

## Lysosomal Redistribution and Increased Fragility of Pancreatic Acinar Cells in Early Stage after Hepatectomy in Rats

TETSUYA HIRANO, TADA0 MANABE and TAKAYOSHI TOBE

First Department of Surgery, Faculty of Medicine, Kyoto University  
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### Abstract

To explore the cellular fragility of pancreatic acinar cells in the early stage after partial hepatectomy, we evaluated the subcellular redistribution of lysosomal enzyme, lysosomal fragility in acinar cells, lactate dehydrogenase (LDH) discharge from acinar cells and the pancreatic water content in rats 4 days after about 70% hepatectomy. Following hepatectomy, there was redistribution of lysosomal enzymes, and more cathepsin B in zymogen pellets (1.3 KP) and less cathepsin B in lysosomal pellets (12 KP) than in sham-operated or normal rats. Soluble cathepsin B activity and LDH discharge also increased significantly after hepatectomy. The pancreatic water content was significantly increased after hepatectomy. These results indicate that the fragility of acinar cells increased in the early stage after hepatectomy.

### Introduction

Pancreatic hormones such as insulin and glucagon have been reported to be important hepatotrophic factors stimulating liver regeneration after hepatectomy<sup>2,3,7,13,16</sup>. Remarkable morphological changes in the endocrine pancreas happen after hepatectomy<sup>7,17</sup>. In our recent study<sup>7</sup>, enlarged acinar cells were found after hepatectomy. In acute viral hepatitis, pancreatic hyperamylasemia has been reported<sup>8</sup>. Hepatectomy and acute hepatitis both cause loss of functional liver mass, and reduced liver function seems to affect the exocrine pancreas. However, there have been few reports on the relationship between hepatectomy and the fragility of pancreatic acinar cells.

In this study, we evaluated the changes in pancreatic lysosomal distribution and the fragility of the acinar cells as well as the water content and the lactate dehydrogenase (LDH) discharge from acinar cells in the early stage after hepatectomy in rats.

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Key words: Pancreatic acinar cells, Partial hepatectomy, Lysosomal fragility, Cathepsin B.

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Present address: First Department of Surgery, Faculty of Medicine, Kyoto University, 54 Shogoin-Kawaracho, Sakyoku, Kyoto 606, Japan.

## Materials and Methods

*Animals:* Male Wister rats weighing 246–315 g were maintained on a standard pellet diet and had free access to tap water. Food was withheld 12 hours before hepatectomy or sham operation.

*Experimental procedures:* Under intraperitoneal pentobarbital anesthesia (25 mg/kg), about 70% hepatectomy was performed on 15 rats by the method of HIGGINS and ANDERSON<sup>5)</sup>. Fifteen other rats were laparotomized and the liver was manipulated but not cut (the sham-operated group). Postoperatively all the animals were allowed free access to food and water until 12 hours before the next stage of the experiment. Another control group consisted of 15 normal rats.

*Pancreatic water content and redistribution of lysosomal enzyme:* Four days after hepatectomy, 5 rats were sacrificed with deep pentobarbital anesthesia, and portions of the pancreas were quickly removed and trimmed of fat. Some portions of the pancreas were homogenized with a glass-teflon homogenizer in buffer (pH=6.5) containing 5 mM MOPS (3-(N-Morpholino) propanesulfonic acid) (Sigma Chemical, St. Louis, MO, U.S.A.), 1 mM MgSO<sub>4</sub>, and 250 mM sucrose. Unbroken cells and debris were removed by low speed centrifugation (150 × g, 15 min, 4°C), and the resulting supernatant was used for subcellular fractionation, as described by TARTAKOFF and JAMIESON<sup>18)</sup> with minor modifications<sup>14)</sup>. That supernatant was centrifuged (1,300 × g, 15 min, 4°C) and the resulting zymogen granule-rich pellet (1.3 KP) harvested. The remaining supernatant was centrifuged (12,000 × g, 12 min 4°C) to obtain a pellet rich in lysosomes and mitochondria (12 KP). The remaining supernatant was ultracentrifuged (105,000 × g, 60 min, 4°C) to obtain a microsomal-rich pellet (105 KP). With all these fractions, as a lysosome enzyme, cathepsin B activity was measured fluorometrically, as described by McDONALD AND ELLIS<sup>9)</sup> and each activity was expressed as the percentage of the total activity.

Another portions of the glands were used for the measurement of the pancreatic water content by comparing the pancreatic weight immediately after sacrifice of the animals with that of the same samples after dessication at 150°C for 48 hours (dry weight); the pancreatic water content was expressed as a percentage of the total pancreatic weight.

*Lactate dehydrogenase (LDH) discharge from dispersed acinar cells.* Four days after hepatectomy, 5 rats were used for the experiment with dispersed acini. Dispersed acini were prepared by collagenase (Cooper Diagnostics, Freehold, NJ, U.S.A.) digestion and gentle shearing, as previously described<sup>12)</sup>. Acini were suspended in HEPES-Ringer buffer<sup>15)</sup> (pH=7.4) with addition of Eagles basal amino acids (Gibco Laboratories, Grand Island, NY, U.S.A.), bovine serum albumin (0.1%) (ICN Immuno Biologicals, Lisle, IL, U.S.A.) and soybean trypsin inhibitor (0.01%) (Cooper Diagnostics, Freehold, NJ, U.S.A.). The acini were incubated in this buffer under O<sub>2</sub>, in a shaking water bath maintained at 37°C. At 30 minute intervals (for 120 min), aliquots were removed and LDH activity in the suspending medium and in the pellet acini was measured by the method of BERGMAYER *et al*<sup>11)</sup>. LDH discharge from acini was expressed as a percentage of the total LDH activity present in the acini at the onset of incubation.

*Lysosomal fragility in the acinar cells:* Five other hepatectomized rats were used for the experiment on lysosomal fragility 4 days after hepatectomy. Portions of the pancreas were homogenized in a glass-teflon homogenizer in 5 mM MOPS buffer as in the redistribution experiment. After the same low speed centrifugation (150 × g, 15 min, 4°C) and 12,000 × g (12 min, 4°C), a combined zymogen-lysosome rich pellet was made. This pellet which was arbitrarily considered to contain 100% lysosomal enzyme activity, was resuspended in 5 mM MOPS buffer and incubated for varying

intervals (30, 60, 90, and 120 min) at 25°C. The samples were then recentrifuged ( $12,000 \times g$ , 12 min, 4°C) to separate the particulate from the soluble lysosomal enzyme activity. Cathepsin B activity in each sample was measured after separation of the pellet and supernatant. Centrifugation and subsequent measurement of particulate and soluble lysosomal enzyme activity identified the rate and extent of in-vitro rupture of lysosomal enzyme-containing organelles. Soluble cathepsin B activity was expressed as a percentage of the total cathepsin B activity, and this figure was used as an index of fragility of the lysosomes.

*Statistical procedures:* The values in this study are presented as means  $\pm$  SEM, and statistical analysis was carried out with Student's t-test. A p-value less than 0.05 was considered to denote significance

## Results

*Pancreatic water content and redistribution of lysosomal enzyme:* Four days after hepatectomy, the pancreatic water content was significantly ( $p < 0.05$ ) greater than in the sham-operated and normal rats (Table I). There was no significant difference between the sham-operated and normal rats. Four days after hepatectomy, cathepsin B activity in the zymogen pellet (1.3 KP) was significantly greater ( $p < 0.05$ ) than in the sham-operated and normal rats (Table II). On the other hand, that in the lysosomal pellet (12 KP) was significantly lower ( $p < 0.05$ ) than in the sham-operated and normal rats.

*Lactate dehydrogenase (LDH) discharge from acinar cells:* Four days after hepatectomy, LDH discharge was almost the same as in the control group before 60 min incubation (Table III). After prolonged incubation (90 min), LDH leakage was significantly ( $p < 0.01$ ) higher in the samples taken

**Table I.** Changes in the pancreatic water content 4 days after partial hepatectomy in rats  
Values = means  $\pm$  SE (no. of rats)

Groups	Pancreatic water content (% of total weight)
Hepatectomy	87 $\pm$ 4 (5)**
Sham operation	76 $\pm$ 2 (5)
Normal rats	74 $\pm$ 2 (5)

\*  $p < 0.05$  vs sham-operated rats.

+  $p < 0.05$  vs normal rats

**Table II.** Changes in subcellular distributions of cathepsin B 4 days after partial hepatectomy in rats  
Values = means  $\pm$  SE (no. of rats)

Groups	Subcellular fractions		
	Zymogen pellet (1.3 KP)	Lysosomal pellet (12 KP)	Microsomal pellet (105 KP)
Hepatectomy	35.3 $\pm$ 2.5 (5)**	33.2 $\pm$ 2.6 (5)**+	7.3 $\pm$ 2.0 (5)
Sham operation	24.9 $\pm$ 2.2 (5)	46.3 $\pm$ 3.5 (5)	5.3 $\pm$ 1.7 (5)
Normal rats	22.3 $\pm$ 1.9 (5)	57.6 $\pm$ 2.8 (5)	6.9 $\pm$ 1.2 (5)

\*  $p < 0.05$  vs sham-operated rats

+  $p < 0.05$ , \*\*  $p < 0.01$  vs normal rats

**Table III.** Changes in LDH discharge from acinar cells 4 days after partial hepatectomy in rats. Values = means  $\pm$  SE (no. of rats)

Groups	30	Incubation time (min)		120
		60	90	
Hepatectomy	3.1 $\pm$ 0.2 (5)	3.9 $\pm$ 0.1 (5)	6.0 $\pm$ 0.1 (5)* <sup>+</sup>	10.9 $\pm$ 0.3 (5)* <sup>+</sup>
Sham operation	3.0 $\pm$ 0.2 (5)	3.5 $\pm$ 0.1 (5)	4.2 $\pm$ 0.1 (5)	7.0 $\pm$ 0.2 (5)
Normal rats	2.8 $\pm$ 0.1 (5)	3.3 $\pm$ 0.2 (5)	3.9 $\pm$ 0.3 (5)	6.3 $\pm$ 0.3 (5)

LDH discharge is expressed as a percentage of total LDH activity in the acini at the onset of incubation.

\*  $p < 0.01$  vs sham-operated rats

+  $p < 0.01$  vs normal rats

**Table IV.** Changes in soluble cathepsin B activity in acinar cells 4 days after partial hepatectomy in rats. Values = means  $\pm$  SE (no. of rats)

Groups	30	Incubation time (min)		120
		60	90	
Hepatectomy	8.7 $\pm$ 2.0 (5)	17.5 $\pm$ 2.0 (5) <sup>+</sup>	34.0 $\pm$ 2.0 (5)* <sup>++</sup>	53.7 $\pm$ 3.0 (5)* <sup>++</sup>
Sham operation	5.4 $\pm$ 1.5 (5)	12.3 $\pm$ 1.1 (5)	17.5 $\pm$ 1.4 (5)	34.0 $\pm$ 1.3 (5)
Normal rats	4.9 $\pm$ 1.3 (5)	10.9 $\pm$ 2.2 (5)	15.8 $\pm$ 2.2 (5)	31.5 $\pm$ 2.3 (5)

Soluble cathepsin B activity is expressed as a percentage of total activity.

\*  $p < 0.01$  vs sham-operated rats

+  $p < 0.05$ , ++  $p < 0.01$  vs normal rats

from the hepatectomized group than in those from the sham-operated and normal rats.

*Lysosomal fragility in the acinar cells:* Incubation of the samples from sham-operated and normal rats at 25°C resulted in a slow but significant increase in soluble cathepsin activity. In contrast, there was a much more rapid and marked increase in soluble cathepsin B activity during incubation of the samples taken from the hepatectomized animals (Table IV).

## Discussion

The endocrine pancreas has been reported to be closely related to liver regeneration<sup>2,6,7,11,13,16</sup>, and there have been many reports on the relationship between partial hepatectomy and pancreatic hormones such as insulin and glucagon<sup>3,4,11</sup>, but few concerning the relationship between partial hepatectomy and the exocrine pancreas, and this remains to be elucidated. In our present study, all the pancreatic water content, lysosomal fragility shown as increased cathepsin B activity in acinar cells and LDH discharge from acinar cells were significantly greater 4 days after hepatectomy than in sham-operated and normal rats. In addition, a redistribution of lysosomal enzyme in acinar cells was noted in the early stage after hepatectomy. These observations suggest that the cellular fragility of acinar cells in the early stage after hepatectomy is related to lysosomal fragility. Increased and crucial lysosomal fragility of acinar cells has also been described in experimental acute pancreatitis<sup>15</sup>. So this lysosomal fragility seems to play an important role in the cellular fragility of acinar cells after partial hepatectomy as it does in experimental acute pancreatitis.

In this study we did not test pancreatic function after hepatectomy, and we tested cellular fragili-

ty only in the early stage after hepatectomy, so we do not know what effect it has on pancreatic function or its duration. However, pancreatic hyperamylasemia has been noted in acute viral hepatitis<sup>8)</sup>, and pathological changes in the pancreas in patients with liver cirrhosis have been also reported<sup>10)</sup>. So this mild pancreatic injury seems to be not uncommon.

As the pancreas itself contains digestive enzymes and lysosomal enzymes such as cathepsin B which can activate trypsinogen, lysosomal fragility in acinar cells may be serious by injury to the pancreas, and after partial hepatectomy the pancreas seems to be especially susceptible to various forms of damage such as hypoxia or trauma. If other factors such as massive bleeding, pulmonary complications, or iatrogenic trauma to the pancreas are added in the early stage after hepatectomy, the risk of severe acute pancreatitis seems to be increased.

Further study will be needed to clarify the pathogenesis of this cellular and lysosomal fragility in acinar cells after partial hepatectomy.

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## 和文抄録

# ラットにおける肝切除後早期の膵腺房細胞の ライソゾームの再分布と脆弱性亢進

京都大学医学部第1外科学教室

平野 鉄也, 真辺 忠夫, 戸部 隆吉

肝切除後早期の膵腺房細胞の脆弱性を明らかにするために、ラットを用い70%肝切除後4日目に膵腺房細胞のライソゾーム酵素の細胞内分布、腺房細胞からのライソゾーム酵素(カテプシンB)、LDHの逸脱、膵の水分含有量を観察した。

その結果、肝切除群では Sham-operation 群、あるいは正常群に較べてカテプシンBの分布が、ライソ

ゾーム分画からチモーゲン分画へ移動することが明らかとなった。また、単離腺房細胞を用いた in-vitro 系では、肝切除群においてカテプシンB、LDHの細胞外への逸脱が有意にみとめられた。さらに肝切除後には膵腺房細胞の水分含有量の増加がみとめられた。

以上の結果より、肝切除後早期には膵腺房細胞の脆弱性が高まることが示唆された。