Laboratories, Hercules, CA, USA), to normalize the samples by protein concentration.

Western blotting

Frozen liver tissue preparations were homogenized with sodium dodecyl sulfate (SDS) sample buffer, clarified by centrifugation, and boiled. The total protein concentration in each tissue sample was quantified by the Bradford method, and 10 μg of total protein per sample were loaded onto 8% SDS-polyacrylamide gels for electrophoresis (PAGE). The separated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories). The membranes were incubated with primary antibody solution consisting of 5 g/L polyclonal antiserum with specificity for mouse CD68 (AbD Serotec, Oxford, UK), and mouse 4-hydroxy-2-noneral (4-HNE) (Japan Institute for the Control of Aging, Shizuoka, Japan). Immunoreactive bands were detected by enhanced chemiluminescence (Amersham Life Science, Buckinghamshire, UK) and quantified using National Institutes of Health imaging software.

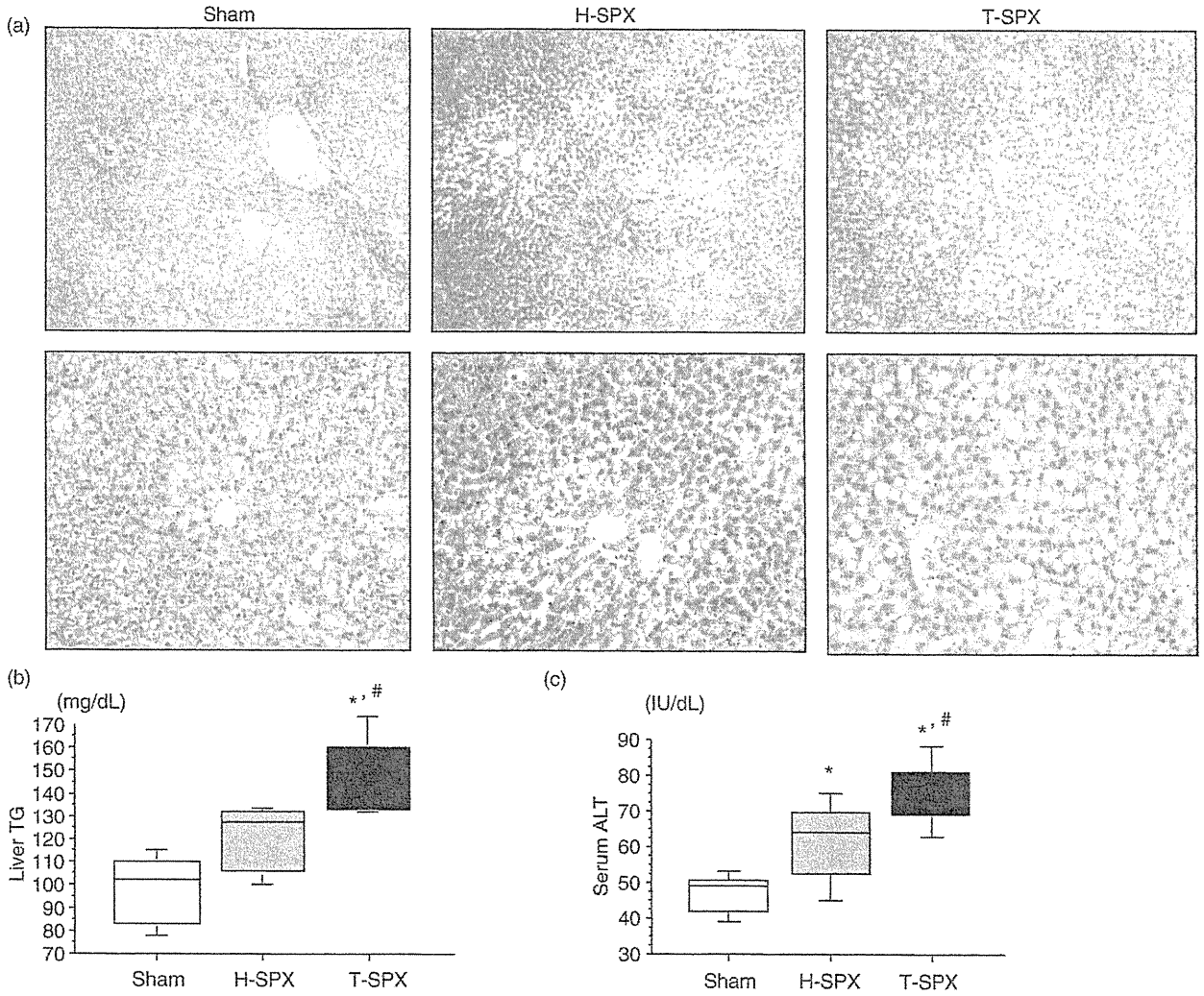

Figure 2 Intrahepatic lipid accumulation and liver injury in high-fat diet-induced obese rats accelerated after the hemisplenectomy (H-SPX) and total splenectomy (T-SPX). Liver sections stained with hematoxylin and eosin (a) at lower (first row: × 100) and higher magnification (second row: × 200) from rats at 12 weeks after splenic surgery. Total liver triglyceride (TG) levels (b) and serum alanine aminotransferase (ALT) levels (c) were measured at 12 weeks after splenic surgery. Data are shown as means ± standard error of the mean (SEM) ($n = 5$ rats/group). $^{*}P < 0.05$ *vs.* Sham, $^{\#}P < 0.05$ *vs.* H-SPX.

Statistical analysis

All data are expressed as means ± standard error (SE). Analysis of variance (ANOVA) and a post hoc Bonferroni test were used to assess multiple comparison differences. The correlations between the markers are assessed by Fisher's *r* method (StatView 4.0; SAS, Cary, NC, USA). A Mann–Whitney *U*-test was used when appropriate.

RESULTS

Effect of SPX on serum TG, FFA and TC levels

WE INVESTIGATED THE effect of SPX on serum levels of TG (Fig. 1a), FFA (Fig. 1b) and TC (Fig. 1c). Serum TG and FFA levels in the SPX groups, including the H-SPX and T-SPX groups, were higher than those of sham controls. In addition, these increases in SPX groups suggested a correlation with splenic remnant volume, which demonstrated that those levels tend to be high when splenic remnant volume is low. There were significant differences between T-SPX and other groups ($P < 0.05$ each). In contrast, serum levels of TC tended to decrease with splenic remnant volume, but this difference was not significant.

Effect of SPX on hepatic histology, liver TG content, and serum ALT level

Liver tissue histology (Fig. 2a), as revealed by H&E staining, and hepatic lipid accumulation, as assessed by liver TG content (Fig. 2b), were evaluated in rats 12 weeks after splenic surgery. In splenectomized rats,

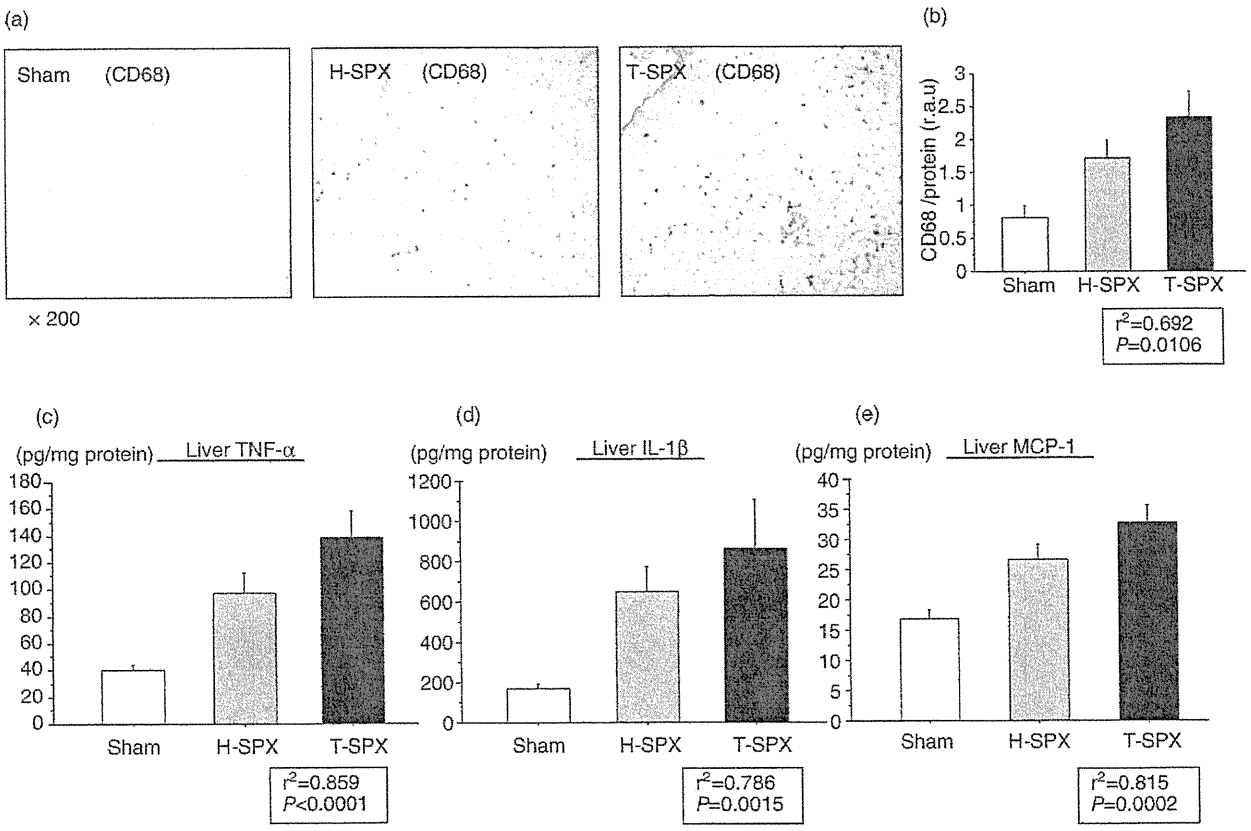

Figure 3 Kupffer cell numbers and hepatic pro-inflammatory cytokine production in high-fat diet-induced obese rats correlated with the residual spleen volume after surgery. Representative immunohistochemical staining using antibody against the specific macrophage marker CD68 in livers from rats at 12 weeks after splenic surgery (×200) (a). Hepatic protein expression was measured by western blotting (b). The protein levels of the hepatic pro-inflammatory cytokines, tumor necrosis factor (TNF)-α (c), interleukin (IL)-1β (d), and monocyte chemotactic protein-1 (MCP-1) (e), were measured at 12 weeks after splenic surgery. Data are shown as means ± standard error of the mean (SEM) ($n = 5$ rats/group).

liver TG content increased as the splenic remnant volume decreased, and a significant increase was found in T-SPX rats compared with the other groups ($P < 0.05$ for each). Liver histological analysis also showed an increased hepatic lipid accumulation after SPX treatment. Liver sections from H-SPX rats showed a marked worsening of steatosis compared with those of sham rats, and further lipid accumulation was observed in T-SPX rats.

Next, we investigated the involvement of SPX in hepatocyte injury by measuring serum ALT levels. The serum ALT levels in H-SPX and T-SPX rats were significantly higher than those of sham controls, and showed a tendency to increase as splenic remnant volume decreased ($P < 0.05$ for each) (Fig. 2c).

Effect of SPX on the KC population and pro-inflammatory cytokine levels in the liver

To examine the effect of SPX on liver KCs, we determined the accumulation of KCs (Fig. 3a) and CD68 protein level (Fig. 3b) in the rat livers. Immunohistochemistry with an anti-CD68 antibody revealed an increase in the number of CD68-positive cells, a marker of activated KCs, in H-SPX and T-SPX rats compared with sham controls. Hepatic expression levels of CD68 protein in H-SPX and T-SPX rats were higher than in sham controls; these increases correlated with a lower residual spleen volume ($r^2 = 0.692$, $P = 0.0106$).

Next, levels of hepatic pro-inflammatory cytokines such as TNF-α (Fig. 3c), IL1-β (Fig. 3d) and MCP-1 (Fig. 3e) were determined in rats 12 weeks after splenic surgery. All pro-inflammatory cytokine levels showed a similar profile to the number of KCs among the three groups. Intrahepatic TNF-α, IL1-β and MCP-1 levels were significantly correlated with resected spleen volume ($r^2 = 0.859$, $P < 0.0001$; $r^2 = 0.786$, $P = 0.0015$; $r^2 = 0.815$, $P = 0.0002$, respectively).

Effect of SPX on liver oxidative stress markers

To evaluate hepatic oxidative stress after SPX, we measured expression of hepatic 4-HNE, a marker of lipid peroxidation, by western blot (Fig. 4). 4-HNE protein levels in H-SPX and T-SPX rats were higher than those of sham controls; this correlated significantly with residual spleen volume ($r^2 = 0.782$, $P = 0.0016$), as well as liver tissue levels of inflammatory markers, such as TNF-α, and expression of KCs.

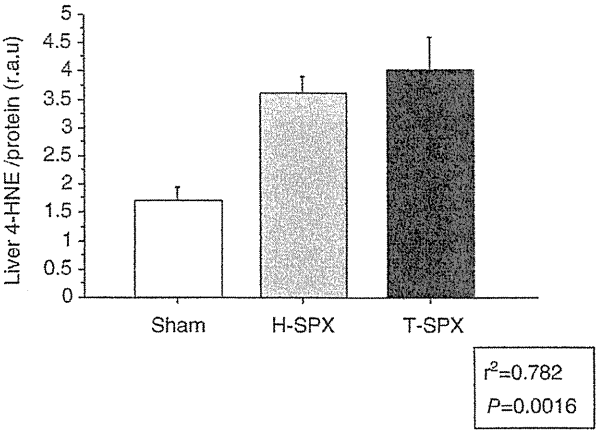

Figure 4 Hepatic oxidative stress increased based on residual spleen volume after splenic surgery in high-fat diet-induced obese rats. Hepatic protein expression of 4-hydroxy-2-noneral (4-HNE), a marker of lipid peroxidation, was assessed by western blotting at 12 weeks after splenic surgery.

Changes in cytokine levels in the residual spleen

In addition, we investigated changes in pro- and anti-inflammatory cytokine levels in the residual spleens of sham and H-SPX rats. The levels of TNF-α (Fig. 5a) and IL-1β (Fig. 5b) were significantly higher in the spleens of H-SPX rats compared with sham controls ($P < 0.05$ for each). In contrast, IL-10 level (Fig. 5c) was decreased by H-SPX. The pro-/anti-inflammatory ratio, assessed by TNF-α/ IL-10 (Fig. 5d) and/or IL-1β/ IL-10 (Fig. 5e), was significantly higher in H-SPX than Sham rats ($P < 0.05$ each).

Effect of SPX on hepatic steatosis and inflammation at 24 weeks after SPX

Based on the above findings, we next examined the livers of the rats at 24W, considered to be a further chronic period after SPX, and evaluate whether these changes depended on time after SPX by comparison with the data at 12W. Liver tissue histology, revealed by H&E staining, showed a marked worsening of steatosis in 24W rats compared with that of 12W rats (Fig. 6a). Intrahepatic fat accumulation assessed by liver TG content of 24W SPX rats was significantly higher than that of 12W SPX rats ($P < 0.05$) (Fig. 6b), indicating that the hepatic steatosis caused by SPX worsened with time after spleen removal. In contrast, although levels of hepatic pro-inflammatory cytokines, such as TNF-α

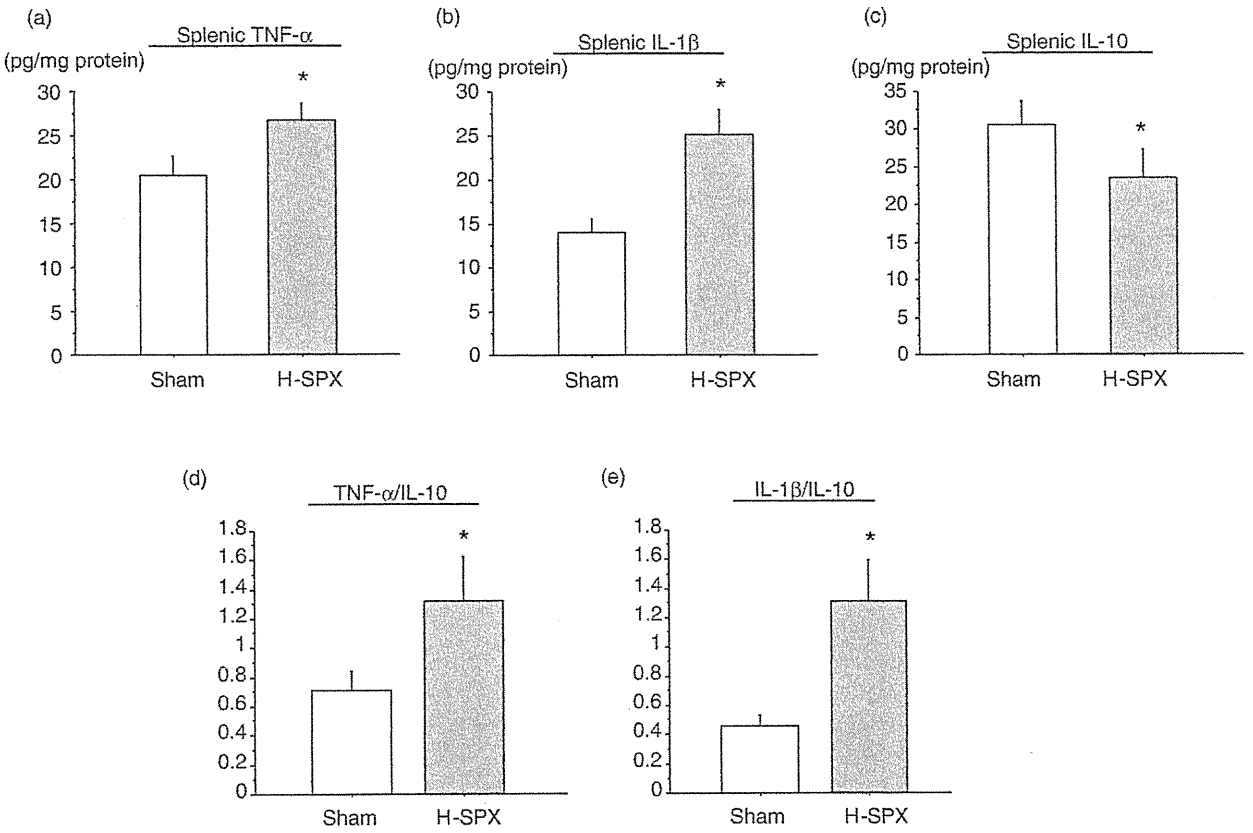

Figure 5 Partial resection of the spleen affected the cytokine level and the balance of pro-/anti-inflammatory cytokines in residual splenic tissue. The protein levels of the splenic pro- and anti-inflammatory cytokines, tumor necrosis factor (TNF)-α (a), interleukin (IL)-1β (b), and IL-10 (c), were measured at 12 weeks after splenic surgery. The splenic pro-/anti-inflammatory ratio was calculated by TNF-α/ IL-10 (d) and IL-1β/ IL-10 (e) at 12 weeks after splenic surgery. Data are shown as means ± standard error of the mean (SEM) ($n = 5$ rats/group). *$P < 0.05$ *vs.* Sham.

(Fig. 6c) and IL-1β (Fig. 6d), in 24W SPX rats were higher than those of SPX rats at 12W, this difference was not significant.

DISCUSSION

WE CONDUCTED A correlational study between diminished splenic function and the development and progression of steatohepatitis in diet-induced obese rats. To investigate this correlation, we prepared a rat model with diminished splenic function by resecting half of the total spleen volume in accordance with the previous demonstration that the phagocytic function of the spleen after partial and total SPX relies highly on the volume of the residual spleen, and its phagocytic function is impaired, as the residual spleen volume is smaller.[19]

The association of a partial or total SPX on systemic lipid metabolism has been studied in various animal models.[13–15] However, the results regarding lipid metabolism after SPX differ among previous reports because of differences in gender, diet, and the period for extracting blood samples from experimental animals to examine serum lipid parameters. SPX worsens systemic dyslipidemia, including increases in serum total cholesterol, TG,[10] low-density lipoprotein cholesterol,[13,15] and decrease in high-density lipoprotein cholesterol[14,15] in rats fed a normal[15] or HF diet.[13,14] Additionally, partially splenectomized animals tend to develop similar changes in lipid metabolism[13,15] but not as clearly as the changes after a total SPX. In this study, our lipid metabolism results after a T-SPX and H-SPX were consistent with these previous reports and showed a significant correlation between the large resected volume of

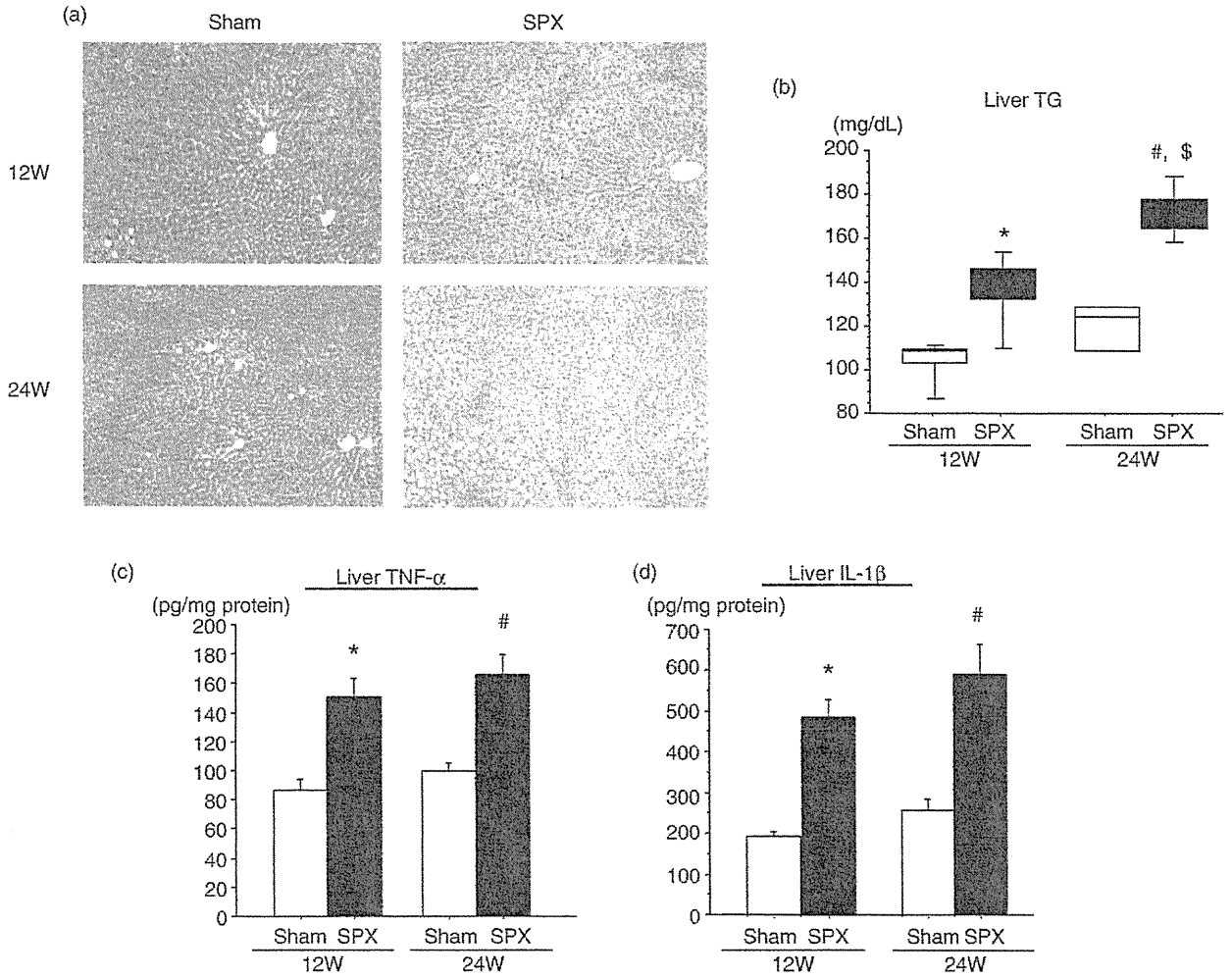

Figure 6 Intrahepatic fat accumulation accelerated with time after spleen removal. Liver sections stained with hematoxylin and eosin (a) at lower magnification (×100) from rats at 12 (first row) and 24 (second row) weeks after splenic surgery. Total liver triglyceride (TG) (b) and tumor necrosis factor (TNF)-α (c), and interleukin (IL)-1β (d) levels were measured at 12 and 24 weeks after splenic surgery and compared. Data are shown as means ± standard error of the mean (SEM) ($n = 5$ rats/group). $^{*}P < 0.05$ *vs.* 12W Sham, $^{\#}P < 0.05$ *vs.* 24W Sham, $^{\$}P < 0.05$ *vs.* 12W SPX.

the spleen and the increased levels of serum TG and free fatty acids.

The effect of SPX on steatohepatitis was suggested in a previous study. Oishi *et al.*[18] demonstrated the significance of SPX in fibrotic non-alcoholic steatohepatitis (NASH) model rats fed a choline-deficient L-amino acid-defined diet, indicating that although hepatic steatosis worsens after SPX, liver fibrosis and the development of neoplastic lesions decrease. These findings were shown in the liver at 4 weeks after SPX, and were considered the early phase. Our results at 12 weeks after SPX are in agreement with this report, in that SPX increased fat accumulation in the liver. Here, we focused on the relationship between residual spleen volume after SPX and the degree not only of fat accumulation but also inflammatory changes in the liver. Notably, we found that the degree of both steatosis and inflammation correlated significantly with less volume of the residual spleen.

Additionally, we measured α-smooth muscle actin (α-SMA) protein expression in the liver to evaluate liver fibrosis in the present model. Although hepatic α-SMA expression tended to be high in both H-SPX and T-SPX rats compared with that in sham controls, no significant

difference was observed among the three groups, and no correlation was found between hepatic α-SMA levels and residual spleen volume (data not shown). Based on these data, we speculated that the impaired splenic function induced by depleting splenic tissue, at least in part, may be a possible factor leading to the development of steatohepatitis except the fibrotic changes.

Another interesting finding of this study is the time course of liver changes after SPX, since further lipid accumulation was evident in the liver at 24W after SPX, as compared with those of 12W, whereas hepatic inflammation was slightly worse, although this was not significant. These findings indicate that acceleration of hepatic steatosis, not inflammatory changes, caused by spleen removal clearly depends on time after SPX.

Previous studies have reported an important relationship between the pathogenesis of steatohepatitis and hepatic natural killer T (NKT) cells,[20–22] indicating that a reduction in hepatic NKT cell numbers occurs in patients with hepatic steatosis. Furthermore, this depletion of hepatic NKT cells leads to hepatic enrichment with NKT cells during fibrotic steatohepatitis, which contributes to the progression of liver fibrosis in patients with steatohepatitis. In contrast, another report demonstrated that SPX does not change the hepatic accumulation and proportion of mouse NKT cells experimentally.[8] Based on these observations, we speculate that no remarkable changes in hepatic NKT cells occurred between obese SPX rats and sham controls in this study. However, there are insufficient data to confirm the influence of SPX on hepatic NKT cells, so further investigations are needed to clarify this relationship directly.

To identify the inducer that caused the liver changes after SPX, we next focused on the condition of the residual spleen after splenic surgery. Our Supplemental Data also indicate that the residual spleen after H-SPX shows remarkable changes in the immune cell population, including splenic macrophages and B cells. That is, H-SPX increased the splenic B cell population in the splenic remnant, whereas no changes occurred in the number of splenic macrophages compared with those in the sham controls (data not shown). These data suggest that an increase in the population of residual splenic B cells may develop to compensate for the splenic B cell depletion caused by the partial reduction in splenic tissue. Also, the total number of macrophages may decrease in the residual spleen (whole tissue), which leads to impaired phagocytic function of the residual spleen after a partial SPX.[19] We speculate that this reduction in splenic macrophage number and subsequent impairment of splenic immune function lead to a compensatory increase and activation of liver KCs, which results in the development of hepatic inflammation. Furthermore, we provide data indicating that increased levels of splenic pro-inflammatory cytokines (such as TNF-α and IL-1β) and a decrease in IL-10, an anti-inflammatory cytokine, are also induced in the remnant spleen compared to those in sham controls. A previous study demonstrated that activated liver KCs induce a downregulation of splenic IL-10 production, indicating that KCs have the potential to regulate splenic cytokine production.[23] Furthermore, IL-10 downregulates pro-inflammatory cytokine production, such as TNF-α and IL-1β. Taken together, activation of liver KCs induced by the depletion in splenic macrophages after H-SPX may reduce splenic IL-10 concentration, resulting in a cytokine imbalance in the residual spleen.

In conclusion, fat accumulation and inflammatory changes in the liver were accelerated in splenectomized rats, and the degree of these changes correlated with splenic remnant volume after partial and total splenic resection, which represents a state of decreased splenic function. Furthermore, hepatic steatosis caused by spleen removal progressed according to the time since surgery. Although further investigations are needed to clarify the relationship between the pathogenesis of steatohepatitis and splenic function, we hope that our findings will provide new insight into preserving the spleen and thus prevent the progression of steatohepatitis in patients with obesity.

ACKNOWLEDGEMENTS

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膵腫瘍との鑑別を要した脾動脈瘤の1例

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成田竜一　向井　亮　石田哲也
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—症例報告—

膵腫瘍との鑑別を要した脾動脈瘤の1例

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要旨：症例は46歳，男性．検診の腹部超音波検査にて膵体部背側に約30mm大の低エコー腫瘤を指摘され，紹介となった．造影CTやMRI，EUSより膵リンパ上皮嚢胞を考えたが，増大傾向にあることや悪性の可能性が否定できずに，膵体尾部・脾臓合併切除術を施行した．最終診断は，脾動脈の解離性動脈瘤の偽腔部分が破綻して生じた仮性動脈瘤であった．

索引用語：脾動脈瘤，膵腫瘍，鑑別診断

はじめに

CTをはじめとする画像診断機器の技術向上にともない，偶然に発見される脾動脈瘤症例が増加している．しかしながら，典型的な脾動脈瘤の画像所見を示さず，診断に苦慮する症例も散見される．今回われわれは，脾動脈に発症した解離性動脈瘤の偽腔部分が破綻し，仮性動脈瘤を生じた1例を経験した．過去に同様の病態を病理組織学的に証明した報告例はなく，画像診断では，膵リンパ上皮嚢胞などの膵腫瘍との鑑別が困難であり，臨床的に示唆に富む症例と思われ，文献的報告を加えて報告することとした．

I　症　例

患者：46歳，男性．

主訴：膵腫瘤の精査．

家族歴：特記すべき事項なし．

既往歴：慢性B型肝炎．

現病歴：平成20年10月に検診で膵体部背側に約30mm大の低エコー腫瘤を指摘された．腹部造影CT検査にて膵嚢胞性腫瘍と診断され，転勤にともない，当院に紹介となった．

入院時現症：身長170cm，体重64.9kg．眼球結膜に黄疸を認めず，眼瞼結膜に貧血を認めず，腹部は平坦，軟，肝，脾を触知せず，表在リンパ節を触知しなかった．

入院時検査所見：血液検査では，B型肝炎のウイルスマーカーが陽性で，AST，ALT，アミラーゼの軽度高値を認めた．また，腫瘍マーカーでは，SPAN-1が32.3U/mlと高値を示した（Table 1）．

腹部超音波所見：病変部は，膵体部背側で脾動脈に接するように存在していた．大きさは30mm弱で，類円形の低エコー腫瘤として描出されていた．病変内部は，全体的にやや高輝度な腫瘤として描出され，単純な嚢胞性病変とは異なる印象を受けた．また，カラードップラー法では，病変部に血流信号は認めなかった（Figure 1）．

1）大分赤十字病院消化器科
2）大分赤十字病院外科
3）大分赤十字病院放射線科
4）大分赤十字病院病理診断科
5）大分大学第1内科
6）医療法人春水会山鹿中央病院消化器科
Corresponding author：永松　秀康（hnagamatsu1737@yahoo.co.jp）

Table 1. 入院時血液検査所見

Hematology		Chemistry	
WBC	6100 /μl	T-bil	0.4 mg/dl
RBC	495×10⁴ /μl	AST	49 IU/l
Hb	15.2 g/dl	ALT	72 IU/l
Ht	45.0 %	ALP	282 IU/l
Plt	14.5 /μl	LDH	177 IU/l
		γ-GTP	47 IU/l
Coagulation		AMY	138 IU/l
PT	90.1 %	BUN	12 mg/dl
		Cr	0.9 mg/dl
Serology		Glu	115 mg/dl
CRP	0.08 mg/dl		
		Tumor markers	
Virus markers		CEA	1.9 ng/ml
HBs-Ag	250.0 IU/ml	CA19-9	19 U/ml
HBs-Ab	<10.0 mIU/ml	DUPAN2	54 U/ml
Hbe-Ag	75.7 S/CO	SPAN-1	32.3 U/ml
Hbe-Ab	0.0 %	Elastase-1	332 ng/dl
Hbc-Ab	11.4 S/CO		
HBV-DNA	7.9 log copy		

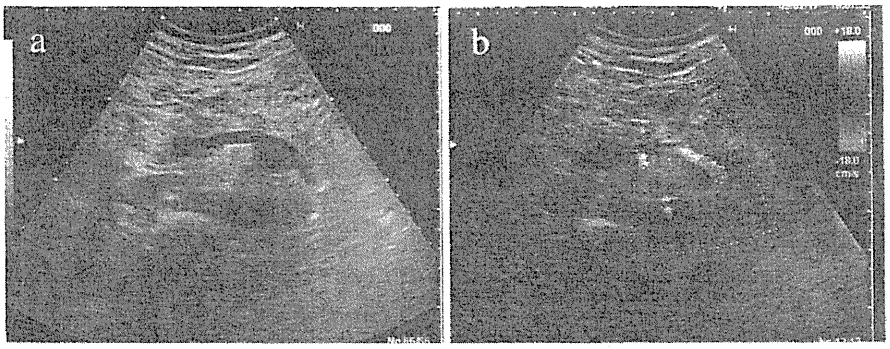

Figure 1. 前医で施行した腹部超音波検査 a）膵体部背側に類円形で内部は全体的にやや高輝度な成分を含む低エコー腫瘤を認めた．b）病変部には，カラードップラーでの血流信号は認めなかった．

腹部CT所見：腹部単純CTでは，腹腔動脈が瘤状に拡張し，脾動脈も高度な口径不整と壁の石灰化を認めた．病変部は径30×20mm大で，前医CTと比較し，軽度増大していた．また，CT値は30～40HUと高値を示した．造影CT（動脈相）水平断像やcurved MPR像では，病変部に造影効果は見られなかった．また，病変部に沿うように非連続性で造影効果の強い領域を認めた．3D CTA像では，病変部は描出されず，拡張した脾動脈が認識された（Figure 2）．以上より，膵炎後の仮性嚢胞や膵腫瘍，後腹膜腫瘍などが鑑別に挙げられた．膵炎後の仮性嚢胞として考えると，病変が膵背側にのみ存在する点や脾静脈の変化に乏しい点が合致しなかった．また，後腹膜腫瘍としては，リンパ管腫なども鑑別に挙げたが，内部の濃度が高く，不均一な点が異なる印象を受

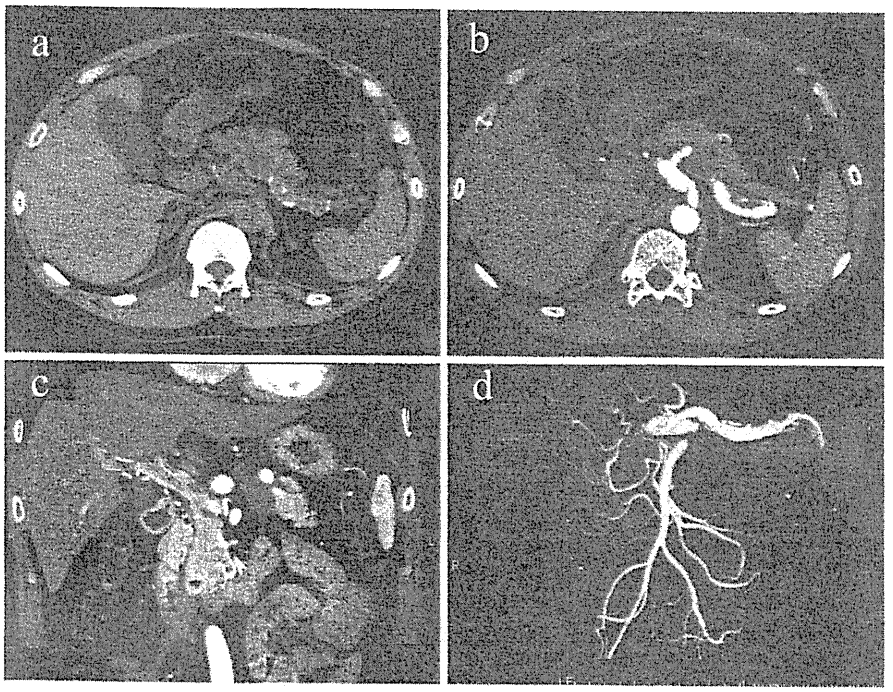

Figure 2. 腹部CT所見 a）単純CTでは，腹腔動脈が瘤状に拡張し，脾動脈も高度な口径不整と壁の石灰化を認めた．病変部は径30×20mm大で，CT値が30～40HUと高値を示した．b）造影CT（動脈相）水平断像では，病変部に造影効果は見られなかった．c）curved MPR像でも脾動脈と病変部の連続性ははっきりしなかった．d）3D CTA像では，病変部は描出されなかった．

けた．膵腫瘍として考えると，solid-pseudopapillary tumorでは，造影効果に乏しい点が合わず，膵リンパ上皮嚢胞を第一に考えた．

腹部MRI所見：病変部の信号はT1WIにて層状の高信号を示し，T2WIでは，高信号と低信号が混在した所見を呈していた（Figure 3）．CT同様に造影効果は判然とはせず，嚢胞性病変を考えたが，T1WIの信号から，内部は角化物質や粘稠成分，出血などが混在する病変と考えた．以上より，CTと同様に膵リンパ上皮嚢胞を第一に考えた．

超音波内視鏡検査所見：病変内部は均一な低エコーではなく，全体的にやや高輝度な成分を混在していたが，明らかな充実成分は指摘できなかった（Figure 4）．

以上より，膵腫瘍であれば，膵リンパ上皮嚢胞を第一に考えたが，病変部が約1年前の前医のCTと比較し，増大傾向にあることや，他の悪性疾患の可能性も否定できないために，手術を行った．

手術所見：腹腔内に腹水，腹膜播種，明らかな肝転移は認めなかった．術中エコーにて病変部は膵体部背側に2cm程度の低エコー腫瘤として認識された．近傍の膵は柔らかく，病変部と膵との連続性ははっきりしなかった．膵腫瘍が否定できず，膵体尾部・脾臓合併切除術を施行した．

病理所見：肉眼所見では，病変部は拡張した脾動脈に接するように存在する嚢胞状腫瘍で，一部で脾動脈との交通部分が確認された．腫瘍の周囲は線維性構造物で囲まれ，膵との境界は比較的明瞭であった（Figure 5）．組織学的検索では，EVG（Elastica van Gieson）染色にて，拡張した脾動脈周囲に外弾性板が認められ，壁の一部に内腔が閉塞し，内弾性板に囲まれた真腔が認められたことにより，拡張部分全体が解離性動脈瘤の偽腔であると考えられた．そして，問題の病変部は，そ

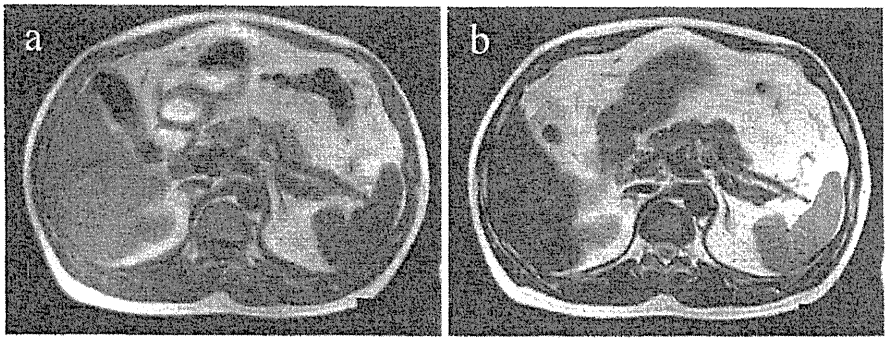

Figure 3. 腹部MRI所見 a)病変部の信号はT1WIにて層状の高信号を示した. b)T2WIでは, 高信号と低信号が混在した所見を呈していた.

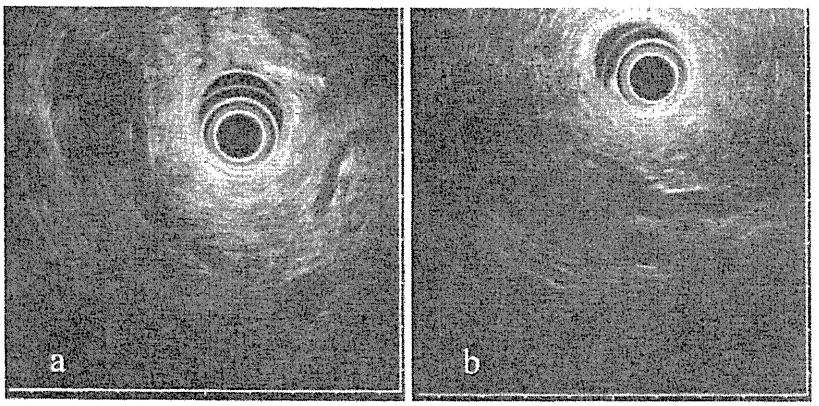

Figure 4. 超音波内視鏡所見 a), b) 病変は脾動脈に接するように存在していた. 病変内部は均一な低エコーではなく, やや高輝度の成分が混在していたが, 明らかな充実成分は指摘できなかった.

の一部が破裂した仮性動脈瘤と診断された (Figure 6). 脾動脈には, 粥状硬化の所見をともなっており, 動脈硬化性動脈瘤が考えられた. 病変部に悪性所見はなく, 脾臓や付属リンパ節に特異的炎症や腫瘍性変化は認めなかった.

II 考 察

内臓動脈瘤の中で, 脾動脈は, 腹部大動脈, 腸骨動脈に続き3番目に発生頻度が高いとされている[1]. 実際の発生頻度は不明だが, おおよそ0.2～10.4%とする報告がある[2][3]. Beaussierが1770年に剖検例で脾動脈瘤を報告したのが最初である[4]. 今までは, 比較的まれとされてきたが, 高分解度の画像機器の普及にともない, 偶然に発見される症例が増加してきている. 脾動脈瘤は, 女性発症が男性の4倍高いといわれる一方で, 動脈瘤破裂は男性がおよそ3倍高いとされている[5]. また, 病因については, 高血圧, 門脈圧亢進症, 肝硬変, 肝移植, 妊娠との関連が報告されている[5]～[7]. 仮性動脈瘤は, 膵炎, 外傷, 医原性, 手術, 消化性潰瘍などが原因といわれ[8], 形態的には, 血管の破綻をきたし周囲組織による線維性被膜にて血管外循環腔が形成されたものとされている. 自験例では, 自覚症状もなく, 以上のような仮性動脈瘤の原因となりうる既往歴はなかった.

脾動脈瘤の診断には, 腹部超音波, カラードップラー法, 造影CT, 腹部血管造影が有用とされ

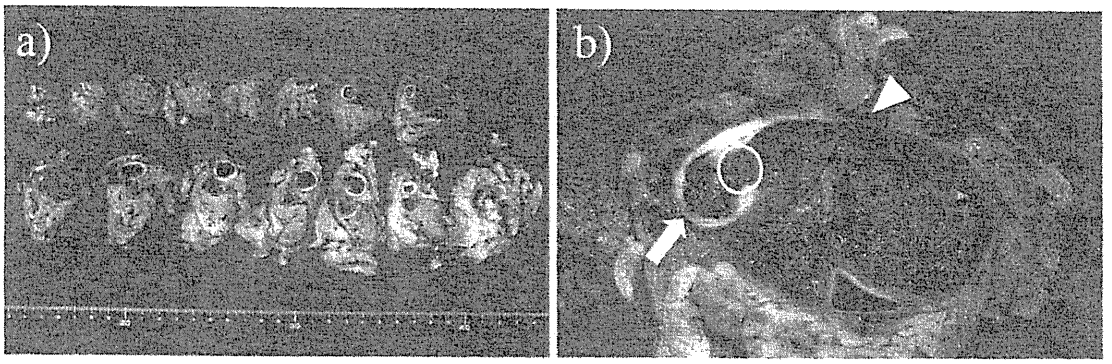

Figure 5. 切除標本 a）全割面像．b）解離性動脈瘤の偽腔（矢印）が破裂（丸印）し，仮性動脈瘤（矢頭）を形成していた．

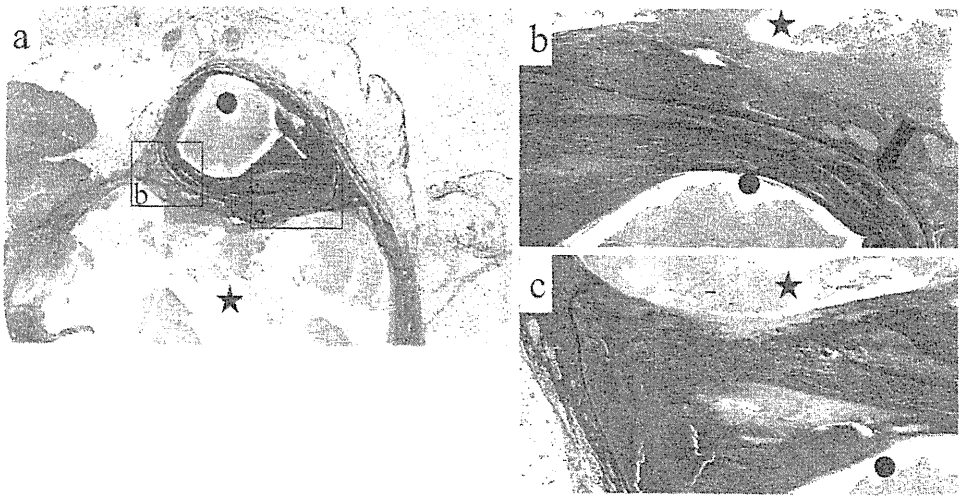

Figure 6. 病理組織標本 a）EVG（Elastica van Gieson）染色（弱拡大図）（×6）．丸印は解離性動脈瘤の偽腔で，星印は仮性動脈瘤を示す．b）aの拡大写真（×20）．解離性動脈瘤の真腔（矢印）は，閉塞していた．c）aの拡大写真（×20）．解離性動脈瘤の破裂部分が確認された．

ている．腹部CTでは，脾動脈に接して，腹部大動脈と同程度に造影されるが，自験例では，造影効果を認めなかった．文献的にも，自験例と同様に，動脈瘤内が血栓で充満されると，CTでの脾動脈瘤診断が困難で，膵腫瘍との鑑別が困難になるとする報告がある[9]．また，MDCTでの再構成画像が，脾動脈もしくは膵組織由来かの鑑別に役立つとする報告があるが[10]，今回のcurved MPR像やCTAでは，脾動脈との連続性は指摘できなかった．また，昨今EUS-FNAが膵腫瘍の診断に利用される機会が増加しているが，自験例のような脾動脈瘤の存在を考慮しないと，思わぬ致命的な結果を招く恐れがある．文献的にも，EUSで膵嚢胞性病変と思われた413病変中4例がCTで動脈瘤と診断されている[11]．

自験例の問題点は，術前に脾動脈瘤の診断が困難であったことが挙げられる．自験例以外の2症例で術前診断が膵尾部嚢胞性腫瘍や胃GISTとされたものがあるが，共通している病理学的特徴は，脾動脈瘤内部が血栓で充満していた点であ

Table 2. 脾動脈瘤に対し，膵切除を施行した12症例（医学中央雑誌1983年から2010年までで自験例を含む）

症例	年齢	性別	脾動脈瘤径(mm)	症状	既往歴	術前診断	術式	報告者	報告年
1	50歳代	女性	22	検診	左背部痛	脾動脈瘤	膵尾部・脾臓合併切除	楠部ら[12]	2009
2	59	男性	50	体重減少	高血圧	胃GIST	膵体尾部・脾臓合併切除	富田ら[13]	2007
3	63	男性	不詳	黒色便・発熱	糖尿病	脾動脈瘤破裂の胃穿破	膵体尾部・脾臓合併切除および胃部分切除	足立ら[14]	2006
4	70	男性	60	吐血	慢性膵炎	脾動脈瘤破裂の膵内穿破	膵体尾部・脾臓合併切除	橋田ら[15]	2006
5	50	女性	40	労作時息切れ	Rendu-Osler-Weber＋症候群	脾動脈瘤	腹腔鏡補助下膵体尾部・脾臓合併切除	中嶋ら[16]	2005
6	68	男性	65	特になし	膀胱癌	脾動脈瘤	膵体尾部・脾臓合併切除	藤田ら[17]	2004
7	54	男性	不詳	不詳	慢性膵炎	脾動脈瘤	膵体尾部・脾臓合併切除	加藤ら[18]	2004
8	76	女性	110	左上腹部不快感	特になし	脾動脈瘤	膵体尾部・脾臓合併切除	堀ら[19]	2003
9	43	男性	不詳	左季肋部痛・背部痛	慢性膵炎	脾動脈瘤破裂	膵体尾部・脾臓合併切除および横行結腸間膜，胃大網切除	沼尻ら[20]	2001
10	57	女性	20	特になし	特になし	膵尾部嚢胞性腫瘍	膵尾部・脾臓合併切除	三松ら[9]	1998
11	48	男性	22	左上腹部痛	不詳	脾動脈瘤破裂	膵体尾部・脾臓合併切除	梅原ら[21]	1995
12	46	男性	30	特になし	慢性B型肝炎	膵腫瘍	膵体尾部・脾臓合併切除	自験例	2011

る．それにより，脾動脈との血流が遮断され，動脈瘤に特徴的な画像所見を呈さなかったものと推察される．さらに，自験例では，脾動脈のほぼ全長にわたり動脈解離が認められ，さらに興味深いことに，この解離性動脈瘤の偽腔の一部が破裂し，仮性動脈瘤を形成していた．今回の病変は，この仮性動脈瘤部分であることが判明した．また，造影CTで非連続性に造影効果が強く見られた領域は，解離性動脈瘤の偽腔部分であることも指摘された．以上のような，脾動脈の解離性動脈瘤破綻による仮性動脈瘤の発生を提示した報告例はない．また，脾動脈瘤の造影効果の差異を病理組織学的に証明した報告例もなく，膵近傍の腫瘍性病変を検討するうえで示唆に富む症例と思われた．

治療に関しては，手術と塞栓術が中心となっている．1983年から2010年までの医学中央雑誌に掲載された報告例（会議録を除く）で，膵切除，脾動脈瘤をキーワードに検索したところ，11文献11症例の報告例が見られた[9][12]~[21]．膵切除を選択した理由の多くは，脾動脈瘤と膵臓の癒着が強固であったことであるが，自験例のように他疾患の可能性が否定できずに膵切除を余儀なくされた例も見られた（Table 2）．

自験例を含む12症例を分析すると，平均年齢は57.6歳（43~76歳）で，男女比は8：4と男性が2倍であった．また，12例中3例，25%が膵炎の合併を認めた．さらに興味深いことに，脾動脈瘤破裂をきたした症例が5例あり，全例男性であった．また，1例は，脾動脈瘤が膵に穿破し，膵管を通じ消化管出血をきたした，いわゆる，hemosuccus pancreaticusの病態を呈していた．

おわりに

以上，自験例のような画像所見を呈する脾動脈

瘤はまれであり，病理学的な検討や文献的考察も加えて報告することとした．

本論文内容に関連する著者の利益相反
：なし

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A case of splenic artery aneurysm simulating a pancreas tumor

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A 46-year-old man was admitted to our hospital for further evaluation of a hypoechogenic mass in the pancreatic body. He had no history of hypertension, pancreatitis, abdominal trauma, or portal hypertension. He had no abdominal symptoms. A contrast-enhanced CT scan demonstrated a hypodense, round shaped mass. EUS and MRI also showed it to be a pancreatic mass. Because of the tumor size of more than 30mm and the possibility of malignancy, distal pancreatectomy was performed. Microscopic findings showed the mass was the dissection of the proximal splenic artery. The true lumen of the dissecting aneurysm was occluded and the false lumen developed fusiform dilatation. Moreover, microscopic findings revealed the rupture of the false lumen complicated by pseudoaneurysm. We finally diagnosed the lesion simulating a pancreatic tumor as the pseudoaneurysm of the splenic artery.

Effect of Vitamin K2 on the Recurrence of Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is characterized by frequent recurrence, even after curative treatment. Vitamin K2, which has been reported to reduce HCC development, may be effective in preventing HCC recurrence. Patients who underwent curative ablation or resection of HCC were randomly assigned to receive placebo, 45 mg/day, or 90 mg/day vitamin K2 in double-blind fashion. HCC recurrence was surveyed every 12 weeks with dynamic computed tomography/magnetic resonance imaging, with HCC-specific tumor markers monitored every 4 weeks. The primary aim was to confirm the superiority of active drug to placebo concerning disease-free survival (DFS), and the secondary aim was to evaluate dose-response relationship. Disease occurrence and death from any cause were treated as events. Hazard ratios (HRs) for disease occurrence and death were calculated using a Cox proportional hazards model. Enrollment was commenced in March 2004. DFS was assessed in 548 patients, including 181 in the placebo group, 182 in the 45-mg/day group, and 185 in the 90-mg/day group. Disease occurrence or death was diagnosed in 58, 52, and 76 patients in the respective groups. The second interim analysis indicated that vitamin K2 did not prevent disease occurrence or death, with an HR of 1.150 (95% confidence interval: 0.843-1.570, one-sided; $P = 0.811$) between the placebo and combined active-drug groups, and the study was discontinued in March 2007. Conclusion: Efficacy of vitamin K2 in suppressing HCC recurrence was not confirmed in this double-blind, randomized, placebo-controlled study. (HEPATOLOGY 2011;54:532-540)

Hepatocellular carcinoma (HCC) is the third-leading cause of cancer death worldwide, claiming 600,000 victims each year. Because of advances in diagnostics and therapeutics, HCC can now be curatively treated, if detected at an early stage. Nevertheless, the long-term prognosis of HCC is not satisfactory, mainly because of its very frequent recurrence, which may occur after a long interval from initial "curative" treatment. Most cases of HCC develop in the liver with cirrhosis or advanced fibrosis.[1-4] Even if HCC nodules have been completely resected or ablated, the remaining liver retains the potential for *de novo* carcinogenesis.[5-7] In addition, precancerous lesions and microscopic metastasis may already exist in the remaining liver.

Adjuvant chemotherapy would be considered for other solid malignancies with high risk of recurrence. However, this is difficult in the case of HCC because few conventional chemotherapeutic agents are effective and hepatotoxicity can be of critical significance, as liver function is often already impaired. A randomized trial was performed with uracil-tegafur as postoperative

Abbreviations: AFP, alpha-fetoprotein; AFP-L3, alpha-fetoprotein lens culinaris agglutinin fraction-3; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer Staging System; CI, confidence interval; CT, computed tomography; DCP, des-gamma-prothrombin; DFS, disease-free survival; ECOG, Eastern Cooperative Oncology Group; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HRs, hazard ratios; IDMC, independent data monitoring committee; MRI, magnetic resonance imaging; RR, risk ratio.

adjuvant therapy, but did not improve recurrence-free survival, and overall survival appeared to be worsened.[8] Safety is clearly a prerequisite to the use of adjuvant therapy agents for HCC. Recently, a randomized trial with peretinoin, a retinoid, in patients with previously treated HCC was conducted. Although recurrence-free survival was higher with high-dose peretinoin than with placebo, there was no statistically significant difference in the predefined primary analysis.

In 2004, Habu et al.[9] reported that the incidence of development of HCC was reduced among cirrhotic women assigned to receive oral vitamin K2 (45 mg/day), originally for the prevention of osteoporosis, compared to controls (risk ratio [RR]: 0.13; 95% confidence interval [CI]: 0.02-0.99) with a limited number of subjects. Des-gamma-carboxy prothrombin (DCP), an abnormal prothrombin produced in vitamin K deficiency, is not only an HCC-specific tumor marker, but also a predictor of portal venous tumor invasion.[10] A number of findings *in vitro* have indicated that vitamin K may play a role in controlling cell growth, including inhibition of growth of HCC cells.[11-15] Vitamin K2 (menatetrenone) reportedly induced differentiation of human myeloid leukemia cells, as well as apoptosis in immature blast cells.[16-18] Vitamin K2 has been widely used for osteoporosis, and its long-term safety has been confirmed.[19-22] Thus, vitamin K2 would be an ideal adjuvant agent, if it could reduce HCC recurrence by preventing *de novo* carcinogenesis or suppressing tumor growth.

In fact, a few small-sized, controlled trials enrolling 45-61 patients have been performed to assess the effects of vitamin K2 on HCC recurrence. Mizuta et al.[23] reported that vitamin K2 reduced HCC recurrence with a multivariate-adjusted RR of 0.27 (95% CI: 0.12-0.60) and, possibly, improved survival. A preventive effect on HCC recurrence was also suggested by Kakizaki et al.,[24] who found an adjusted RR of 0.45 (95% CI: 0.10-2.05) for recurrence, although they failed to observe survival benefits. Another study failed to detect a reduction of HCC recurrence.[25] Although these previous results were inconsistent, considering the urgent need for prevention of HCC recurrence, we judged that the effect of vitamin K2 on HCC recurrence deserved evaluation in a larger scale, randomized, controlled trial. The present study was, therefore, performed as a multicenter, placebo-controlled, double-blind trial enrolling 548 patients at 31 study sites in Japan.

Patients and Methods

Patients. Candidate participants were those who had received curative treatment, in the form of local ablation or surgery, for primary HCC or first intrahepatic recurrence. Diagnosis of HCC was based on

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histopathologic examination or typical findings on dynamic computed tomography/magnetic resonance imaging (CT/MRI) (i.e., hyperattenuation in the arterial phase with washout in a later phase[26]). Inclusion criteria were the following: 20 years of age or older; performance status (Eastern Cooperative Oncology Group; ECOG) 0-2; and compensated liver function (albumin, $\geq$2.8 g/dL; total bilirubin, <2.0 mg/dL; prothrombin time activity, $\geq$40%). Exclusion criteria included the following: previous systemic or hepatic arterial chemotherapy; extrahepatic metastasis; portal vein invasion; interferon treatment within the previous 2 years or a sustained virologic response; uncontrollable encephalopathy, ascites, or plural effusion; a history of gastrectomy or extensive resection of the digestive tract; malabsorption of lipophilic agents, including a history of cholecystectomy; comorbidity with severe cardiovascular, hematological, or renal disease; a history of cancer other than HCC within 5 years; administration of warfarin; administration of vitamin K preparations within the previous 6 months; pregnant or breast-feeding women, or women with childbearing potential or intention; and ongoing participation in other clinical studies.

Assignment. The study was conducted as a multicenter, three-armed, randomized, placebo-controlled, double-blind, comparative, clinical study. Patients who met all criteria were enrolled and randomly assigned in double-blind fashion to receive 45 or 90 mg/day of oral vitamin K2 or a placebo with dynamic allocation, based on the modified minimization method by the registration center, which randomly allocated each patient a randomized study-drug number in the order of registration with a preset computer algorithm, adjusting for balance within each study site and across total registration, considering factors that may affect HCC recurrence (i.e., primary or recurrent HCC, medical ablation or surgical resection, hepatitis C virus (HCV)-related or -unrelated disease, and concomitant administration of glycyrrhizic acid).[27] The investigators, study sponsor, and patients remained blinded to the allocated drug during the study. The protocol was approved by the ethics committee of each participating institution. Patients were well informed of the details of the study and agreed to participate with written informed consent. This trial was conducted in conformity with CONSORT statements and in accord with the Declaration of Helsinki and good clinical practice and is registered as NCT00165633 at Clinicaltrial.gov.

Vitamin K2/Placebo Administration. Each patient took one of the identical capsules (Eisai Co., Ltd., Tokyo, Japan), containing 15 or 30 mg of menatetrenone, vitamin K2 with four isoprenoids, or a placebo, according to group assignment, three times a day after each meal. Medications for chronic hepatitis, such as glycyrrhizic acid and ursodeoxycholic acid, were continued but could not be newly commenced. Antiviral therapies (i.e., interferon, ribavirin, and nucleos(t)ide analogues, such as lamivudine) could not be administered during the study. Vitamin K2/placebo administration was discontinued when recurrent HCC was detected.

Sample Size. The sample size was determined based on previous reports on HCC recurrence among patients who received vitamin K2 and those who did not. Although a previous study reported an adjusted HR of 0.27 (95% CI: 0.12-0.60),[23] the study was conducted in a small number of subjects and the 95% CI ranged widely. We considered 30% risk reduction clinically significant, and the 30% risk reduction was conservatively adopted. Median disease-free survival (DFS) was considered to be 2 years in the placebo group, and the HR in the combined active drug groups was assumed to be 0.67-0.70. Assuming that DFS function followed an exponential distribution, a total of 240-360 events were required to detect the effect of vitamin K2 on DFS, with a one-sided significance level of 2.5%, power of 90%, and an allocation ratio of 1:2 (placebo group:combined active drug groups). To observe the number of events during the follow-up of 3-3.5 years, 180 patients were required in each group (540 in total), assuming loss of information in 5% patients.

DFS. The primary endpoint was DFS, defined as the interval between randomization and either diagnosis of HCC recurrence (i.e., intrahepatic lesions adjacent to or distant from previously treated nodules, and extrahepatic metastasis), cancer other than HCC, or death from any cause. Patients who survived without HCC recurrence or cancer other than HCC at the end of the study were censored on the day of last CT/MRI examination showing no recurrence.

Assessment of Recurrence. HCC recurrence was surveyed every 12 weeks with dynamic CT/MRI, together with ultrasonography. HCC-specific tumor markers, including alpha-fetoprotein (AFP), AFP lens culinaris agglutinin fraction-3 (AFP-L3), and DCP, were monitored every 4 weeks, and dynamic CT/MRI was additionally performed when recurrence was suspected by an increase in tumor marker levels. HCC recurrence was diagnosed by hyperattenuation in the arterial phase and hypoattenuation in the portal venous or equilibrium phase of dynamic CT/MRI. Tumor biopsy was performed when findings on CT/

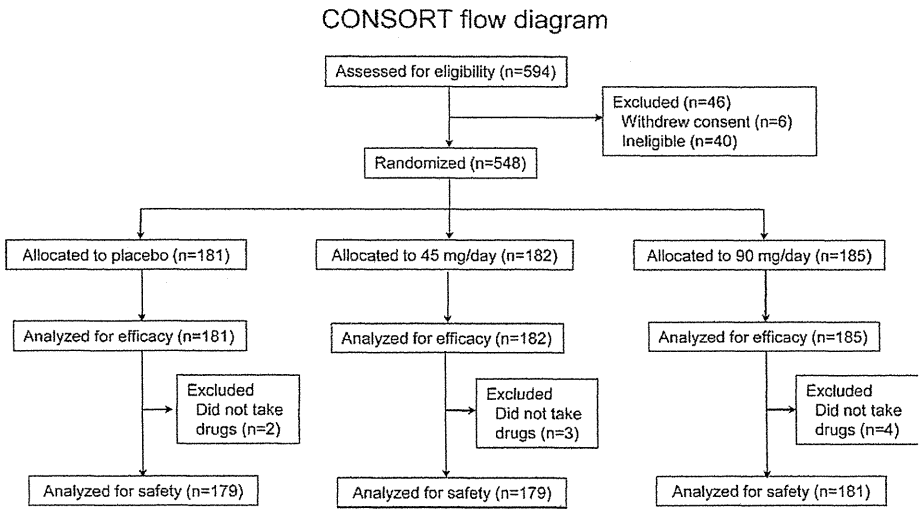

Fig. 1. CONSORT flow diagram.

MRI were equivocal. The presence of recurrence was finally judged by an independent review committee, which thoroughly reviewed the diagnostic imagings in blind fashion. The day of recurrence was defined at the time of first detection of recurrence.

Assessment of Safety. Safety was assessed at 4-week intervals by interview, physical examination, and laboratory tests. Adverse events were defined as any untoward or unintended events that occurred in a subject receiving a study drug. Serious adverse events were defined as those that resulted in death or required hospitalization. Adverse drug reactions were defined as adverse events possibly related to the study drug.

Statistical Analysis. The primary aim of this study was to confirm the superiority of active drug to placebo concerning DFS, and the secondary aim was to evaluate the dose-response relationship between the two active drug groups. DFS rate and median DFS were calculated using the Kaplan-Meier method. Superiority and dose-response relationship were evaluated by the log-rank test, using score statistics with contrast coefficients (−2, 1, and 1) and (0, −1, and 1), respectively, for placebo, 45-mg/day, and 90-mg/day groups. HRs were calculated using Cox's proportional hazards regression model. Adverse events and adverse drug reactions were tabulated based on groups and compared with placebo by Fisher's exact test.

Two interim analyses by the independent data monitoring committee (IDMC) were scheduled. The first was planned 1 year after the commencement of registration to assess safety. The second was planned when 160 events were recorded to assess significance of effect by the finding of $P < 0.005$ (one-sided) or futility. Alpha spending was, for this interim analysis, defined as 0.5% (one-sided), and the overall significance level of statistical tests for the primary aim was maintained at one-sided 2.5%, adjusted for multiplicity associated with interim analyses by the method of Lan and DeMets.[28] The rule for stopping for reasons of futility was defined as follows: The Bayesian predictive probability[29] of detecting a significant effect on observation of 360 events was less than 5%, or the number of events required to assure 50% conditional power exceeded 360. If the IDMC decided to continue the trial, the final required number of events (maximum, 360 events) was to be recalculated to assure 80% conditional power, with the overall significance level maintained for recalculation of the required number of events by Cui's method.[30]

Significance levels for homogeneity among the groups were two-sided 15%, and others were two-sided 5%.

Results

A total of 548 patients were enrolled at 31 study sites in Japan and randomly assigned between March 2004 and September 2005 (Fig. 1). Tumor biopsy was performed in 14 patients, whereas diagnosis was obtained radiologically in remaining patients.[26] Efficacy (i.e., DFS) was assessed among 548 patients (placebo group: 181; 45-mg/day group: 182; 90-mg/day group: 185). Safety was assessed among 539 patients, excluding nine patients who never took drugs. Two patients took drugs at a dose different from that allocated. They were included in the group of allocated dose in the efficacy analysis, but in the group of actually received dose in the safety analysis.