論　文　概　要（Thesis Abstract）

○ 論 文 題 目: Platelets stimulate liver regeneration in a rat model of partial liver transplantation.

（部分肝移植のラットモデルにおいて血小板は肝再生を刺激する）

○ 指 導 教 員: 小田　竜也

人間総合科学研究科　　疾患制御医学　専攻　消化器外科　教授

（所　　属）: 筑波大学大学院人間総合科学研究科
疾患制御医学専攻

（氏　　名）: 梁　宸

Purpose:

Living donor liver transplantation is sometimes associated with impaired regeneration and severe ischemia/reperfusion injury in the graft, leading to postoperative complications and decreased patient survival. Platelets were previously reported to stimulate liver regeneration in models of hepatectomy, but the role of platelets in living donor liver transplantation is controversial as platelets are supposed to aggravate ischemia/reperfusion in the liver. Although recent studies suggested that perioperative thrombocytopenia is associated with poor graft regeneration after living donor liver transplantation, it is not fully understood whether thrombocytopenia is the cause or result. In this study, we examined the role of platelets in a rat model of partial liver transplantation, and demonstrated the positive impacts of platelets on partial liver transplantation. We further investigated the potential mechanisms underlying these processes.

Material and method:

Male Lewis rats (250-300 g) were used for both donor and recipients, and partial liver transplantation was performed to mimic living donor liver transplantation. Rats with partial liver transplantation were divided into 3 groups: phosphate-buffered saline administration (control group); Thrombopoietin administration (TPO group); Thrombopoietin administration and Kupffer cell depletion (KDTPO group).

In experiment I, 30% partial liver transplantation was performed. Total bilirubin, aminotransferases and ascites were measured to evaluate small-for-size syndrome. Real-time quantitative PCR, ELISA, western blot and histological analyses were performed to assess the conditions of regeneration and ischemia/reperfusion injury in the liver graft. Additionally, we depleted Kupffer cells in both donor and recipient rats to analyze the changes in platelet-related liver regeneration;

In experiment II, we performed 20% partial liver transplantation in rats, and evaluated the survival rates between the control and TPO groups.

Result:

The platelet levels in rats were significantly increased after thrombopoietin administration.

In experiment I, small-for-size syndrome was improved in rats with thrombopoietin administration. The tissue and serum levels of regeneration-related cytokines, such as IL-6, IGF-1, HGF and TNF-α, increased significantly in the TPO group compared with those in the control group; The phosphorylation of proliferative signaling pathways, Stat3, Erk 1/2, Akt and NF-κB, were significantly elevated. As a result, the condition of regeneration was improved, which were shown by the increased expression levels of cyclin D1, Ki67-labeling index, mitotic index and liver/body weight ratio. Platelets did not aggravate ischemia/reperfusion injury, as there was no change in oxidative stress, their downstream signaling pathways, necrosis or apoptosis in the liver grafts between the control and TPO groups. After Kupffer cell depletion, the platelet-induced attenuation of serum aminotransferases, increased serum and tissue levels of IL-6 and TNF-α, and proliferation-related signaling pathways were abolished.

In experiment II, after 20% partial liver transplantation, the 7-day survival rate was improved in the TPO group compared with that in the control group.

Discussion:

Living donor liver transplantation is different from partial hepatectomy in that the partial liver graft in living donor liver transplantation requires proliferation under I/R injury.

On the aspect of liver regeneration, thrombocytosis induced significant accumulation of platelets in the liver graft after transplantation, along with elevated levels of HGF, IGF-1, IL-6 and TNF-α. This revealed that platelets were efficient carriers of proliferative growth factors and stimulators of Kupffer cells, which led to subsequent activation of downstream proliferative transcription cascades. The increases in liver/body weight ratio, Ki67-labeling index and mitotic index indicated that liver regeneration was accelerated under thrombocytosis. We considered that these positive impacts of thrombocytosis eventually improved the survival rates in the rat model of 20% partial liver transplantation. After KC depletion, a decrease of platelet

accumulation in the liver graft was observed, and the elevations in proliferative cytokines and signaling pathways under thrombocytosis were abolished, indicating that hepatic regeneration could be delayed in the absence of Kupffer cells.

For ischemia/reperfusion injury, although platelets are considered to be related to the generation of reactive oxygen species, the oxidative stress-related factors did not increase under thrombocytosis, and ischemia/reperfusion injury was not aggravated. TNF-α is a pleiotropic cytokine possessing two opposite effects on hepatocytes, namely, promoting proliferation and inducing ischemia/reperfusion injury. Since TNF-α-mediated ischemia/reperfusion injury could be inhibited by Akt, and platelet-derived IGF-1 induced the phosphorylation of Akt after partial hepatectomy, we hypothesized that the platelet-derived IGF-1 enhanced the phosphorylation of Akt, and consequently prevented TNF-α-mediated hepatic ischemia/reperfusion injury.

Conclusion:

Our results suggested that platelets stimulated graft regeneration and prolonged survival without aggregating ischemia/reperfusion injury after partial liver transplantation, and Kupffer cells vitally contributed to platelet-derived regeneration. Platelet therapies to increase perioperative platelet counts may improve the outcomes after living donor liver transplantation.