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ORIGINAL ARTICLE

Platelets prevent acute liver damage after extended hepatectomy in pigs

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Abstract
Background/Purpose Platelets develop tissue repair and promote liver regeneration. We investigated whether platelets prevented acute liver damage after extended hepatectomy in pigs.
Methods Thrombocytosis was induced by the following two methods; afterwards 80% hepatectomy was performed in pigs. In the first method, the pigs received administration of thrombopoietin [TPO (+) group], and they were compared with a control group [TPO (−) group]. In the second method, the pigs received a splenectomy [Sp (+) group], and theywere compared with another control group [Sp (−) group]. Platelet counts, biochemical examination of blood, and histopathological findings of the residual liver were examined.
Results Serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin (T-Bil) levels were significantly decreased in the thrombocytotic groups compared with the control groups in the early period after hepatectomy. In the histopathological findings, hemorrhagic necrosis with a bile plug was observed in the control groups, but this phenomenon was not observed in the thrombocytotic groups. On transmission electron microscopy, the sinusoidal endothelial lining was destroyed and detached into the sinusoidal space with enlargement of Disse's spaces in the thrombocytotic groups, but these findings were not observed in the control groups.
Conclusion An increased number of platelets prevents acute liver damage after extended hepatectomy.

Keywords Platelet · Extended hepatectomy · Acute liver damage · Thrombopoietin · Splenectomy

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Introduction

It is known that platelets stick to injured tissue and initiate the healing response [1]. Recently, it has been reported that platelets promote tissue repair in ischemic liver injury [2]. We have demonstrated that platelets induced hepatocyte proliferation and promoted liver regeneration after massive hepatectomy [3–8]. An increased number of platelets rescued mice that were 90% hepatectomized, a level which is usually considered as fatal [5]. Thrombopoietin (TPO), which is a primary regulator of megakaryopoiesis and thrombopoiesis, prevents the progression of liver fibrosis caused by chronic liver damage and promotes liver regeneration after hepatectomy [6]. However, it is unclear whether platelets have a protective effect against liver damage after extended hepatectomy. Liver failure after hepatectomy is induced by severe damage to hepatocytes and failure of regeneration. Therefore, reduction of liver damage following hepatectomy would improve the surgical outcome [9].

The anatomy of the porcine liver is similar to that of the human liver, especially in the hepatic segment, and porcine

liver is useful for mimicking human liver surgery [10, 11]. In addition, it is easier to collect blood samples sequentially from the same animal, unlike the way blood samples are collected from rats or mice [11, 12]. Also, concerns have recently been raised about the welfare and care of dogs or monkeys being used for medical research. As a result, the use of pigs to complement the use of dogs or monkeys has attracted notice [13]. For these reasons, the porcine model is thought to be the best for the clinical application of new therapies. Extended hepatectomy in the pig causes portal hypertension, followed by liver damage [12, 14, 15]. It was reported that 85–90% liver resection in pigs induced more severe liver damage than 70–80% liver resection and had a lethal effect by causing acute liver failure [11]. Xia et al. [12] reported that pigs in which 85% liver resection was performed died during the early period after hepatectomy. In the present study, we performed 80% hepatectomy in pigs and investigated the effect of increased numbers of platelets on liver function after the operation.

Methods

Animals

Chinese miniature pigs, weighing 41.5 ± 9.0 kg, and young Landrace/White (LW) pigs, weighing 30.1 ± 6.7 kg, were used. Each pig was housed in a cage or a pen (1.5 m × 2 m) and the room temperature was maintained at 23°C. The animal was allowed ad libitum access to food and water for several days. Before surgery, the animal was fasted overnight, but allowed free access to water. This experiment was performed in the laboratories of Jichi Medical University and in the National Institute of Agrobiological Sciences. All experiments complied with the Guidelines for Care and Use of Laboratory Animals at the University of Tsukuba.

Anesthesia

Miniature pigs were anesthetized in the following way. Induction of anesthesia was performed through the administration of xylazine (4 mg/kg body weight) and ketamine (10 mg/kg body weight) intramuscularly. After atropine sulfate (1.0 mg/body) was administered, oral tracheal intubation was performed and anesthesia was maintained using 2% isoflurane. LW pigs were similarly anesthetized except that intramuscular injection of xylazine (4 mg/kg body weight) and midazolam (0.5 mg/kg body weight) was used for induction, and 2% isoflurane with nitrous oxide was used for maintenance. A catheter for central venous access was placed through the jugular vein for the administration of medicine and blood sampling after the operation. During the operation, lactate Ringer solution was administered at a rate of 10 ml/kg/body weight/h with famotidine (20 mg/body) and cefazolin sodium (1 g/body).

Hepatectomy procedure

In this study, 80% hepatectomy was performed in the pigs. In the preliminary studies, in pigs in which 75% hepatectomy was performed, the procedure did not cause portal hypertension followed by severe liver damage, and the pigs in which 85% hepatectomy was performed died in the early period after the hepatectomy (data not shown). So we considered that 80% hepatectomy was appropriate in this study. A midline laparotomy was performed. The cystic artery and cystic duct were ligated and divided. Glisson sheaths of the left lobe and medial lobe were identified and divided. The left and middle hepatic veins were ligated with liver parenchyma with transfixing sutures. Parenchymal resection was performed and the left and medial lobes were removed. Because the weight of the left plus medial lobes is 75% of the total liver weight [14], additional hepatectomy is needed for an 80% hepatectomy. Five percent of the total liver weight could be calculated from the resected liver weight, and equivalent to 5% of the total liver was resected from the right lateral lobe. In the preliminary experiment, the pigs that received 80% hepatectomy suffered portal hypertension but did not die during the experiment

Postoperative management

After the hepatectomy, parenteral nutrition solution (Aminofluid; Otsuka Pharma, Tokyo, Japan) was administered for 5 days. The volume of infusion was 40 ml/kg body weight/day for the first 3 days and 20 ml/kg body weight/day for the last 2 days with famotidine (20 mg/day). After the hepatectomy, the pigs were fed and watered freely. The animals in each group were killed on days 2 and 7 after the total hepatectomy.

Thrombocytosis

To induce thrombocytosis, two models were prepared. In the first model, Chinese miniature pigs received administration of thrombopoietin (TPO); pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF) (20 μg/kg body weight; donated by Kyowa Hakko Kirin, Tokyo, Japan) was given intramuscularly 5 days before the hepatectomy [TPO (+)] group. In previous studies, the number of platelets reached a peak on day 5 after the administration of TPO [3, 5, 6, 8, 16]. It was reported that the genomic structure of the *TPO* gene is

conserved between humans, pigs, and mice [17]. Indeed, the number of platelets was increased by the administration of TPO to Chinese miniature pigs in our preliminary study. In the second model, splenectomy was performed in young LW pigs because the number of available Chinese miniature pigs was limited. Splenectomy was performed 7 days before the hepatectomy in the Sp (+) group because there was a peak in the number of platelets on day 7 after splenectomy. In our preliminary study, the number of platelets in young LW pigs was not increased by the administration of TPO. The increase in platelet numbers lasted for 9 days after the administration of TPO in Chinese miniature pigs and 9 days after splenectomy in young LW pigs (data not shown). In each model, a control group was set up: without the administration of TPO; TPO (−) for the TPO (+) group, and without splenectomy; Sp (−) for the Sp (+) group. The number of pigs in each group was 5.

Blood analysis

The number of platelets was measured with a platelet count analyzer (Micros abc; Horiba ABX, Montpellier, France) before the hepatectomy. The serum levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin (T-Bil) were examined with a serum multiple biochemical analyzer (Fuji Drichem; Fuji Film, Tokyo, Japan) before the hepatectomy, at 2 and 6 h after the hepatectomy, and up to postoperative day 7.

Histopathological examination

Liver specimens were taken at 2 h after hepatectomy, and on days 2 and 7 after hepatectomy after the animals were killed, and were fixed in 10% buffered formalin and paraffin-embedded. Thick paraffin sections were stained with hematoxylin–eosin, using standard histological techniques. On day 7, histological analysis was carried out, using a semiquantitative scoring system for the following features: cholestasis, ballooning, and hepatocyte necrosis (Table 1) [18].

Transmission electron microscopy

Liver tissue taken by laparotomy under anesthesia at 2 h after hepatectomy was cut into small pieces (approximately 1 mm^3) and the specimens were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer. The specimens were dehydrated through a graded series of ethanol, passed through propylene oxide, and embedded in EPON 812. Ultrathin sections mounted on copper grids were stained with uranyl acetate and lead citrate and observed with a Hitachi H-7000 transmission electron microscope (Hitachi, Tokyo, Japan).

Table 1 Semiquantitative scoring system for histological features

Feature	Scoring system
Cholestasis	0. No
	1. Yes
Ballooning	0. No
	1. Yes
Necrosis	0. None
	1. Small foci
	2. Confluent areas
	3. Bridging necrosis

Liver regeneration, apoptosis

At 2 h after hepatectomy and on days 2 and 7 after hepatectomy, pigs were killed and the regenerated liver weights were measured to calculate the liver/body weight ratio, and the proliferating cell nuclear antigen (PCNA) labeling index (PCNA staining kit; ZYMED, San Francisco, CA, USA) in the liver tissues was measured to evaluate the degree of liver regeneration.

To detect apoptotic cells in liver tissue, a terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay (In situ Apoptosis Detection Kit; Takara Bio, Tokyo, Japan) was performed at 2 h after hepatectomy and on day 2 after hepatectomy.

Statistics

Values of parameters were expressed as means ± SD. Statistical significance was determined by unpaired *t*-test. *p* values of less than 0.05 were regarded as statistically significant.

Results

Characteristics of experiments

All pigs in both models survived until they were killed. Body weight, estimated liver weight, resected liver weight, estimated residual liver weight, liver resection rate, operation time, and blood loss, with or without TPO administration, and with or without splenectomy, are indicated in Tables 2 and 3, respectively. Operation time in the Sp (+) group was significantly longer than that in the Sp (−) group, and blood loss in the Sp (+) group was significantly

Table 2 Characteristics of Chinese miniature pigs in the experiment with or without thrombopoietin (TPO) administration

Parameters	TPO (−) ($n = 5$)	TPO (+) ($n = 5$)
Body weight (kg)	41.7 ± 11.9	43.3 ± 3.0
Estimated total liver (g)	556.8 ± 119.1	566.0 ± 70
Weight of resected liver (g)	442.6 ± 93.1	450.0 ± 58.3
Estimated residual liver (g)	114.2 ± 26.8	116.3 ± 12.6
Resection rate of the liver (%)	79.6 ± 1.1	79.4 ± 0.9
Operation time (min)	201 ± 40	180 ± 38
Blood loss (ml)	340 ± 222	425 ± 53

TPO (+), hepatectomy was performed after platelet numbers were increased by administration of TPO. TPO (−), hepatectomy only was performed. Data are expressed as means ± SD

Estimated total liver = (weight of left and medial lobes) × 100/75

Estimated residual liver = (estimated total liver) − (weight of resected liver)

Table 3 Characteristics of young Landrace/White (LW) pigs in the experiment with or without splenectomy (Sp)

Parameters	Sp (−) ($n = 5$)	Sp (+) ($n = 5$)
Body weight (kg)	31.4 ± 5.8	28.5 ± 7.1
Estimated total liver (g)	726.7 ± 95.8	699.0 ± 153.4
Weight of resected liver (g)	580.3 ± 76.5	555.4 ± 118.9
Estimated residual liver (g)	146.3 ± 21.4	141.6 ± 31.7
Resection rate of the liver (%)	79.9 ± 1.3	79.6 ± 1.1
Operation time (min)	168 ± 18	209 ± 34*
Blood loss (ml)	358 ± 102	410 ± 219*

Sp (+), hepatectomy was performed after platelets were increased by splenectomy. Sp (−), hepatectomy only was performed. Data are expressed as means ± SD

Estimated total liver = (weight of left and medial lobes) × 100/75

Estimated residual liver = (estimated total liver) − (weight of resected liver)

* $p < 0.05$ versus Sp (−) (unpaired *t*-test)

larger than that in the Sp (−) group. There were no significant differences among the other characteristics in these two experimental groups.

Platelet count

Before hepatectomy, the number of platelets was significantly higher in the TPO (+) and Sp (+) groups compared with the levels in each of the control groups (Fig. 1).

Liver damage

Serum AST levels in the TPO (+) group were significantly lower than those in the TPO (−) group on day 2 after hepatectomy (Fig. 2a). Serum ALT levels in the Sp (+) group were significantly lower than those in the Sp (−) group on day 2 after hepatectomy (Fig. 2b). Serum ALP levels in the TPO (+) group were significantly lower than those in the TPO (−) group at 6 h and on day 2 after hepatectomy, and those in the Sp (+) group were significantly lower than those in the Sp (−) group at 6 h and on day 1 after hepatectomy (Fig. 2a, b). Serum T-Bil levels in the TPO (+) group were significantly lower than those in the TPO (−) group on days 1 and 2 after hepatectomy (Fig. 2a).

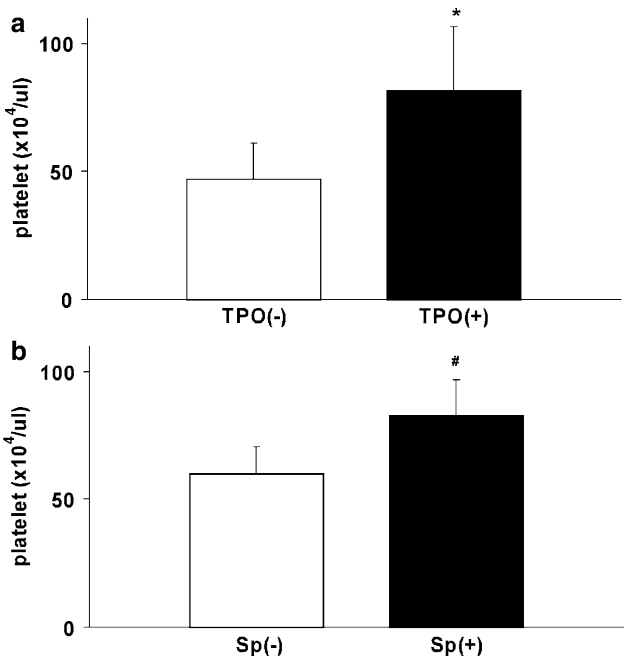

Fig. 1 Platelet count before hepatectomy with or without thrombopoietin (*TPO*) administration (**a**) and with or without splenectomy (*Sp*; **b**). Data are expressed as means ± SD. *$p < 0.05$ versus TPO (−) group, #$p < 0.05$ versus Sp (−) group

Histological findings

Light microscopic photographs of the liver tissue, stained with hematoxylin–eosin, are shown in Fig. 3. At 2 h after hepatectomy, no serious change was observed in any groups (data not shown). In the TPO (−) group, necrotic changes with vacuolization and cholestasis were observed on days 2 and 7 after hepatectomy. However, few changes were observed in the TPO (+) group (Fig. 3a). Necrotic changes with vacuolization were also observed in the Sp (−) group, but the structure was mostly preserved in the Sp (+) group (Fig. 3b). Cholestasis, ballooning, and hepatocyte necrosis were recognized in zone 2 in the TPO (−) group but these findings were not observed in the TPO (+) group ($p < 0.05$) (Fig. 4a). Ballooning and necrosis were recognized in zone 2 in the Sp (−) group, but these findings

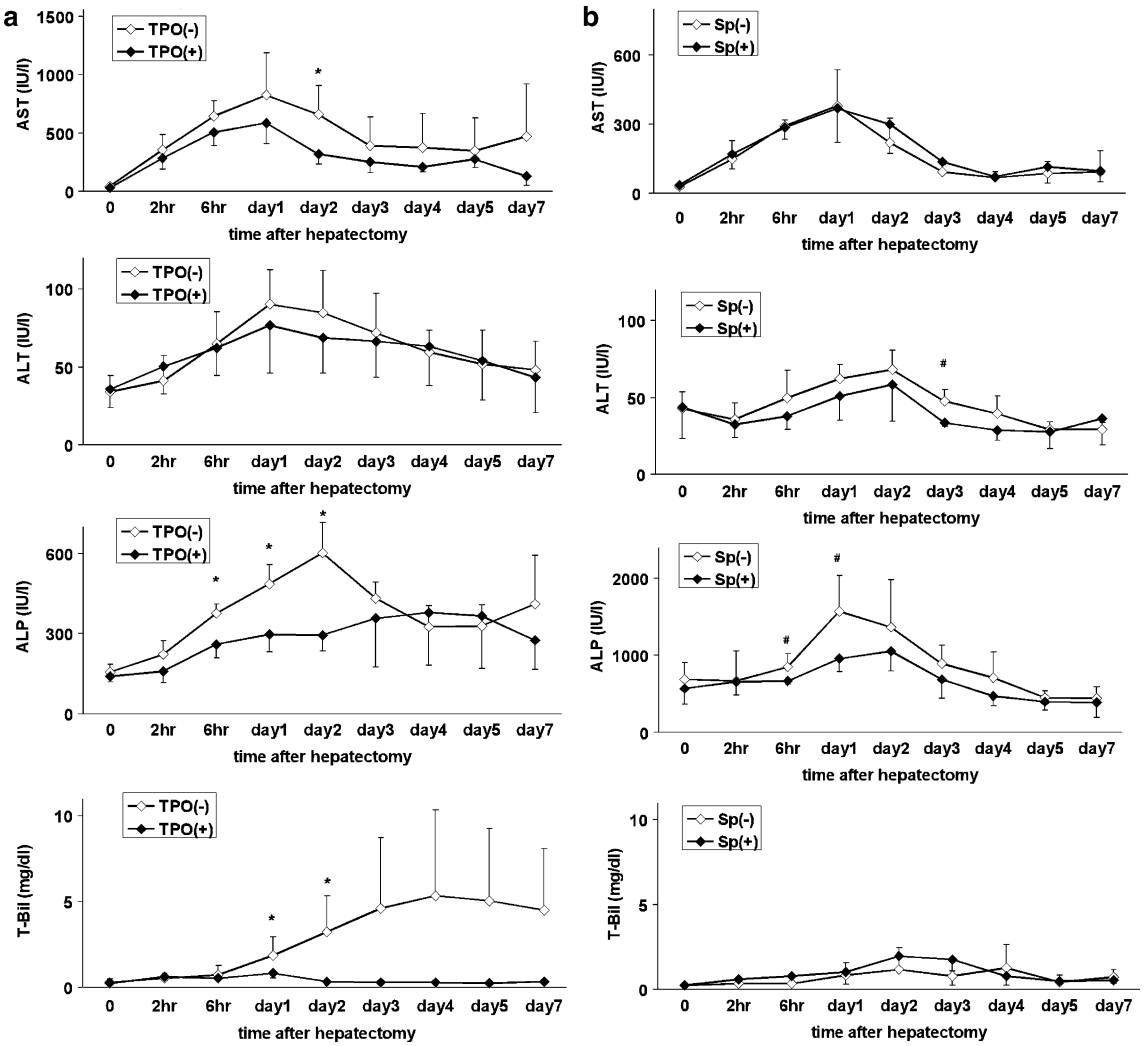

Fig. 2 Changes in serum levels of biochemical data. Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin (T-Bil) were measured until day 7 after hepatectomy with or without TPO administration (**a**) and with or without splenectomy (**b**). Data are expressed as means ± SD. *Asterisk* indicates significant differences between TPO (−) and TPO (+) groups at that time point, and *hash* indicates significant differences between Sp (−) and Sp (+) groups at that time point ($p < 0.05$ for both)

were not observed in the Sp (+) group ($p < 0.05$) (Fig. 4b).

Transmission electron microscopy

In the TPO (−) group, the sinusoidal endothelial lining was destroyed and detached into the sinusoidal space with the enlargement of Disse's spaces, and the cytoplasm of sinusoidal endothelial cells was swollen with secondary lysosomes at 2 h after hepatectomy. In contrast, the structure of the endothelial lining in the TPO (+) group was well preserved (Fig. 5a). Severe destruction, i.e., deterioration of the endothelial lining, detachment of endothelial cells, and secondary lysosomes were recognized in the Sp (−) group. But these findings were not observed in the Sp (+) group (Fig. 5b).

Liver regeneration, apoptosis

The liver/body weight ratio and the PCNA labeling index showed no significant difference between the TPO (+) and TPO (−) groups on days 2 and 7 after hepatectomy (Figs. 6a, 7a). There were also no significant differences between the Sp (+) and Sp (−) groups (Figs. 6b, 7b).

TUNEL-positive cells were not observed at 2 h or on day 2 after hepatectomy in any groups (data not shown).

Discussion

With the advances in surgical techniques and anesthesia, extended hepatectomy is safer than before [19]; however, surgeons are still fearful of liver failure. After extended

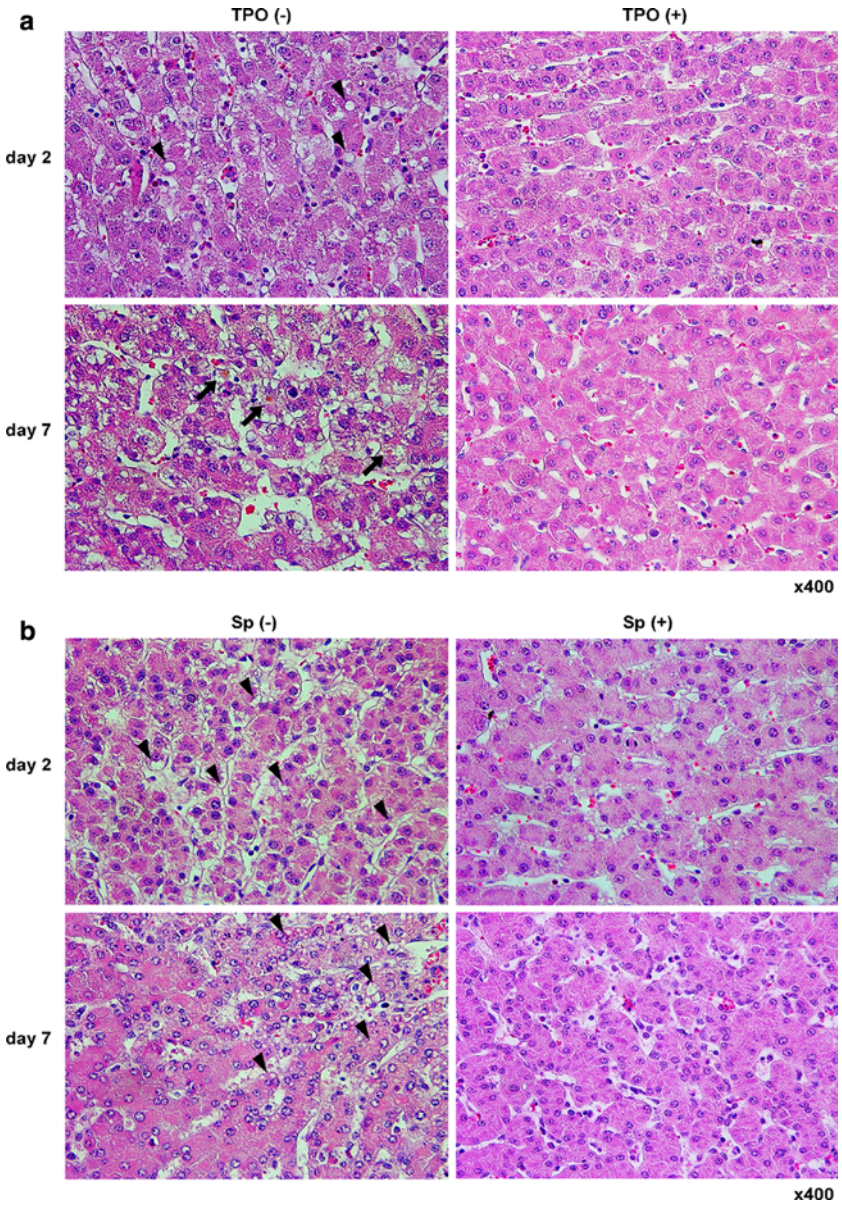

Fig. 3 Light microscopic findings of the liver tissues on days 2 and 7 after hepatectomy with or without TPO administration (**a**) and with or without splenectomy (**b**) (H&E, ×100; H&E, ×400). Necrotic change with vacuolization (*arrow heads*) and cholestasis (*arrows*) were observed in the TPO (−) group. In contrast, few serious changes were observed in the TPO (+) group. Necrotic change with vacuolization (*arrow heads*) was also observed in the Sp (−) group, but was only slightly observed in the Sp (+) group

hepatectomy, the hepatic vascular bed immediately decreases and vascular resistance in the residual liver increases, consequently leading to high portal pressure. This "shear stress" caused by portal hypertension induces severe damage to sinusoidal endothelial cells and disruption of the sinusoidal lining, which disturbs the sinusoidal microcirculation, and this is one of the major causes of acute liver failure [12, 14, 15, 20–22]. It has been reported that portal pressure was increased by 83–85% hepatectomy, resulting in a lethal effect in pigs [12, 14]. In the present study we performed 80% hepatectomy, because in this model high portal pressure and severe liver damage were observed, but the effects were not lethal. We performed extended hepatectomy in two different strains of pigs; that is, Chinese miniature pigs and LW pigs. In particular, the Chinese miniature pigs suffered from icterus, which is similar to clinical liver failure after hepatectomy.

Platelets accumulate in injured tissue and release key mediators of hemostasis and promote the healing response [1]. In the liver, accumulation of platelets occurs immediately after hepatectomy or other types of liver injury, such as ischemia/reperfusion (I/R) and administration of lipopolysaccharide [3, 23–26]. In our previous studies, we have demonstrated that platelets promote liver regeneration after hepatectomy [3–5]. However, the protective effect of platelets against liver damage caused by extended hepatectomy was unknown. In a recent study, tissue repair was delayed in platelet-depleted animals with normothermic I/R injury [2]. That is, platelets have a protective effect on

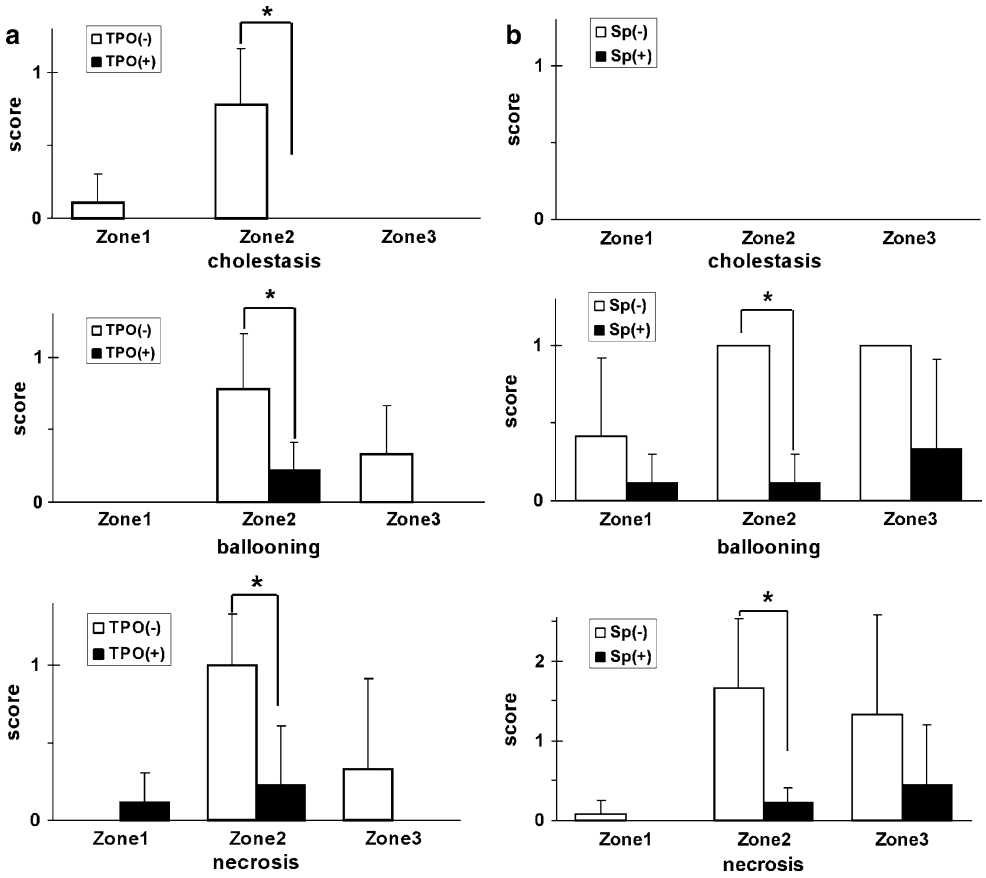

Fig. 4 Semiquantitative scoring for cholestasis, ballooning, and hepatocyte necrosis. Cholestasis, ballooning, and hepatocyte necrosis were recognized in zone 2 in the TPO (−) group but these findings were not observed in the TPO (+) group ($p < 0.05$) (**a**). Ballooning and necrosis were recognized in zone 2 in the Sp (−) group, but these findings were not observed in the Sp (+) group ($p < 0.05$) (**b**)

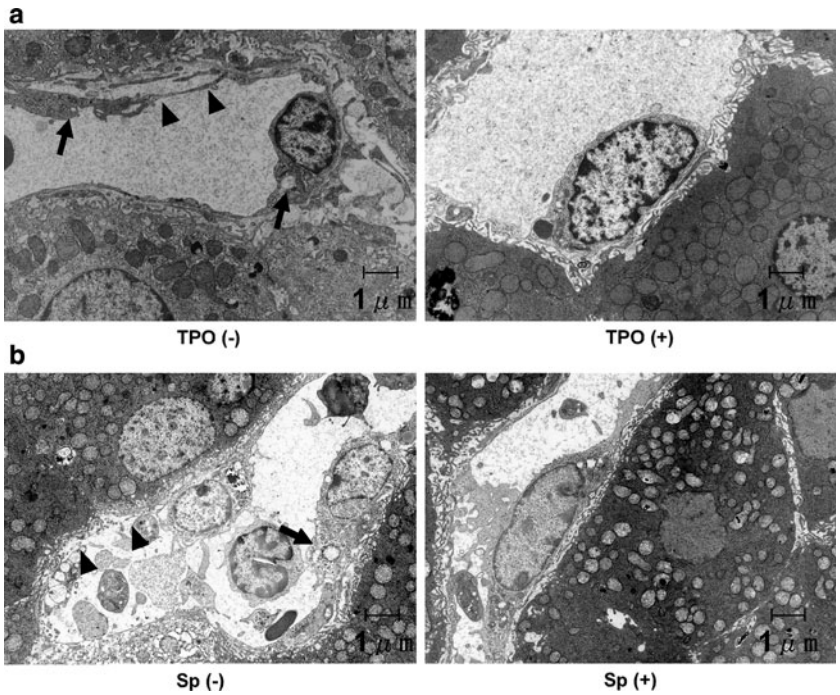

Fig. 5 Transmission electron microscopic findings at 2 h after hepatectomy (×6000). In the TPO (−) group (**a**) and the Sp (−) group (**b**), the sinusoidal endothelial lining was destroyed and detached into the sinusoidal space with enlargement of Disse's spaces (*arrow heads*), and the cytoplasm of sinusoidal endothelial cells was swollen with secondary lysosomes (*arrows*). In contrast, in the TPO (+) group (**a**) and the Sp (+) group (**b**), the sinusoidal endothelial cells were well preserved

tissue repair after normothermic hepatic ischemic injury [2]. In the clinical setting, it is reported that the number of platelets is related to recovery from postoperative liver dysfunction and platelets play a critical role after hepatectomy [27]. In the present study, disruption of the sinusoidal lining and necrotic change caused by extended

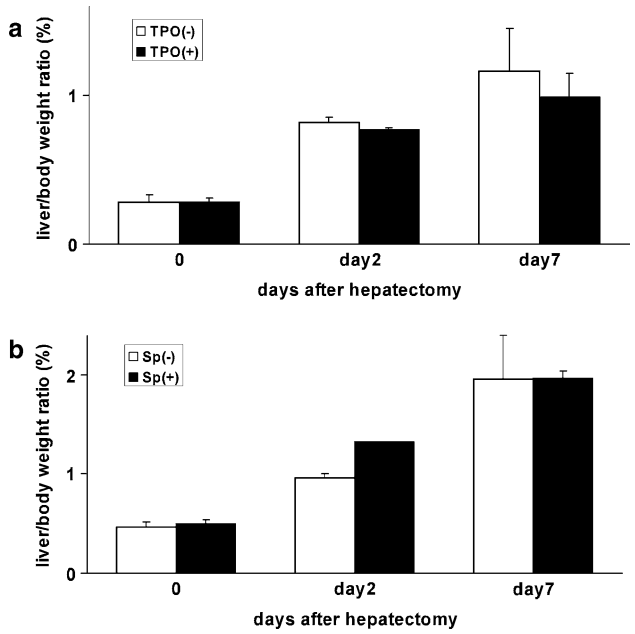

Fig. 6 Changes in the liver/body weight ratio on days 0, 2, and 7 after hepatectomy with or without TPO administration (**a**) and with or without splenectomy (**b**). Data are expressed as means ± SD

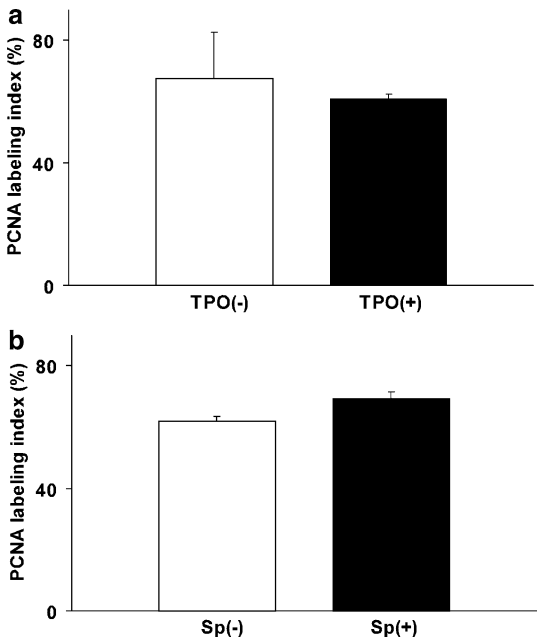

Fig. 7 Proliferating cell nuclear antigen (PCNA) labeling index on day 2 after hepatectomy with or without TPO administration (**a**) and with or without splenectomy (**b**). Data are expressed as means ± SD

hepatectomy was observed in the residual liver, and biochemical markers related to liver damage were increased in the control groups. In contrast, these phenomena were not observed in the thrombocytotic groups. These results suggest that platelets prevent acute liver damage by protecting sinusoidal endothelial cells after extended hepatectomy. It is known that apoptosis of sinusoidal endothelial cells is induced by partial hepatectomy [28, 29]. However, in the present study, there were no apoptotic cells in the liver tissue after extended hepatectomy. We performed TUNEL staining only at two time points, i.e., on days 2 and 7 after hepatectomy; therefore, it remains possible that apoptosis of liver tissue was not detected. On the other hand, it was reported that liver failure after extended hepatectomy originated not only from apoptosis but also from necrosis caused by various factors, i.e., hemodynamic changes, ischemia, sepsis, and cholestasis [30]. Additional research is needed to clarify the mechanisms of the protective effects of platelets on sinusoidal endothelial cells.

It is well known that administration of TPO or splenectomy is performed to induce thrombocytosis. TPO, which is produced by the hepatocytes in the liver, is a primary regulator of megakaryopoiesis and thrombopoiesis, leading to platelet production [31]. In the present study, administration of TPO increased the number of platelets and prevented acute liver damage after extended hepatectomy. However, it is not clear whether the protective effect was due to the increased number of platelets or to the TPO itself. There is no study in which TPO was reported to prevent liver damage. Further detailed investigation will be required to reveal the mechanism of the effect of TPO administration. On the other hand, TPO did not increase platelet numbers in young LW pigs. It was reported that the TPO level and platelet count were higher in human infants than in adults [32]. It is likely that the TPO dosage in the present study was insufficient for young LW pigs.

The influence of splenectomy on liver damage has been described in several reports [33–35]. Jiang et al. [33] reported that prior splenectomy inhibited apoptosis and inflammation of liver tissue, and ameliorated acute liver damage induced by hepatic I/R. In a clinical setting, splenectomy improved biochemical markers of liver function in patients with chronic hepatitis C [34]. Glanemann et al. [35] reported that splenectomy significantly reduced shear-stress-induced liver injury due to a reduction of portal venous blood flow in 90% hepatectomized rats. In the present study, there were no significant differences in portal venous pressure after hepatectomy between the Sp (−) and Sp (+) groups (data not shown). The results of the present study indicate that thrombocytosis induced by splenectomy itself may have a protective effect on the liver other than by bringing about a reduction in portal pressure. However, performing splenectomy prior to hepatectomy creates additional surgical stress in the patient, and hepatectomy would then be difficult because of postoperative adhesions. In the present study, significantly greater blood loss and prolongation of operation time were observed in the Sp (+) group than in the Sp (-) group. In addition, the spleen is well known to be the largest lymphoid organ, and

asplenia predisposes the patient to infection [36, 37]. For these reasons, performing splenectomy for clinical applications is the subject of discussion.

Liver regeneration after hepatectomy is an efficient and well-regulated process [38]. Recently, it was reported that platelets have a strong effect on the initiation of liver regeneration [3, 39]. We previously reported the strong effects of platelets on liver regeneration and clarified the mechanism in both in vivo and vitro studies [3, 4]. In the present study, there were no significant differences in the parameters of liver regeneration; i.e., liver/body weight ratio and PCNA labeling index, between thrombocytotic and the control groups. It is reported that the potential for liver regeneration is different between 67% hepatectomized rats and 82% hepatectomized rats [40]. Genetic responses related to apoptosis, nitric oxide metabolism, oxidative stress, and the cell cycle, which are associated with liver damage and liver regeneration, vary with the degree of hepatic resection in the residual liver of the pig [41]. It is likely that the degree of hepatic resection influenced liver regeneration in the present study.

Conclusion

Increases in the number of platelets protect the sinusoidal lining from disturbance and prevent acute liver damage after extended hepatectomy. In the near future, the induction of thrombocytosis induced by the administration of TPO or by splenectomy could be a new therapy in hepatic surgery; i.e., extended hepatectomy for the prevention of liver damage. In the clinical setting, thrombopoietin injection, as well as platelet transfusion, will be applicable for increasing platelet numbers before hepatectomy.

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References

1. Diegelmann RF, Evans MC. Wound healing: an overview of acute, fibrotic, and delayed healing. Front Biosci. 2004;9:283–9.
2. Nocito A, Georgiev P, Dahm F, Jochum W, Bader M, Graf R, et al. Platelets and platelet-derived serotonin promote tissue repair after normothermic hepatic ischemia in mice. Hepatology. 2007;45:369–76.
3. Murata S, Ohkohchi N, Matsuo R, Ikeda O, Myronovych A, Hoshi R. Platelets promote liver regeneration in early period after hepatectomy. World J Surg. 2007;31:808–16.
4. Matsuo R, Ohkohchi N, Murata S, Ikeda O, Nakano Y, Watanabe M, et al. Platelets strongly induce hepatocyte proliferation with IGF-1 and HGF in vitro. J Surg Res. 2008;145:279–86.
5. Myronovych A, Murata S, Chiba M, Matsuo R, Ikeda O, Watanabe M, et al. Role of platelets on liver regeneration after 90% hepatectomy in mice. J Hepatol. 2008;49:363–72.
6. Murata S, Hashiomoto I, Nakano Y, Myronovych A, Watanabe M, Ohkohchi N. Single administration of thrombopoietin prevents progression of liver fibrosis and promotes liver regeneration after hepatectomy in cirrhotic rats. Ann Surg. 2008;248:821–8.
7. Hoshi R, Murata S, Matsuo R, Myronovych A, Hashimoto I, Ikeda H, et al. Freeze-dried platelets promote hepatocyte proliferation in mice. Cryobiology. 2007;55:255–60.
8. Murata S, Matsuo R, Ikeda O, Myronovych A, Watanabe M, Hisakura K, et al. Platelets promote liver regeneration under conditions of Kupffer cell depletion after hepatectomy in mice. World J Surg. 2008;32:1088–96.
9. Helling TS. Liver failure following partial hepatectomy. HPB (Oxford). 2006;8:165–74.
10. Court FG, Wemyss-Holden SA, Morrison CP, Teague BD, Laws PE, Kew J, et al. Segmental nature of the porcine liver and its potential as a model for experimental partial hepatectomy. Br J Surg. 2003;90:440–4.
11. Court FG, Laws PE, Morrison CP, Teague BD, Metcalfe MS, Wemyss-Holden SA, et al. Subtotal hepatectomy: a porcine model for the study of liver regeneration. J Surg Res. 2004;116:181–6.
12. Xia Q, Lu TF, Zhou ZH, Hu LX, Ying J, Ding DZ, et al. Extended hepatectomy with segments I and VII as resection remnant: a simple model for small-for-size injuries in pigs. Hepatobiliary Pancreat Dis Int. 2008;7:601–7.
13. Tanaka H, Kobayashi E. Education and research using experimental pigs in a medical school. J Artif Organs. 2006;9:136–43.
14. Wang HS, Ohkohchi N, Enomoto Y, Usuda M, Miyagi S, Asakura T, et al. Effect of portocaval shunt on residual extreme small liver after extended hepatectomy in porcine. World J Surg. 2006;30:1–9.
15. Iida T, Yagi S, Taniguchi K, Hori T, Uemoto S. Improvement of morphological changes after 70% hepatectomy with portocaval shunt: preclinical study in porcine model. J Surg Res. 2007;143:238–46.
16. Shibuya K, Akahori H, Takahashi K, Tahara E, Kato T, Miyazaki H. Multilineage hematopoietic recovery by single injection of pegylated recombinant human megakaryocyte growth and development factor in myelosuppressed mice. Blood. 1998;91:37–45.
17. Gurney AL, Kuang WJ, Xie MH, Malloy BE, Eaton DL, de Sauvage FJ. Genomic structure, chromosomal localization, and conserved alternative splice forms of thrombopoietin. Blood. 1995;85:981–8.
18. Neil DA, Hubscher SG. Histologic and biochemical changes during the evolution of chronic rejection of liver allografts. Hepatology. 2002;35:639–51.
19. Makuuchi M, Imamura H, Sugawara Y, Takayama T. Progress in surgical treatment of hepatocellular carcinoma. Oncology. 2002;62:74–81.
20. Niiya T, Murakami M, Aoki T, Murai N, Shimizu Y, Kusano M. Immediate increase of portal pressure, reflecting sinusoidal shear stress, induced liver regeneration after partial hepatectomy. J Hepatobiliary Pancreat Surg. 1999;6:275–80.
21. Sato Y, Koyama S, Tsukada K, Hatakeyama K. Acute portal hypertension reflecting shear stress as a trigger of liver regeneration following partial hepatectomy. Surg Today. 1999;29:1–9.
22. Macario DK, Entersz I, Abboud JP, Nackman GB. Inhibition of apoptosis prevents shear-induced detachment of endothelial cells. J Surg Res. 2008;147:282–9.
23. Nakamura M, Shibazaki M, Nitta Y, Endo Y. Translocation of platelets into Disse spaces and their entry into hepatocytes in response to lipopolysaccharides, interleukin-1, and tumor necrosis factor: the role of Kupffer cells. J Hepatol. 1998;28:991–9.

24. Endo Y, Nakamura M. The effect of lipopolysaccharide, interleukin-1 and tumor necrosis factor on the hepatic accumulation of 5-hydroxytryptamine and platelets in the mouse. Br J Pharmacol. 1992;105:613–9.
25. Endo Y, Nakamura M. Active translocation of platelets into sinusoidal and Disse spaces in the liver in response to lipopolysaccharides, interleukin-1 and tumor necrosis factor. Gen Pharmacol. 1993;24:1039–53.
26. Nakano Y, Kondo T, Matsuo R, Hashimoto I, Kawasaki T, Kohno K, et al. Platelet dynamics in the early phase of postischemic liver in vivo. J Surg Res. 2008;149:192–8.
27. Alkozai EM, Nijste MW, de Jong KP, de Boer MT, Peeters PM, Slooff MJ, et al. Immediate postoperative low platelet count is associated with delayed liver function recovery after partial liver resection. Ann Surg. 2010;251:300–6.
28. Greene AK, Wiener S, Puder M, Yoshida A, Shi B, Perez-Atayde AR, et al. Endothelial-directed hepatic regeneration after partial hepatectomy. Ann Surg. 2003;237:530–5.
29. Beldi G, Wu Y, Sun X, Imai M, Enjyoji K, Csizmadia E, et al. Regulated catalysis of extracellular nucleotides by vascular CD39/ENTPD1 is required for liver regeneration. Gastroenterology. 2008;135:1750–60.
30. Garcea G, Maddern GJ. Liver failure after major hepatic resection. J Hepatobiliary Pancreat Surg. 2009;16:145–55.
31. Kaushansky K. Thrombopoietin: the primary regulator of platelet production. Blood. 1995;86:419–31.
32. Ishiguro A, Nakahata T, Matsubara K, Hayashi Y, Kato T, Suzuki Y, et al. Age-related changes in thrombopoietin: reference interval for serum thrombopoietin levels. Br J Haematol. 1999;106:884–8.
33. Jiang H, Meng F, Li W, Tong L, Qiao H, Sun X. Splenectomy ameliorates acute multiple organ damage induced by liver warm ischemia reperfusion in rats. Surgery. 2007;141:32–40.
34. Elsebae MM, Abu-Zekri NB. A study of the effect of splenectomy on hepatic functional reserve and structural damage in patients with chronic hepatitis C virus infection by non-invasive serum markers. A prospective study. Int J Surg. 2008;6:362–6.
35. Glanemann M, Eipel C, Nussler AK, Vollmar B, Neuhaus P. Hyperperfusion syndrome in small-for-size livers. Eur Surg Res. 2005;37:335–41.
36. Cadili A, de Gara C. Complication of splenectomy. Am J Med. 2008;121:371–5.
37. Dickerman JD. Bacterial infection and the asplenic host: a review. J Trauma. 1976;16:662–8.
38. Michalopoulos GK, DeFrances MC. Liver regeneration. Science. 1997;276:60–6.
39. Lesurtel M, Graf R, Aleil B, Walther DJ, Tian Y, Jochum W, et al. Platelet-derived serotonin mediates liver regeneration. Science. 2006;312:104–7.
40. Weinbren K, Woodward E. Delayed incorporation of ^{32}P from orthophosphate into deoxyribonucleic acid of rat liver after subtotal hepatectomy. Br J Exp Pathol. 1964;45:442–9.
41. Mortensen KE, Conley LN, Hedegaard J, Kalstad T, Sorensen P, Bendixen C, et al. Regenerative response in the pig liver remnant varies with the degree of resection and rise in portal pressure. Am J Physiol Gastrointest Liver Physiol. 2008;294:G819–30.