regeneration-associated genes also were less activated in the ICI group. Moreover, portal venous flow, determined by fluorescent microsphere injection, was significantly lower in the ICI group compared with vehicle group. These changes correlated with the attenuated expression of endothelial nitric oxide synthase mRNA in both superior mesenteric arteries and veins. In conclusion, these results indicate that the estrogen’s contribution on hepatic regeneration following PBL is at least partly mediated through maintaining mesenteric blood flow by mesenteric endothelial nitric oxide synthase upregulation rather than directly activating liver regeneration in the liver.

portal vein embolization; nonligated lobe; estrogen receptor; endothelial nitric oxide synthase; mesenteric flow

PREOPERATIVE PORTAL VEIN EMBOLIZATION (PVE) has been performed widely before extended hepatectomy for hepatoma (21), metastatic colorectal cancer (16), and biliary cancer (27). After PVE for the hepatic lobe to be resected, the nonembolized lobe enlarges and ensures the safety of major hepatectomy. Patients in the PVE group were better than the non-PVE group with respect to lower concentration of postoperative peak bilirubin, fewer occurrence of liver failure, and shorter hospital stay (5, 12). Interestingly, a report analyzing 84 consecutive cases of PVE demonstrated that female sex is preferable in the process of hepatic regeneration (14), indicating that gender or sex hormone milieu has some influence on the regeneration rate following PVE. A better hepatic regeneration rate in female subjects was also observed after major hepatectomy (31). However, these studies were retrospective analyses of patients and thus the precise mechanism with regard to the effect of gender or sex hormone in the process of hepatic regeneration following PVE is not known.

Estrogen, a representative female sex hormone, has been shown to modulate hepatic regeneration in experimental hepatectomy models (4, 6–8). Serum concentrations of estradiol were elevated following major hepatectomy, whereas those of testosterone were decreased both in animals (7) and humans (8). Nuclear estrogen binding 48 h after 70% partial hepatectomy was elevated although no alterations in affinity of the receptor for estrogen have been observed (4, 6). In contrast, total and nuclear androgen receptor content demonstrated a massive decline after hepatectomy (7). These results indicate that female sex hormones promote whereas male sex hormones suppress hepatocyte proliferation. However, no study has to date demonstrated the role of estrogen in the process of hepatic regeneration following portal branch ligation (PBL), which simulates clinical PVE and is another hepatic regeneration model.

The mesenteric circulation deteriorates under various stressful conditions (3), leading to an impaired hepatic blood flow due to a decreased portal venous flow. In this regard, estrogen has been shown to be protective in maintaining the mesenteric blood flow (3, 19), and thus it acts as a hepatoprotective agent (22). During the liver regeneration after PBL, maintaining the blood flow in the portal venous system is important because the flow rate in the portal venous system directly correlates with the regeneration rate of the nonligated lobe after PBL (10). However, no study has examined whether estrogen plays an role in maintaining the mesenteric blood flow and promoting hepatic regeneration following PBL.

The aim of this study, therefore, was to determine whether estrogen plays any role in the alteration of mesenteric blood flow and hepatic regeneration rate after hemihepatic portal vein occlusion using a model of rat PBL. The effect of estrogen receptor (ER) blockade was also examined by use of the specific estrogen receptor antagonist ICI 182,780.
Liver tissue specimens were surgically resected from the nonligated lobes. The specimens were embedded in optimum cutting temperature (OCT) compound (Miles, Elkhart, IN) for immunohistochemistry. Frozen liver tissue specimens embedded in OCT compound were stained with Ventana Medical Systems’ Anti-ER Primary Antibody (clone 6F11), using an autounimunostainer (VENTANA HX system, Ventana, Tucson, AZ) according to the manufacturer’s recommendations. Quantification of the data of light microscopic localization of ER in the liver tissue sections was performed by image analysis using Adobe Photoshop (Adobe Systems, San Jose, CA) and Scion Image Beta 4.03 for Windows software (Scion, Frederick, MD). Three microphotographs with low power were taken in each section for portal (zone 1) and pericentral (zone 3) areas and were subjected to the image analysis. A threshold of ER expression intensity was set up, the staining area above the threshold was extracted in a photograph, and the integrated area was calculated. The integrated area calculated for each of the three photographs in a liver tissue section was averaged.

**Determination of tissue mRNA expression by comparative quantitative real-time RT-PCR.** The mRNA levels of hepatocyte growth factor (HGF), interleukin (IL)-6, tumor necrosis factor (TNF)-α, c-fos, c-jun, c-myc, and endothelial nitric oxide synthase (eNOS) in the liver were determined by comparative quantitative real-time PCR using the MX3000P Real-Time PCR System (Stratagene, La Jolla, CA). Total RNA was isolated from liver tissues by using Qiagen RNasey mini kit (Qiagen, Germany) according to the manufacturer’s protocol. cDNA was generated from the total RNA samples by using a SuperScript III reverse transcriptase reagent (Invitrogen, Carlsbad, CA). Each reaction was performed in a 20-µl reaction mixture containing of cDNA, 2×PCR Master Mix (Applied Biosystems, Foster City, CA), and each probe and primer set. TaqMan gene expression assays (Applied Biosystems) for HGF, IL-6, TNF-α, c-fos, c-jun, c-myc, eNOS, and 18S rRNA (endogenous control) were purchased as a probe and primer set (HGF, Rn00566673_m1; IL-6, Rn00664200_m1; TNF-α, Rn01525860_g1; c-fos, Rn00582193_m1; c-jun, Rn00572991_s1; c-myc, Rn00561507_m1; eNOS, Rn02136234_s1; 18S rRNA, Hs99999901_s1). The reaction mixture was denatured for one cycle of 10 min at 95°C, and was incubated for 40 cycles (denaturing for 15 s at 95°C and annealing and extending for 1 min at 60°C). All samples were measured by using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s instructions.

**Chemicals.** A high-affinity estrogen receptor antagonist, ICI 182,780, was purchased from Tocris Cookson (Ballwin, MO). All other chemicals were purchased from Sigma.

**Animal and surgical procedure of PBL.** Male Wister rats (280–320 g) were purchased from SLC (Tokyo, Japan). The animals were kept in a temperature- and humidity-controlled environment in a 12-h light-dark cycle, and they were allowed free access to water and diet at all times. All rat experiments were approved by the University Committee on Animal Research and received humane care in accordance with National Institutes of Health publication 86-23 “Guide for the Care and Use of Laboratory Animals.” The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg) following which the abdomen was opened by a subcostal incision. The branch of the portal vein feeding the left lateral and median lobes (equivalent to 70% of the liver) was carefully dissected without producing any injury to the hepatic artery and the bile duct. A 7-0 poly(hexafluoropropylene-vinylidene fluoride) suture (PRONOVA, Ethicon, Cincinnati, OH) was placed around the portal branch. A suture knot was made without ligation, and both ends of the suture were passed out from the peritoneal cavity through both sides of the flank. Subsequently, the peritoneal cavity was closed layer by layer by continuous suture. Under light anesthesia, the portal branch can be ligated by pulling both ends of the suture after 1 wk, when the effects of laparotomy disappeared (18). Thereafter, the animals were killed and sampling was performed on days 2 and 7 after PBL or sham operation. Upon death, the ligation of the portal vein was verified. If it was incomplete, the animal was omitted from the study.

**Measurement of plasma estradiol levels.** Plasma estradiol levels were measured by using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s instructions.

**Biochemical assay of blood sample.** Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by standard laboratory methods.

**Western blotting analysis for estrogen receptors.** Whole liver tissue was homogenized in RIPA lysis buffer [10 mM Tris·HCl pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.004% sodium azide, PMSF, sodium orthovanadate, and protease inhibitor cocktail (Santa Cruz Biotechnology, Santa Cruz, CA)]. The lysate was centrifuged at 10,000 g for 10 min, and the clear supernatant was collected. Protein quantification of samples was performed with the Lowry assay (Bio-Rad Laboratories, Hercules, CA). In experiments determining levels of ER-α and -β protein, 30 μg of protein were used for electrophoresis. After quantification, protein samples were boiled for 3 min in loading buffer and separated by SDS-PAGE on the precast gel e-PAGEL (15%) (ATTO, Tokyo, Japan), and proteins were electroblotted onto Immobilon-P transfer membrane (Millipore, Bedford, MA). Membranes were washed in Tris-buffered saline with 0.1% Tween 20 (TBST) for 5 min and blocked in 5% powdered skim milk in TBST for 1 h, washed in TBST, and incubated overnight with anti-ER-α and -β antibodies (Santa Cruz Biotechnology) at a 1:250 dilution in 0.5% powdered skim milk in TBST. Membranes were washed in TBST and incubated for 1 h with a horseradish peroxidase-conjugated secondary antibody (Cell Signaling Technology, Danvers, MA). After three washes with TBST for 10 min each, membranes were treated with enhanced chemiluminescence reagent (Amersham Biosciences, Piscataway, NJ) for 1 min and exposed to X-ray film (Fuji Photo Film, Tokyo, Japan). Densitometric quantification of Western blot signal intensity of the films was performed by using a digital camera and densitometric analysis program (Gel-Pro analyzer: Media Cybernetics, Silver Spring, MD).

**Immunohistochemistry for estrogen receptors.** Liver tissue specimens were surgically resected from the nonligated lobes. The specimens were embedded in optimum cutting temperature (OCT) compound (Miles, Elkhart, IN) for immunohistochemistry. Frozen liver tissue specimens embedded in OCT compound were stained with Ventana Medical Systems’ Anti-ER Primary Antibody (clone 6F11), using an autounimunostainer (VENTANA HX system, Ventana, Tucson, AZ) according to the manufacturer’s recommendations. Quantification of the data of light microscopic localization of ER in the liver tissue sections was performed by image analysis using Adobe Photoshop (Adobe Systems, San Jose, CA) and Scion Image Beta 4.03 for Windows software (Scion, Frederick, MD). Three microphotographs with low power were taken in each section for portal (zone 1) and pericentral (zone 3) areas and were subjected to the image analysis. A threshold of ER expression intensity was set up, the staining area above the threshold was extracted in a photograph, and the integrated area was calculated. The integrated area calculated for each of the three photographs in a liver tissue section was averaged.
were tested in duplicate, and average values were used for quantification. Analysis was performed using MxPro Software version 2.00 (Stratagene) according to the manufacturer’s instructions. The comparative cycle threshold method was used for quantification of gene expression. The average of the sham group was set as onefold induction, and other data were adjusted to that baseline.

**Measurement of the hepatic blood flow.** The procedure for the measurement of hepatic blood flow was described by Yokoyama et al. (36). Briefly, the rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). The right carotid artery was cannulated with a PE-50 catheter and it was advanced to the left ventricle. The left femoral artery was also cannulated with a PE-50 catheter for the measurement of mean arterial pressure, heart rate, and blood sampling during the fluorescent microsphere injection study. The reference blood sample was withdrawn from the femoral artery for 60 s at a rate of 1.0 ml/min. Ten seconds after the beginning of blood withdrawal, ~75,000 fluorescent microspheres (15 ± 3 μm in diameter) were injected into the left ventricle at the rate of 20 μl/s for 20 s. The spleen, stomach, small intestine, large intestine, and liver were harvested carefully. After the tissues were digested with 5–7 ml of 2.3 M ethanolic KOH with 0.5% Tween-80, the microspheres were recovered by sedimentation as described previously (36). Finally, 3 ml of ethoxyethyl acetate were added to the pellet to dissolve the fluorescent microspheres. All blood and tissue samples were centrifuged at 2,000 g for 20 min and the supernatant was measured on the same day by using a CytoFluor (Applied Biosystems, Foster City, CA).

Flow to each organ was calculated by the following equation:

\[
Q_{org} (\text{ml/min}) = (FL_{org}/FL_{ref}) \times R (\text{ml/min})
\]

where \(Q_{org}\) is the blood flow rate of the sample, \(FL_{org}\) is the fluorescence reading of the sampled organ, \(FL_{ref}\) is the fluorescence reading of the reference blood sample, and \(R\) is the withdrawal rate of the reference blood flow sample. The hepatic arterial flow rate was

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**Fig. 2.** Western blotting analysis of estrogen receptor (ER)-α and -β in the nonligated lobe on day 2 after PBL. The right lobe of the liver samples from sham-operated rats was used as a control. Densitometric values of Western blotting were standardized against those of β-actin and expressed as the fold increase and the value for the sham liver was arbitrarily set at 1.0, and other data were adjusted to that baseline. Data are means ± SE of 6 animals in each group. No significant difference was observed between sham and the nonligated lobe on day 2 after PBL.

**Fig. 3.** Zonal ER expression pattern was evaluated by the immunohistochemistry and densitometric analysis in sham liver and nonligated lobe on day 2 after PBL. Densitometric values of immunohistochemistry were expressed as the fold increase when the value for the periportal area (zone 1) of sham liver was arbitrarily set at 1.0, and other data were adjusted to that baseline. Data are means ± SE of 3–4 animals in each group. The difference between zone 1 and 3 or sham and on day 2 after PBL was determined by Student’s t-test. *P < 0.05 vs. zone 1.
calculated from samples of the liver. Portal venous inflow was calculated from the sum of arterial blood flow to the stomach, small intestine, large intestine, and spleen as described by Vorobioff et al. (33). The cardiac output (CO), cardiac index (CI), and total peripheral resistance (TPR) were calculated by the following equations

\[
\text{CO} \, (\text{ml/min}) = (F \text{Linj}/F \text{Lref}) \times R
\]

\[
\text{CI} \, (\text{ml}^{-1} \cdot \text{kg}^{-1}) = \frac{\text{CO}}{\text{body wt (kg)}}
\]

\[
\text{TPR} \, (\text{dyn} \cdot \text{s} \cdot \text{cm}^{-5}) = \left[\frac{\text{MAP (mmHg)} / \text{CO (ml/min)}}{80 \times 10^3}\right]
\]

where FLinj is the fluorescence reading of the injected suspension.

Statistical analysis. There were 5–12 animals in each group. The results were presented as means ± SE. One-way ANOVA followed by the Student-Newman-Keuls test for multiple comparisons was used to determine the significant differences among the experimental groups. When criteria for parametric testing were violated the appropriate nonparametric (Mann-Whitney U-test) test was used. Student’s t-test was used to compare two groups. A P value <0.05 was considered to indicate a significant difference.

RESULTS

Plasma estradiol, ALT, and AST levels. Plasma estradiol levels increased significantly on day 2 after PBL compared with sham. However, the levels did decrease and did not show any significant difference from shams on day 7 after PBL (Fig. 1). These changes were harmonized with the change in plasma AST and ALT levels, which also showed significant elevation on day 2 after PBL and returned to normal levels within 7 days after PBL (Fig. 1).

Immunoblotting for ER following PBL. Since a significant elevation of plasma estradiol levels were observed on day 2 after PBL, we measured the expression of estrogen receptor (ER)-α and -β by Western blotting in the nonligated lobes in additional studies. Right lobe of the liver samples from sham-operated rats was used as a control. Densitometric values of Western blotting were standardized against those of β-actin and expressed as the fold increase when the value for the sham liver was arbitrarily set at 1.0. Unexpectedly, there was no significant difference between sham and day 2 after PBL in the expression of both ER-α and -β in the nonligated lobe (Fig. 2). These results suggest that estrogen-estrogen receptor activation in the liver does not appear to play an important role on day 2 after PBL.

Immunohistochemistry for ER following PBL. To further investigate whether there is an alteration in zonal ER expression pattern after PBL, we performed immunohistochemistry. The images in perportal area (zone 1) and pericentral area (zone 3) were microscopically captured randomly and the intensity of ER expression was digitally analyzed with Scion Image Beta 4.03 for Windows software (Scion, Frederick, MD). The expression of ER was mainly observed in the nucleus of hepatocytes, and the expression was significantly higher in perportal area (zone 1) compared with pericentral area (zone 3) both in sham and day 2 after PBL (Fig. 3). However, no difference was observed in the total amount and the pattern of expression between sham and day 2 after PBL. These results, together with the results of Western blotting, indicate that the expression of ER did not show any alteration after PBL not only as the total amount in the liver but also as in the zonal expression pattern.

Effect of estrogen receptor blockade on the volume change of the liver following PBL. We also examined the effects of estrogen receptor blockade on the volume change of the liver after PBL. The high affinity ER-α and -β antagonists ICI 182,780 (2 mg/kg body wt subcutaneously) (ICI group) or ethanol as vehicle (Vehicle group) was used 1 day before and daily after PBL. The dose of ICI 182,780 was determined from the previous report, which showed a substantial physiological effect in rats (13). The weights of nonligated lobe (right and caudate lobes) and ligated lobe (left and middle lobes) were measured from shams, and on days 2 and 7 after PBL, respectively. The weight of each lobe was expressed as a proportion to the body weight (Fig. 4). The weight of the nonligated lobe on days 2 and 7 after PBL were significantly higher than sham both in Vehicle and ICI groups. On day 7 after PBL, the relative weight of nonligated lobe grew almost three times as much as that of sham in the Vehicle group, whereas the ICI group showed significantly lower weight compared with the Vehicle group. The weight of the ligated lobe decreased less than one-third as much as that of sham both in Vehicle and ICI groups and showed no significant difference between these two groups.

Real-time RT-PCR for hepatic regeneration-associated factors following PBL. We next examined the activation of liver regeneration-associated factors in the nonligated lobe for both
in Vehicle and ICI groups. The expression of hepatic regeneration-associated factors in the nonligated lobe following PBL was detected by real-time RT-PCR. IL-6 and TNF-α, as triggering factors of liver regeneration (1), HGF, as a representative growth factor for liver regeneration (28), and c-fos, c-jun, and c-myc, as immediate early genes (23), were examined. All of these factors were significantly upregulated on day 2 following PBL with vehicle treatment compared with sham (Fig. 5). However, the expression of IL-6, TNF-α, and c-jun on day 2 did not show any upregulation in the ICI group. Moreover, the expression of all examined genes in the ICI treatment group was significantly lower at day 7 following PBL compared with the Vehicle treatment group at the same time point. ICI treatment alone for sham animals did not show any significant effect on the expression of liver regeneration-associated factors.

**Measurement of hepatic blood flow.** We hypothesized that the lower regeneration rate and attenuated expression of liver regeneration-associated factors in the ICI group are due to the lower blood flow rate to the liver. To test this hypothesis, we measured the organ blood flow rate using fluorescent microsphere technique for the rats on day 7 after PBL. Portal venous flow was calculated as a sum of total intestinal and splenic blood flow. Systemic hemodynamics also was monitored during the experiments. There was no significant difference between the Vehicle and ICI groups with respect to systemic hemodynamics parameters (Table 1). Interestingly, total intestinal flow was significantly lower in the ICI group compared with the Vehicle group (Fig. 6). This difference was further reflected in the differences in portal venous flow and total hepatic flow between these two groups. In contrast, the hepatic arterial flow and splenic flow did not show any difference. We therefore concluded that the estrogen’s contribution to the hepatic regeneration in the nonligated lobe was at least partly mediated through the maintenance of intestinal blood flow, which subsequently constitutes portal venous flow.

**Expression of eNOS in the mesenteric vessels.** Several reports have shown that estrogen upregulates eNOS expression in the endothelium of blood vessels and contribute to produce nitric oxide (NO) (9, 11, 17, 32). Therefore, as the final experiment, we measured the expression of eNOS transcripts in mesenteric vessels on days 2 and 7 following PBL. The expression of eNOS transcripts was significantly lower in ICI treatment group in the superior mesenteric artery on days 2 and 7 after PBL and portal vein on day 7 after PBL (Fig. 7). Therefore, we concluded that the effect of estrogen receptor antagonist was at least partly responsible for the inhibition of eNOS expression and thus lower production of NO, which could lead to the lower portal venous flow.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle</th>
<th>ICI</th>
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<tbody>
<tr>
<td>CO, ml/min</td>
<td>69.40 ± 6.24</td>
<td>65.55 ± 3.65</td>
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<tr>
<td>CI, ml/min·kg⁻¹</td>
<td>244.03 ± 17.13</td>
<td>235.32 ± 10.94</td>
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<tr>
<td>MAP, mmHg</td>
<td>98.98 ± 4.21</td>
<td>101.26 ± 3.94</td>
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<tr>
<td>TPR, dyn·s/m²</td>
<td>11.88 ± 1.12</td>
<td>12.50 ± 0.75</td>
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Values are means ± SE. CO, cardiac output; CI, cardiac index; MAP, mean arterial pressure; TPR, total peripheral resistance. ICI, estrogen receptor antagonist ICI 182, 780.
are means calculated from the sum of portal venous flow and hepatic arterial flow. Data arterial flow was calculated from samples of the liver. Total hepatic flow was calculated from the sum of portal venous flow and splenic flow. The hepatic arterial flow was calculated from samples of the liver. Total hepatic flow was calculated from the sum of portal venous flow and hepatic arterial flow. Data are means ± SE of 5 animals in each group. The difference among the groups was determined by one-way ANOVA. *P < 0.05 vs. Vehicle.

DISCUSSION

PVE is widely used before major hepatectomy to induce hypertrophy of the future liver remnant and to reduce the risks of major hepatectomy (5, 14, 26, 27). Several reports have analyzed the factors that affect the outcome of PVE in human clinical studies (5, 34). Pathophysiology of PVE is also extensively studied in an animal PBL model (24, 25). However, none of the previous studies have evaluated the role of estrogen in the process of hepatic regeneration following PBL. Because estrogen has been shown to modulate liver regeneration, we first sought to determine the changes in plasma estradiol levels and the expression of estrogen receptor in the liver.

Unexpectedly, the expression of estrogen receptor did not show any change in the liver after PBL, despite the significantly elevated plasma estradiol levels on day 2 after PBL. However, our study using the blockade of estrogen receptors by ICI 182,780 demonstrated significantly lower regeneration rate in the nonligated lobe following PBL compared with vehicle treatment. The representative liver regeneration-associated genes, such as IL-6, TNF-α, HGF, c-fos, c-jun, and c-myc, were also less activated in ICI treatment group. On the basis of these observations, we hypothesized that estrogen contributes to liver regeneration by increasing total hepatic blood flow. Measurement of mesenteric blood flow by microspheres demonstrated significantly lower portal venous flow in the ICI group, although arterial flow was almost identical with vehicle treatment group. Furthermore, the level of eNOS transcripts, which is known to be upregulated by estrogen (30) and contribute to generate vasodilator NO (20), in the superior mesenteric artery and portal vein was significantly lower in the ICI group. We therefore concluded that the role of estrogen in the process of hepatic regeneration following PBL is at least partly mediated through the enhancement of portal flow by producing vasodilators in the mesenteric vessels rather than directly activating estrogen-estrogen receptor interaction in the liver. To the best of our knowledge, this is the first report that examined the role of estrogen on hepatic regeneration from the aspect of mesenteric blood flow regulation.

In the previous study by Francavilla et al. (6), nuclear estrogen binding 48 h after partial hepatectomy was found to be elevated fivefold over normal values with no alterations in affinity of the receptor for estrogen. These changes were paralleled with increases in nuclear deoxyribonucleic acid synthesis and mitotic indexes in the liver. These results strongly implied that estrogen directly activates hepatic regeneration after hepatectomy. However, no significant upregulation of ER was observed in either the nonligated and ligated lobe following PBL in our study, and that was confirmed by Western blotting. Immunohistochemistry showed ER expression in the hepatocyte nucleus predominantly in the periportal area (zone 1). Neither the expression pattern nor the density of expression showed any change after PBL. It should be noted that the levels of ER immunostaining do not necessarily correlate with ER receptor activity. However, our results indicated that the role of ER following PBL is minor. The other reason of the differences in the results of Francavilla et al. and ours could be due to the difference in the models used by them and by us (i.e., partial hepatectomy and PBL). However, the process of hepatic regeneration of the nonligated lobe should be similar to that of the remnant liver after partial hepatectomy (24). Further investigations are therefore required to elucidate the precise role of estrogen or ER in the liver in the process of hepatic regeneration.

It is intriguing which ER receptor type (i.e., ER-α or ER-β) is more important in the process of hepatic regeneration. To our best knowledge, there is no report which differentiates the role of each receptor type in hepatocyte proliferation. Previous report showed that hepatocytes exclusively express ER-α,
whereas ER-β is expressed in cholangiocytes and hepatic stellate cells (2, 37). Our immunostaining showed a predominant ER expression in the hepatocyte nucleus, which may be exclusively ER-α (Fig. 3). Therefore, we believe that the ER-α is more important than ER-β in the process of hepatocyte proliferation following PBL.

The role of estrogen was further examined in the in vivo study using specific ER antagonist. The specific ER antagonist was administered daily 1 day before and after PBL. Interestingly, the rate of hepatic regeneration was significantly attenuated with the blockade of ER. Furthermore, all of the hepatic regeneration associated genes studies were less activated by ICI treatment. These results, taken together with the observation of no alteration of ER expression in the liver following PBL, imply that estrogen contributes to the liver regeneration following PBL through some indirect mechanism. We thought one of the possibilities was the role of estrogen in maintaining the mesenteric blood flow. To test this hypothesis, we used fluorescent microsphere technique, which allowed us to measure organ blood flow in multiple organs simultaneously. As expected, the hepatic blood flow was significantly lower in the ICI group compared with vehicle group on day 7 following PBL. This was mainly due to a significantly lower intestinal blood flow under those conditions. Nevertheless, the reason why a decrease in hepatic blood flow lead to a decrease in liver regeneration associated gene expression is still unclear. We previously reported that the endothelial stretch, induced by an increased blood flow to the nonligated lobe after PBL, is an important trigger of IL-6 production from the hepatic endothelial cells (15, 18, 29). It is possible that a decrease in blood flow leads to a weak stretch of endothelial cells and attenuates the activation of liver regeneration associated signals. Although this could be one of the explanations, further study is required to elucidate the precise mechanism.

The portal venous flow rate was determined by the total vascular resistance in the liver and mesenteric organs. Nitric oxide, a gaseous vasodilator, has been shown to be one of the most important regulators of hepatic and mesenteric blood flow (35). Additionally, estrogen is known to influence vascular relaxation through the upregulation of eNOS in the endothelium (9, 11, 32), which constantly produces NO. In view of this, we further evaluated eNOS mRNA expression in the mesenteric vessels whether these are responsible for the decreased blood flow following ICI treatment. The eNOS mRNA expression was significantly attenuated in mesenteric vessels (in both arteries and veins) in the ICI group, indicating less eNOS expression. However, because of technical difficulties we could not measure the levels of circulating NO levels in the mesenteric blood flow. Nonetheless, we do believe that our observations could be one of the explanations for the decreased portal venous flow in the ICI group.

The reason we used male animals in this study was that we intended to characterize the role of estradiol, which is increased in the serum after PBL even in the male animals. One possible problem with male rats is that the enzyme systems that metabolize and inactivate estrogens are downregulated by a variety of stressors, resulting in increases in serum estradiol. Therefore, the increase in serum estradiol could be a result of nonspecific response after PBL stress. To overcome this problem and further elucidate the role of estradiol in the process of hepatic regeneration, the experiment using female rats that are ovariectomized with and without estradiol and ICI treatment should be performed in the future.

The model we used of PBL simulates clinical PVE, which is used to enlarge the liver to be resected before major hepatectomy (21). Interestingly, a report has shown that female sex is a preferable factor in the outcome of hepatic regeneration rate after PVE. Multiple regression analysis of 84 PVE patients showed that male sex was one of the negative factors in hepatic hypertrophy rate following PVE (14). Another report by Shan et al. showed that female sex had a positive correlation with increased percentage of liver volume after major hepatectomy (31). It could be therefore be postulated that female sex hormone may be advantageous for the process of hepatic regeneration after PVE. However, the patients included in those studies were in their 60s, and thus the circulating sex hormone concentrations might have been low. We therefore are not certain whether female sex hormones are truly a contributing factor in those clinical models. Furthermore, it remains unknown whether estradiol administration in the male or postmenopausal female patients is beneficial with respect to liver regeneration after PVE. A well-controlled prospective randomized study is therefore required to answer this question.

In summary, our study demonstrated inhibitory effects of ER blockade on the hepatic regeneration of the nonligated lobe following PBL, despite a lack of alteration of ER expression pattern in the liver. ER blockade was associated with a significantly attenuated mesenteric blood flow and less eNOS mRNA expression in the mesenteric vessels. These results therefore indicate that estrogen promotes liver regeneration following PBL at least in part through facilitating mesenteric blood flow, which might be mediated by eNOS upregulation in the mesenteric vessels.

REFERENCES


