ORIGINAL ARTICLE

Basic Study

Evaluation of safety for hepatectomy in a novel mouse model with nonalcoholic-steatohepatitis

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Abstract

AIM

To investigate whether the liver resection volume in a newly developed nonalcoholic steatohepatitis (NASH) model influences surgical outcome.

METHODS

For establishment of a NASH model, mice were fed a high-fat diet for 4 wk, administered CCl_4 for the last 2 wk, and administered T0901317 for the last 5 d. We divided these mice into two groups: A 30% partial hepatectomy (PH) of NASH liver group and a 70% PH of NASH liver group. In addition, a 70% PH of normal liver group served as the control. Each group was evaluated for survival rate, regeneration, apoptosis, necrosis and DNA expression after PH.

RESULTS

In the 70% PH of NASH group, the survival rate was significantly decreased compared with that in the control and 30% PH of NASH groups ($P < 0.01$). 10 of 32 mice in the NASH 70% PH group died within 48 h after PH. Serum aspartate aminotransferase (AST) levels and total bilirubin (T-Bil) in the NASH 70% PH group were significantly higher than the levels in the other two groups (AST: $P < 0.05$, T-Bil: $P < 0.01$). In both PH of NASH groups, signaling proteins involved in regeneration were expressed at lower levels than those in the control group ($P < 0.01$). The 70% PH of NASH group also exhibited a lower number of Ki-67-positive cells and higher rates of apoptosis and necrosis than the NASH 30% PH group ($P < 0.01$). In addition, DNA microarray assays showed differences in gene expression associated with cell cycle arrest and apoptosis.

CONCLUSION

The function of the residual liver is impaired in fatty liver compared to normal liver. A larger residual volume is required to maintain liver functions in mice with NASH.

Key words: Hepatectomy; Liver regeneration; Residual liver; Liver proliferation; Nonalcoholic steatohepatitis

Core tip: We report whether the liver resection volume in the nonalcoholic steatohepatitis (NASH) model influences surgical outcome. The population of patients with NASH has been increasing. However, few animal models fully reflect both the histopathology and pathophysiology of NASH in humans. We established a novel experimental NASH model that exhibited the same characteristics as NASH in humans. This study elucidates the metabolism of the residual liver after a hepatectomy with NASH. Compared with normal liver, the residual NASH liver function is impaired, especially its regenerative ability. Therefore, a larger residual volume is required to maintain liver function in NASH liver after partial hepatectomy.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is observed in 20%-40% of the general population, and its incidence continues to increase in industrialized countries[1,2]. NAFLD includes several diseases, such as simple liver steatosis, nonalcoholic steatohepatitis (NASH), and cirrhosis. NASH is characterized by hepatic steatosis, lobular inflammation, and abnormal glucose tolerance. In NASH, continuous inflammation contributes to hepatocellular carcinoma (HCC)[3,4]. The cause of HCC is frequently infection with hepatitis B virus and hepatitis C virus (HCV). New antiviral medications for hepatitis are currently being used in clinics; therefore, the number of patients with virus-related HCC is expected to decrease in the future[5-9]. By contrast, the number of patients with NASH-related HCC has been increasing recently, and this trend is expected to continue because no effective treatments are available[10].

Steatosis is a risk factor for postoperative liver failure[11,12]. A number of clinical studies revealed that steatosis caused severe mortality and morbidity after liver resection compared with normal liver following liver resection[11,12]. In experimental models, hepatectomy of fatty livers resulted in suppressed liver regeneration and survival rates[13-16]. However, the influence of hepatectomy on NASH livers has not been extensively evaluated.

Hepatectomy is a standard and most effective therapy for HCC patients. Postoperative liver failure is a serious complication after hepatectomy, and its occurrence correlates with the volume and function of the residual liver[17-20]. To prevent liver failure after hepatectomy, the liver resection volume is limited according to preoperative liver function[21-23]. For promotion of regeneration and maintaining liver function preserving sufficient residual liver volume enables the prevention of liver failure[24,25]. Thus, the degree of liver regeneration is dependent on the volume of the residual liver. Although several NASH models, such as the methionine- and choline-deficient (MCD) model and high-fat (HF) diet model, have been reported, few models completely reflect the histopathology and pathophysiology of NASH in humans[26,27]. The disadvantages of the MCD model are that MCD mice exhibit severe body weight loss with the absence of insulin resistance. The HF diet model is not suitable for researching the pathogenesis of NASH because a longer period of time is required for presentation of NASH characteristics, and hepatic fibrosis is weaker than that observed in human NASH.

Thus, the ability of regeneration in the NASH liver has not yet been assessed in an experimental model. For the same reasons, the effect of hepatectomy on NASH liver has not been clearly elucidated in previous reports. We established a novel experimental NASH model that indicates similar histopathological and pathophysiological characteristics as those of NASH in humans[28]. The aim of this study was to investigate whether a difference in liver resection volume in a novel NASH model influences surgical outcomes.

MATERIALS AND METHODS

Animals
Six-week-old male C57BL/6J mice were obtained from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) and were acclimated for one week before the start of the experiment. Mice were maintained under a 12-h light-dark cycle and had free access to standard chow and tap water. The animal experiments were performed in a humane manner after receiving approval from the Institutional University Experiment Committee of the University of Tsukuba and in accordance with the Regulations for Animal Experiments at the university and Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science, and Technology.

NASH mouse model protocol
NASH mice were fed an HF diet (60 kcal% fat; D12492, Research Diets, Inc., New Brunswick, NJ, United States) for 4 wk, intraperitoneally injected with CCl$_4$ (Wako Pure Chemical Industries, Ltd., Osaka, Japan) twice a week for the final 2 wk, and intraperitoneally injected with T0901317 (Cayman Chemical Co., Ann Arbor, MI, United States) solubilized in DMSO for the final 5 d. The CCl$_4$ dose was 0.1 mL/kg, and the T0901317 dose was 2.5 mg/kg[28].

Surgical procedure and anesthesia
We categorized the mice into three groups: (1) 70% partial hepatectomy (PH) of normal liver mice as the control; (2) 30% PH of NASH liver group; and (3) 70% PH of NASH liver group. The normal liver mice have been not added any reagent and the histology and pathology have been not change. In 30% PH and 70% PH of the NASH liver group, liver specimens were evaluated by an experienced pathologist in a blinded fashion, the histology and pathology finding in the NASH severity of each groups have resulted in no difference in the NAFLD activity scores[28]. All mice received the hepatectomy 48 h after the final administration of CCl$_4$ and T0901317. In the 70% PH groups, the left and middle lobes of the liver were removed by using a single ligature, whereas only the left lobe was removed in the 30% PH group[29]. Hepatectomy was performed under ether anesthesia.

Liver tissue collection
Blood samples were collected from the orbital capillary and centrifuged at 3000 rpm for 10 min to isolate the serum. Each sample was stored at -80 ℃ until analysis. Mice of each group were sacrificed at 6 h and 12 h after PH. Then, the liver was quickly removed and weighed. The liver specimen was immediately fixed in 10% neutral-buffered formalin for further histological examination. Survival rates were evaluated in the NASH 70% PH group (n = 32) and NASH 30% PH group (n = 27).

Histology and immunohistochemistry
Fixed liver tissues were processed and embedded in paraffin using standard methods. Then, liver tissues were sliced into 2-μm thick paraffin sections and stained with hematoxylin and eosin (HE) to evaluate necrosis. Necrotic areas were detected by morphological features, and the ratio of necrosis/total area was calculated in 20 random intralobular fields. Liver proliferation was assessed by Ki-67 staining. Apoptosis was detected by TUNEL staining. TUNEL staining and Ki-67 staining were performed using an antibody kit (New History Science Laboratory Co., Ltd., Tokyo, Japan). The ratio of positive/total hepatocytes was calculated in 20 random intralobular fields.

Immunoblotting
Liver tissue extracts were prepared from specimens that were frozen in liquid nitrogen. We evaluated the expression of signaling proteins involved in liver regeneration, including AKT, STAT3, and ERK1/2, by western blotting. We compared the expression levels of these proteins in each group 6 h after PH. Immunoblots were developed using polyclonal antibodies against phospho-AKT (9271), total AKT (9272), phospho-STAT3 (9131), total STAT3 (9132), phospho-ERK1/2 (9101), and total ERK1/2 (9102) (Cell Signaling Technology, Beverly, MA, United States).

Gene expression analysis
Liver tissue samples were freshly collected and immediately frozen at -30 ℃ until investigation. Frozen liver samples were homogenized, and total RNA was isolated from whole cells using a NucleoSpin® RNA kit (Takara Bio, Inc., Otsu, Japan). RNA concentrations were determined by measuring the absorbance at 260/280 nm with a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Inc., Wilmington, DE, United States). Synthesis of complementary DNA was performed using AMV Reverse Transcriptase (Promega, Corp., Madison, WI, United States) and random primers (Takara Bio, Inc., Otsu, Japan). Briefly, a mixture of 1 mmol/L dNTPs (Fermentas Life Sciences, Inc., Burlington, ON, Canada), 0.025 μg/mL random primers, 0.25 U/mL reverse transcriptase, and 500 ng of total RNA was incubated at 30 ℃ for 10 min, 37 ℃ for 60 min, 95 ℃ for 5 min and

Table 1 Serum parameters of normal liver and nonalcoholic steatohepatitis groups after partial hepatectomy

	6 h after PH				12 h after PH			
	AST	ALT	T-Bil	IL-6	AST	ALT	T-Bil	IL-6
Normal 70%PH	2343.3 ± 6160.4	1828.3 ± 990.4	1.43 ± 0.5	2036.0 ± 1470.9	2976.7 ± 1395.7	2053.3 ± 886.2	2.0 ± 0.9	731.5 ± 483.7
NASH 30%PH	1610.0 ± 3700.6	1507.1 ± 563.5	0.76 ± 0.2	1511.5 ± 284.8	1841.3 ± 619.1	1522.9 ± 537.9	0.9 ± 0.7	692.8 ± 211.1
NASH 70%PH	3064.3 ± 1289.8[a]	2422.9 ± 1194.8	2.57 ± 1.36[b]	3026.2 ± 2127.5	4067.5 ± 2059.2[a]	2403.8 ± 1111.8	3.85 ± 0.96[b]	987.9 ± 550.7

Data are presented as the mean ± SD (n = 5-7). [a]$P < 0.05$, [b]$P < 0.01$ *vs* other groups. NASH: Nonalcoholic steatohepatitis; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; PH: Partial hepatectomy; T-Bil: Total bilirubin; IL-6: Interleukin-6.
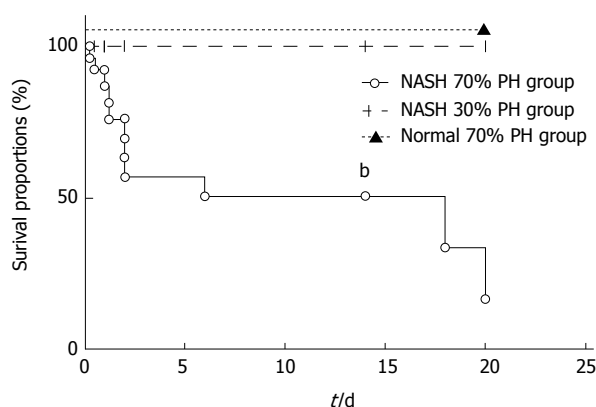

Figure 1 Survival rate after partial hepatectomy. Survival rates of the 30% PH and 70% PH groups were evaluated by the Kaplan-Meier method. All mice in the 30% PH group survived. In contrast, 80% of the NASH 70% PH group died by 20 d. n = 27 in NASH 30% PH group; n = 32 in NASH 70% PH group. [b]$P < 0.01$. PH: partial hepatectomy; NASH: nonalcoholic steatohepatitis.

4 ℃ before storage at -80 ℃.

RT-PCR primers were designed using Primer Express Software for Real-time PCR ver. 3.0 (Applied Biosystems, Inc., Foster City, CA, United States) based on the sequences available in GenBank. Primers were purchased from Takara Bio, Inc. (Otsu, Japan). *GADD45A* primer sequences were 5'-CCTGCACTGTGTGCTGGTGA-3' and 5'-CCACTGATCCATGTAGCGACTTTC-3'. *PDE4B* primer sequences were 5'-CCCATCAGCAGTTAAGGACAGGA-3' and 5'-TGGGCAGAACTAGGGACTCAAGA-3'.

Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as an endogenous control. RT-PCR was performed using SYBR-Green Real-Time PCR Master Mix-Plus (Toyobo Co., Ltd., Osaka, Japan) and an Applied Biosystems 7300 real-time PCR system (Applied Biosystems, Inc., Foster City, CA, United States) as recommended by the manufacturer's instructions[28].

Microarray analysis

DNA microarray analysis was conducted on RNA samples isolated from liver tissue in the control group and novel NASH model group. Labeled cRNA was synthesized from 100 ng of total RNA using a GeneChip® 3' IVT Plus Reagent Kit (Affymetrix, Inc., Santa Clara, CA, United States) according to the manufacturer's protocol. Fragmented and labeled cRNA (7.5 μg) was hybridized to an Affymetrix Mouse MG-430 PM Array Strip (Affymetrix) for 16 h at 45 ℃. The strips were washed and stained using a GeneAtlas Fluidics Station 400 (Affymetrix),

and the resulting images were scanned using a GeneAtlas Imaging Station (Affymetrix). Probe-level analysis, including background subtraction and quantile normalization, was conducted using a robust multiarray average algorithm (RMA) using Affymetrix Expression Console Software 1.4 (Affymetrix). The gene expression profile of the novel NASH model was compared with the HF group. Genes exhibiting differences in expression with an increase of greater than 1.4-fold and a decrease of less than 0.65-fold were classified as differentially expressed genes[28].

Statistical analysis

All data are expressed as the mean ± SD. Statistical analyses were conducted using PRISM. Mann-Whitney U test was used for comparing between two groups. P-values less than 0.05 were considered significant. The Kaplan-Meier estimator was used for survival rate evaluation.

RESULTS

Survival rate

The survival rate of the NASH 70% PH group was significantly lower than that of the NASH 30% PH group ($P < 0.01$) (Figure 1), and 10 of 32 mice in the NASH 70% PH group died within 48 h after PH. On the other hand, all mice in the NASH 30% PH group survived.

Liver function

At 6 and 12 h after PH, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were high in all three groups. AST levels in the NASH 70% PH group were significantly higher than the levels in the other two groups (AST: $P < 0.05$). Total bilirubin (T-Bil) in the normal liver and NASH 30% PH groups did not change, but the values only significantly increased in the NASH 70% PH group ($P < 0.01$) (Table 1).

Liver proliferation assay

Many more Ki-67-positive hepatocytes were observed in the NASH 30% PH group than in the preoperative NASH liver ($P < 0.05$). On the other hand, fewer Ki-67-positive cells were noted in the NASH 70% PH group than in the preoperative NASH liver ($P < 0.01$). Additionally, significantly fewer Ki-67-positive cells were noted in the 70% PH group than in the NASH 30% PH
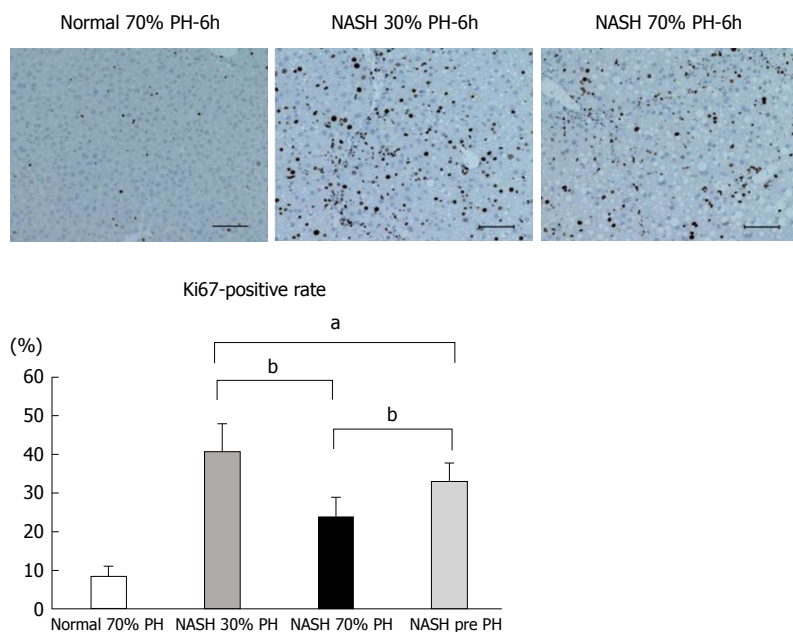
Normal 70% PH-6h   NASH 30% PH-6h   NASH 70% PH-6h

Ki67-positive rate

Figure 2 Ki-67 staining and proliferation score. Proliferation was evaluated by Ki-67 staining. We compared preoperative nonalcoholic steatohepatitis (NASH) groups with NASH 30% PH and NASH 70% PH groups 6 h after PH. Ratios of Ki-67-positive/total hepatocytes were calculated. Significantly fewer Ki-67-positive cells were noted in the 70% PH group than in the NASH 30% PH group. Ratios (%) are expressed as the mean ± SD. *n* = 2 per groups, 10 fields per sample. [a]$P < 0.05$; [b]$P < 0.01$. Scale bar: 100 μm. PH: partial hepatectomy; NASH: nonalcoholic steatohepatitis.

group ($P < 0.01$) (Figure 2).

Liver regeneration signal

In the normal 70% PH liver group, *i.e.*, the control group, expression of AKT, STAT3, and ERK1/2 phosphorylation was observed. In the both NASH 30% PH and 70% groups, phosphorylation of AKT, STAT3, and ERK1/2 was significantly lower than in the control group ($P < 0.01$) (Figure 3).

Histological assay

The number of TUNEL-positive cells in the NASH 70% PH group was significantly higher than in the other groups ($P < 0.01$). The TUNEL-positive rate of normal liver was significantly higher than that in the NASH 30% PH group ($P < 0.01$) (Figure 4A). The area of necrosis in the NASH 70% PH group was significantly larger than that in the NASH 30% PH group ($P < 0.01$). In both NASH groups, the necrotic area was significantly larger than that in the normal liver group ($P < 0.01$) (Figure 4B).

Microarray assay

mRNAs in the NASH 70% PH group with the highest fold-change (> 1.4 or < 0.70) in expression and with *P*-values <0.05 were selected and compared with those in the NASH 30% PH group (Table 2). *PDE4B*, *SLC20A1*, *CXADR*, *GADD45A*, *ZSWIM6*, and *C15orf39* were expressed at higher levels in the NASH 70% PH group. *PDE4B* and *GADD45A* are associated with cell cycle arrest and apoptosis. Using qPCR, *GADD45A* and *PDE4B* mRNA expression was significantly different

between the two groups (*GADD45A*: $P < 0.01$, *PDE4B*: $P < 0.05$) (Figure 5).

DISCUSSION

NAFLD/NASH is a common hepatic disorder that causes HCC[1-4]. Recently, the population of patients with NASH and NASH-related HCC has been increasing[1,2,10]. Hepatectomy is the first-line treatment for patients with HCC[21]. After hepatectomy, the incidences of mortality and morbidity are dependent on the volume and function of the residual liver[17-20]. Previous reports have demonstrated that steatosis impaired liver regeneration and caused liver dysfunction after hepatectomy[11,12]. NASH has been proposed to cause liver failure rather than steatosis because NASH presents with not only steatosis but also fibrosis, inflammation, and insulin resistance. However, regarding NASH animal models, few models completely reflect the histopathology and pathophysiology of NASH in humans. Therefore, the effect on the residual liver under NASH conditions has not been appropriately evaluated[26,27]. In our previous study, we established a novel experimental NASH model that exhibited histopathological and pathophysiological findings similar to that of NASH in humans[28]. In this study, new NASH mice were received 30% PH or 70% PH, and the influence of liver resection volume on the residual liver function in NASH liver was investigated. Our results indicated that the survival rate after PH in NASH liver strongly correlated with resected liver volume and was attributed to the proliferative ability and the rates of apoptosis and necrosis compared
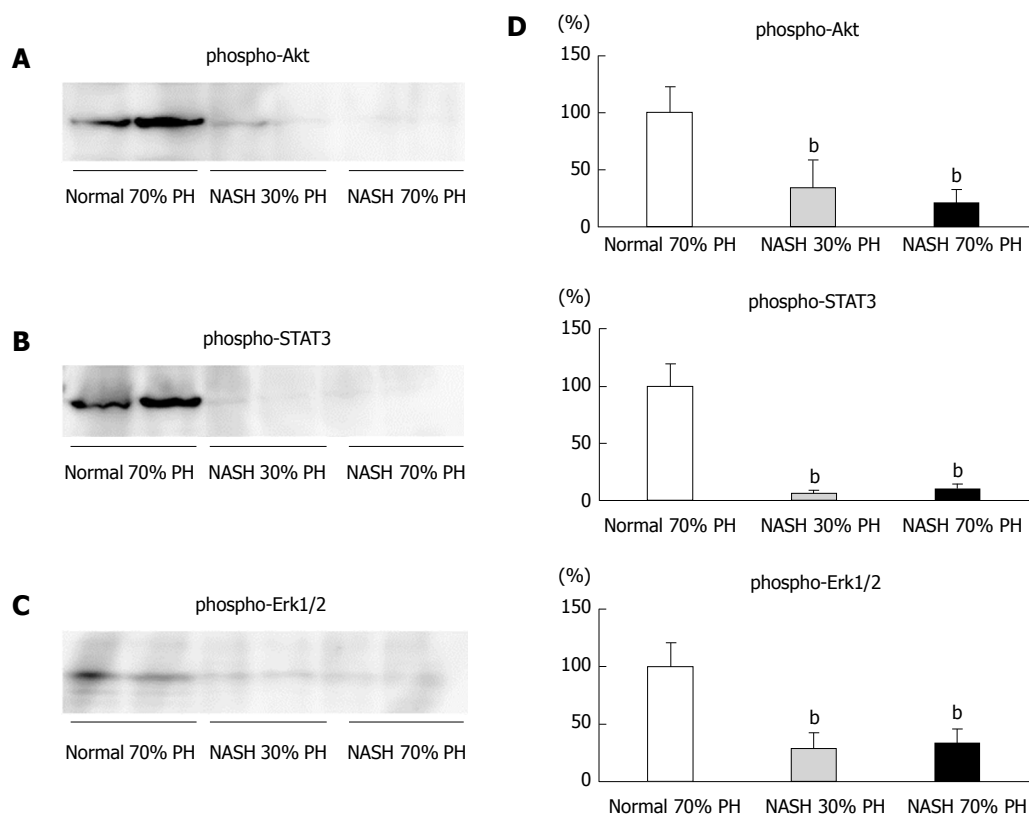
Figure 3 Protein assay. Expression of phosphorylated (A) Akt, (B) STAT3, and (C) ERK1/2 in normal and nonalcoholic steatohepatitis liver groups 6 h after PH. Expression levels were detected by western blot analysis. Ratios of densitometry were calculated by each score/score of control 70% PH (D). Data are expressed as the mean ± SD. *n* = 5-7 per groups. [b]*P* < 0.01 *vs* other groups.

with those in the normal liver. Even 30% of PH NASH residual liver could not offer sufficient liver function, and the volume of the functional residual liver significantly decreased due to less cell proliferation, apoptosis and necrosis after hepatectomy. Based on these results, we hypothesized that the residual liver volume that can support sufficient function in a normal liver could not maintain liver function in NASH. Our results suggested that to avoid liver dysfunction after hepatectomy in NASH, resection volume should be carefully determined and not the same as that in patients with normal liver.

In patients with PH, fatty liver causes a high rate of mortality and morbidity compared with normal liver[11]. In NAFLD patients, postoperative complications also increase in a manner that is similar to patients with fatty liver[12,30]. In animal models with PH, the survival rate of a fatty liver model decreased compared with a normal liver even with the same residual volume[13,15,16,31]. In this study, the survival rate after PH remarkably decreased in the NASH 70% PH group, and 30% of the deaths occurred within the first 48 h after PH. This result supported the previous reports, *i.e.*, outcome of PH significantly influences the survival rate of fatty mice[13,15,16,31]. In NASH liver, it was assumed that other characteristics, *i.e.*, fibrosis, inflammation, and insulin resistance, caused increased liver function deterioration. It was hypothesized that the residual liver volume of the small group, *i.e.*, 30% of residual volume of the

NASH liver, could not maintain sufficient liver function for survival after PH.

Liver regeneration occurred in cases with acute injury and/or liver resection[19]. In normal liver after PH, cell proliferation was observed in a small residual liver but not in a large residual liver[24]. Large residual livers have sufficient volume to maintain liver function, whereas small residual livers are unable to maintain liver function. Therefore, promotion of cell proliferation occurs in the small residual liver[24]. Ki-67 protein is expressed during the G1, G2, and S phases of cell division[32,33]. In this study, the number of Ki-67-positive cells in the residual liver in the 30% PH of NASH liver group was higher than in the preoperative NASH liver. On the other hand, the number of Ki-67-positive cells in the residual liver of the 70% PH of NASH liver group was significantly decreased. These results suggested that NASH hepatocytes would not have insufficient proliferation ability after large amount of PH, such as 70%.

Signaling pathways of liver regeneration are promoted by cytokines, *i.e.*, interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha, and growth factors[20]. The expression of AKT, STAT3, and ERK1/2 protein play an important role in liver regeneration, and the IL-6/STAT3 signaling pathway accelerates liver proliferation[16,24,34-36]. STAT3 was expressed at high levels in a liver with steatosis; however, these phenomena did not induce liver regeneration[37]. The NASH liver received continuous
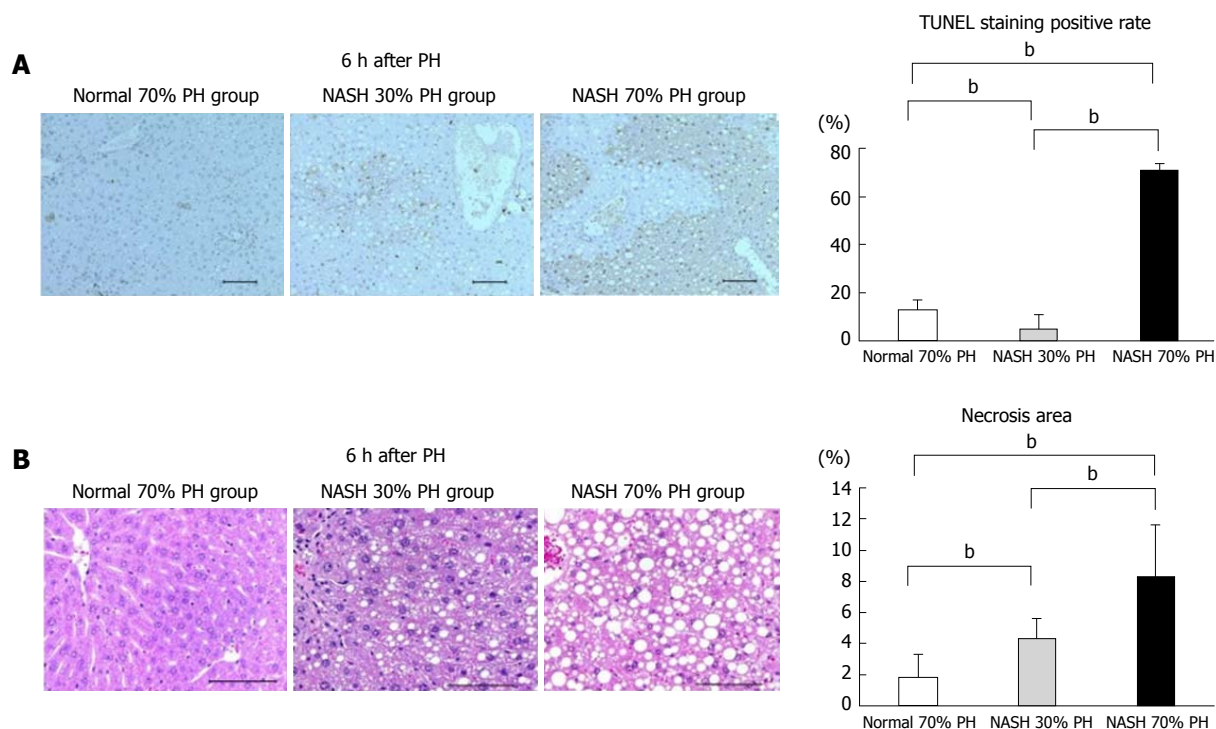
Figure 4 Histopathological findings. A: Apoptosis was evaluated by TUNEL staining. Ratios of TUNEL-positive/total hepatocytes were calculated; B: Necrosis was evaluated by HE staining. Ratios of necrosis morphological feature/total area were calculated. Apoptosis and necrosis were higher in the NASH 70% PH group than in the other groups. Data are expressed as the mean ± SD. *n* = 2 per groups, 10 fields per sample. [b]*P* < 0.01. Scale bar: 100 μm. PH: Partial hepatectomy; NASH: Nonalcoholic steatohepatitis.

Table 2 Gene expression microarray

Function	Gene name	Gene abbreviation	Fold-change (> 1.4)	*P* value (< 0.05)
Regulate the cellular concentrations of cyclic nucleotides	Phosphodiesterase 4B, cAMP-specific	*PDE4B*	2.102691211	0.0063
Growth arrest	Growth arrest and DNA-damage-inducible, alpha	*GADD45A*	1.672778704	0.013
Signal transduction	Coronin 1C	*CORO1C*	1.489851976	0.031
Stimulates expression of cytokines, including IL6, MIF and VEGFA	Hypoxia inducible lipid droplet associated	*HILPDA*	1.481465541	0.011
EGF-like growth factor	Heparin binding EGF-like growth factor	*HBEGF*	1.432049736	0.043
Cell-cell junctions	Membrane associated guanylate kinase, WW And PDZ domain containing 1	*MAGI1*	1.430522907	0.0222
Innate immune system	Ankyrin repeat and SOCS box containing 13	*ASB13*	0.515175325	0.0042
Apoptosis and autophagy	TIA1 cytotoxic granule-associated RNA binding protein-like 1	*TIAL1*	0.609948905	0.027
Gene expression	Nucleic acid binding protein 1	*NABP1*	0.6558797325	0.042
Cell cycle	S-phase kinase-associated protein 2, E3 ubiquitin protein ligase	*SKP2*	0.6588204077	0.044
Mitochondrial metabolism	Translocase of inner mitochondrial membrane 9 homolog (yeast)	*TIMM9*	0.6598542503	0.042
Cytokine signaling in immune system	B-cell CLL/lymphoma 6	*BCL6*	0.6760241074	0.0097
Cell cycle	Mutated in colorectal cancers	*MCC*	0.6761191711	0.0076
Gene expression	Zinc finger protein 519	*ZNF519*	0.6864230003	0.028
Gene expression	RNA binding motif protein, X-linked	*RBMX*	0.6983542235	0.012

In total, 6 genes were overexpressed greater than 1.4-fold in the nonalcoholic steatohepatitis (NASH) 70% PH group compared with their expression in the NASH 30% PH group, and 7 genes were downregulated by less than 0.70-fold in the NASH PH group compared with their expression in the NASH 30% PH group (*n* = 2). PH: Partial hepatectomy.

damage and stress *via* inflammatory cytokines and cells; therefore, NASH liver was considered to exhibit limited proliferation, which was only induced by hepatectomy[38]. In this study, the expression of transcriptional factors induced by cytokines for liver regeneration was not recognized in the residual livers of the NASH PH groups. No significant difference was noted in the activation of these proteins in both NASH PH groups, but liver cell proliferation was significantly higher in the 30% PH NASH liver group than in the 70% PH NASH liver group.
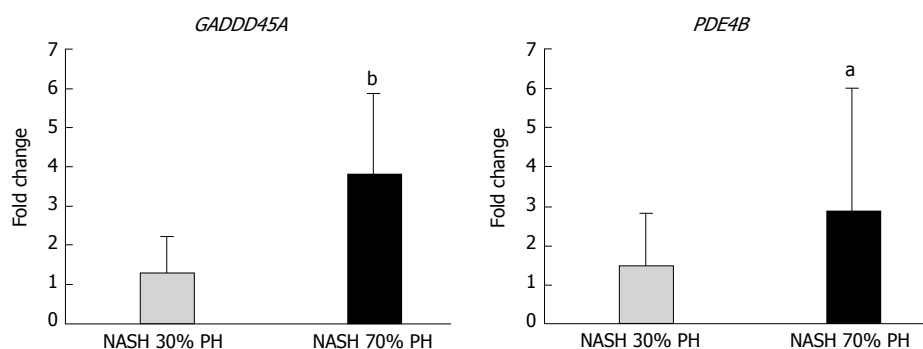
Figure 5 Gene expression of microarray. *GADD45A* and *PDE4B* expression correlated with the cell cycle. RT-PCR demonstrated a significant difference between the groups. Data are expressed as the mean ± SD. *n* = 5-7 per groups. [a]*P* < 0.05; [b]*P* < 0.01. PH: partial hepatectomy; NASH: nonalcoholic steatohepatitis.

Although we need to confirm these findings in future studies, the consistent decrease in liver proliferation exists, especially in the 70% PH of NASH liver group, *i.e.*, a large hepatectomy volume, which reduces the survival rate.

In general, the stress of liver resection promotes apoptosis in the residual liver[13,39], and liver damage after hepatectomy is considered to be the result of apoptosis to some degree[38]. The degree of liver damage also depends on the extent of the liver resection volume[37,39]. The STAT3 and AKT signaling pathways not only promote liver regeneration but also inhibit apoptosis[38]. In this study, STAT3 and AKT expression was significantly suppressed, and the number of TUNEL-positive cells was higher in the NASH PH groups than in the control. These results suggest that the difference in the expression of regenerative signaling proteins affected the degree of apoptosis. Resection of a large volume of the liver also enhanced necrosis[38]. In this study, microarray analysis revealed *GADD45A* upregulation in the NASH 70% PH group. *GADD45A* promotes apoptosis and cell cycle arrest[13]. The differences in the survival rate between 30% or 70% PH in the NASH groups are inversely proportional to the incidence of Ki-67-positive cells, apoptosis, and necrosis. *GADD45A* upregulation correlates with the differences between the NASH groups and the low survival rate in small residual NASH liver after 70% PH.

In conclusion, residual NASH liver dysfunction after hepatectomy is attributed to a reduction in liver regeneration and cell proliferation. These findings suggest that the resection volume is a more limiting factor in patients with NASH than in those with a normal liver. Regarding liver surgery, the risk of complications for patients diagnosed with NASH by liver biopsy should be determined before hepatectomy. Further studies are needed to clarify therapeutic agents for NASH using our novel NASH model.

ARTICLE HIGHLIGHTS

Research background
The population of patients with nonalcoholic steatohepatitis (NASH) and NASH-related hepatocellular carcinoma (HCC) has been increasing. However,

few animal models fully reflect both the histopathology and pathophysiology of NASH in humans, therefore, the metabolism of the residual liver after a hepatectomy with NASH has not been clarified. We succeeded to establish a novel experimental NASH model that had same characteristics of histopathology and pathophysiology of NASH in humans.

Research motivation
In NASH, continuous inflammation contributes to HCC. The cause of HCC is frequently infection with hepatitis B virus and hepatitis C virus (HCV). New antiviral medications for hepatitis are currently being used in clinics; therefore, the number of patients with virus-related HCC is expected to decrease in the future. By contrast, the number of patients with NASH-related HCC has been increasing recently, and this trend is expected to continue because no effective treatments are available

Research objectives
The aim of this study was to investigate whether a difference in liver resection volume in a novel NASH model influences surgical outcomes.

Research methods
To establishment of a NASH model, mice were fed a high-fat diet for 4 wk, administered CCl_4 for the last 2 wk and administered T0901317 for the last 5 d. These mice were divided into two groups: A 30% partial hepatectomy (PH) of NASH liver group and a 70% PH of NASH liver group (control). Evaluate the survival rate, regeneration, apoptosis, necrosis and DNA expression level after PH.

Research results
In the 70% PH of NASH group, the survival rate was significantly decreased compared with that in the control and 30% PH of NASH groups (*P* < 0.01). 10 of 32 mice in the NASH 70% PH group died within 48 h after PH. serum aspartate aminotransferase (AST) levels and total bilirubin (T-Bil) in the NASH 70% PH group were significantly higher than the levels in the other two groups (AST: *P* < 0.05, T-Bil: *P* < 0.01). In both PH of NASH groups, signaling proteins involved in regeneration were expressed at lower levels than those in the control group (*P* < 0.01). The 70% PH of NASH group also exhibited a lower number of Ki-67-positive cells and higher rates of apoptosis and necrosis than the NASH 30% PH group (*P* < 0.01). In addition, DNA microarray assays showed differences in gene expression associated with cell cycle arrest and apoptosis.

Research conclusions
The residual NASH liver dysfunction after hepatectomy is attributed to a reduction in liver regeneration and cell proliferation. A larger residual volume is required to maintain liver functions in mice with NASH.

Research perspectives
This study suggests that the resection volume is a more limiting factor in patients with NASH than in those with a normal liver. Regarding liver surgery, the risk of complications for patients diagnosed with NASH by liver biopsy

should be determined before hepatectomy. Further studies are needed to clarify therapeutic agents for NASH using our novel NASH model.

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