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Research Report

Beneficial effects of ventromedial hypothalamus (VMH) lesioning on function and morphology of the liver after hepatectomy in rats

Eun Young Lee[a,b], *Shuji Inoue*[a,c,*], *Akira Senoo*[c], *Hiroyuki Shimizu*[c], *Yoko Suzuki*[c], *Noriko Ishizuka*[c], *Nobuo Imazeki*[c], *Kahoru Sasaki*[c], *Masako Kako*[c], *Toshimasa Osaka*[d], *Takashi Miki*[b]

[a]*Department of Nutrition and Physiology, Faculty of Home Economics, Kyoritsu Women's University, Tokyo 101-8433, Japan*
[b]*Department of Medical Physiology, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan*
[c]*Faculty of Health Care, Kiryu University, Midori 379-2392, Japan*
[d]*National Institute of Health and Nutrition, Tokyo 162-8636, Japan*

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ABSTRACT

Liver has a high regenerative capacity and restores its mass and function shortly after partial hepatectomy through increased proliferation and metabolic modification of hepatocytes. The proliferation of hepatocytes can be triggered by its mass reduction after hepatectomy or by the neural factors including lesioning of the ventromedial hypothalamus (VMH). In the present study, we examined the effect of VMH lesioning on liver regeneration in hepatectomized rats by evaluating liver function and morphology. We found that functional deficits caused by partial hepatectomy [prolonged prothrombin time (PT), increased indocyanine green (ICG) retention, and decrease in PAS (periodic Acid-Schiff staining)-positive hepatocytes] were restored by VMH lesioning at 1 week after the surgery, whereas these alterations disappeared at 4 weeks. Morphologically, lipid microdroplets, which are considered to be important for maintaining contiguous liver function via supplying fuel for cell proliferation, were found to accumulate in hepatocytes of the hepatectomized rats at early period (1 day) after partial hepatectomy. Interestingly, such lipid microdroplets were also detected in the VMH lesioned rats and the more abundantly in the VMH lesioned, hepatectomized rats up to 1 week after the surgery. In conclusion, our results suggest that VMH lesioning in rats promotes recovery of liver anatomically and functionally after partial hepatectomy by promoting cell proliferation process.

* *Corresponding author at:* Faculty of Health Care, Kiryu University, 606-7 Azami, Kasakake, Midori, Gunma, 379-2392, Japan. Fax: +81 277 48 9122.

E-mail addresses: eylee@restaff.chiba-u.jp (E.Y. Lee), ishuji@kiryu-u.ac.jp (S. Inoue), senoo-a@kiryu-u.ac.jp (A. Senoo), shimizu-hi@kiryu-u.ac.jp (H. Shimizu), yksuzuki@nih.go.jp (Y. Suzuki), ishizuka-no@kiryu-u.ac.jp (N. Ishizuka), imazeki-no@kiryu-u.ac.jp (N. Imazeki), sasaki-ka@kiryu-u.ac.jp (K. Sasaki), kako-ma@kiryu-u.ac.jp (M. Kako), osaka@nih.go.jp (T. Osaka), tmiki@faculty.chiba-u.jp (T. Miki).

Abbreviations: VMH, ventromedial hypothalamus; PT, prothrombin time; ICG, indocyanine green; PAS, periodic acid-Schiff reaction; PCNA, proliferating cell nuclear antigen.

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1. Introduction

The liver is the largest organ in the body and plays an essential role in regulation of energy homeostasis, synthesis of most secreted proteins in the blood, excretion of bile acids required for lipid absorption, and elimination of extrinsic or intrinsic toxic substances (Kuntz and Kuntz, 2008). Impairment of liver function leads to liver failure, which is a fatal condition if not treated properly. A decline in liver function may be caused by dysfunction of existing hepatocytes or by a decrease in the number of functioning hepatocytes.

A common cause of loss of hepatocytes in human disease is surgical resection of part of the liver harboring malignant tumors. Although the loss of liver mass would cause attenuation of liver function, liver possesses a high regenerative capacity. In rodents, liver restores its mass within a week, even if 2/3 of the liver is resected (Higgins and Anderson, 1931). The mechanism of liver regeneration has been studied extensively. Liver regeneration is a sequential process involving various genes that promote liver regeneration. These include hepatocyte growth factor (HGF), ligands of the epidermal growth factor receptor (EGFR) such as EGF, TGFα, and HB-EGF; tumor necrosis factor (TNF); and interleukin-6 (IL-6) (Michalopoulos, 2007).

Despite the highly regenerative properties of hepatocytes, surgery is unsuccessful in some cases because of excessive liver loss due to massive hepatectomy (Michalopoulos, 2010). Therefore, a method to promote liver regeneration is desirable. In addition to the internal mechanism in the liver, proliferation of hepatocytes is also regulated by organs outside the liver. We previously reported that VMH lesioning increased DNA synthesis mainly through cholinergic nerves (Kiba et al., 1992) and that the expression levels of genes involved in neural development and immune system were increased in the liver of VMH lesioned rats (Kiba et al., 2009). We also reported that regeneration of liver after partial hepatectomy was facilitated by VMH lesioning along with increased DNA synthesis, as assessed by thymidine uptake in the residual liver (Kiba et al., 1994). However, the effects of VMH lesioning on the function and morphology of the hepatectomized liver have not been reported. In this study, we examined functional parameters [serum albumin level, prothrombin time, and indocyanine green (ICG) retention rate], the morphology of the liver [staining with methylene blue using epoxy resin embedded sections or with periodic acid-Schiff reaction (PAS) using ordinary paraffin sections], and proliferating cell nuclear antigen (PCNA), a marker of cell proliferation in rats subjected to simultaneous VMH lesioning and/or partial hepatectomy. In order to observe the accumulation of lipid microdroplets in the livers of VMH lesioned rats, the rats were maintained under pair-feeding conditions to exclude the effect of hyperphagia caused by VMH lesioning. The morphological alterations were examined by both light- and electron-microscopy.

2. Results

2.1. Changes in body weight after the surgery

The body weights in the 4 experimental groups before and after the surgery (1 or 4 weeks) are shown in Fig. 1. The body weights of sham VMH lesioned and sham hepatectomized (SV-SH, designated as controls) and sham VMH lesioned and hepatectomized (SV-H) rats returned to basal levels within a week after the surgery, while those of VMH lesioned and sham hepatectomized (V-SH) (342.2±12.1 g, n=6), and VMH lesioned and hepatectomized (V-H) (330.6±8.9 g, n=6) rats at 1 week after the surgery were significantly higher compared with SV-SH (279.4±1.5 g, n=6) and SV-H (274.1±8.1 g, n=6) rats, respectively (Fig. 1A). Four weeks after the surgery, the body weights of the VMH lesioned rats (V-SH and V-H rats) were further increased compared with their counterparts (SV-SH and SV-H rats, respectively) (Fig. 1B). We also measured food intake at 1 week after the surgery, and found that V-SH and V-H rats ate more than SV-SH and SV-H rats (Fig. 1C). These results are consistent with the previous reports that VMH lesioning caused obesity associated with hyperphagia in animals (Inoue et al., 1977a; King, 2006).

2.2. Effects of VMH lesioning and partial hepatectomy on weights of liver and other tissues

Liver weights of the free feeding rats were measured at 1 week after the surgery. Although approximately 70% of the liver was removed by the hepatectomy, the liver weight of the SV-H rats had recovered to 86% of that of the SV-SH rats within a week after the surgery (Fig. 2A). In V-SH rats, VMH lesioning increased the liver weight by 50% compared with that of SV-SH rats (Fig. 2A). Similarly, VMH lesioning in hepatectomized rats had the same effect on liver weight, with a 64% increase (V-H vs. SV-H).

In addition to the effect on liver weight, VMH lesioning caused a significant increase in the weight of the pancreas and stomach (Fig. 2B). However, we found only a marginal increase in the weight of the heart or kidney and none for the lung. These results correspond to previous findings showing increased weights of the pancreas and stomach as well as liver in VMH lesioned rats (Kiba et al., 1993, 1996) and mice (Suzuki et al., 2011).

2.3. Effect of VMH lesioning on liver function after partial hepatectomy

We next evaluated liver function at 1 and 4 weeks after the surgery. There were no differences in serum aspartate aminotransferase (AST) among the 4 groups of rats (Fig. 3A). The albumin levels at 1 week tended to be lowered (by 16%) by hepatectomy (2.44±0.07 g/dl in SV-SH; 2.04±0.09 g/dl in SV-H, n=6), but the difference was not significant. However, the serum albumin levels in V-SH rats (3.0±0.11 g/dl) at 1 week were significantly higher (P<0.01) than those in SV-SH rats, suggesting that VMH lesioning increased serum albumin at this time point (Fig. 3B). At 4 weeks after the surgery, the same trends for serum albumin were present in the 4 groups (Fig. 3B).

We also examined prothrombin time (PT) to assess liver function. Hepatectomy exacerbated PT at 1 week after the surgery (17.2±0.17 sec in SV-H rats vs. 15.6±0.27 sec in SV-SH rats, n=5, P<0.01) (Fig. 3C). In contrast, VMH lesioning alone did not affect PT (16.0±0.35 sec in V-SH rats vs. 15.6±0.27 sec in SV-SH rats, not significant). However, VMH lesioning reversed the elongated PT caused by hepatectomy (16.2±0.18 sec in V-H rats;