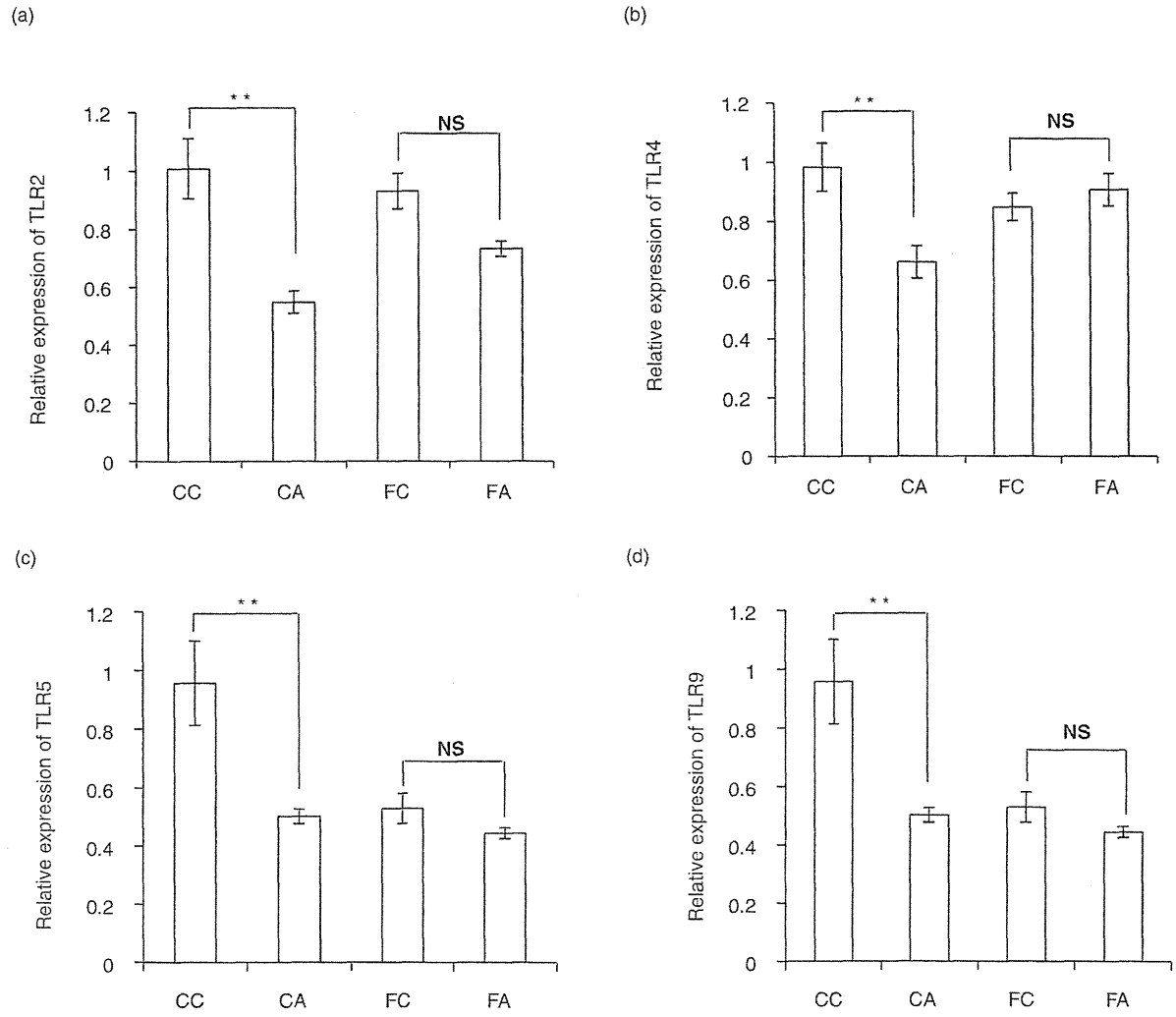

Figure 6 mRNA expression of Toll-like receptors in the small intestine of high fat diet (HFD)-fed and control mice with or without antibiotic administration. Toll-like receptors (TLRs) expression was not altered by antibiotic treatment in non-alcoholic fatty liver disease (NAFLD) small intestine (TLR2 (a), TLR4 (b), TLR5 (c), and TLR9 (d)). (*$P < 0.05$, **$P < 0.01$) (NS, not significant).

demonstrates upregulation of TLR signal pathways and may be more sensitive to the ligands of intestinal microbial components. Bertola *et al.* reported that the expression of TLRs and certain cytokines/chemokines in genes was upregulated in biopsy specimens of morbidly obese patients with histologically normal liver or severe steatosis with or without NASH,[30] and our findings from this animal study concur with this.

Although many studies have examined the association between TLR signal pathways and the pathogenesis of NASH, these reports focused mainly on hepatocytes, Kupffer cells, and hepatic stellate cells.[6,10,12,31] However, small intestinal microbiota are the cause of liver damage in NASH, and to our knowledge, this is the first report to investigate the expression of intestinal TLR signal pathways in NAFLD model and to reveal a discrepancy in TLRs expression in the gut-liver axis. The microbial TLR signal pathway and downstream cytokines were downregulated in the small intestine of NAFLD model and this downregulation may contribute to imbalance of the immune system and, consequently, alter the microflora component ratio and induce SIBO with NAFLD.

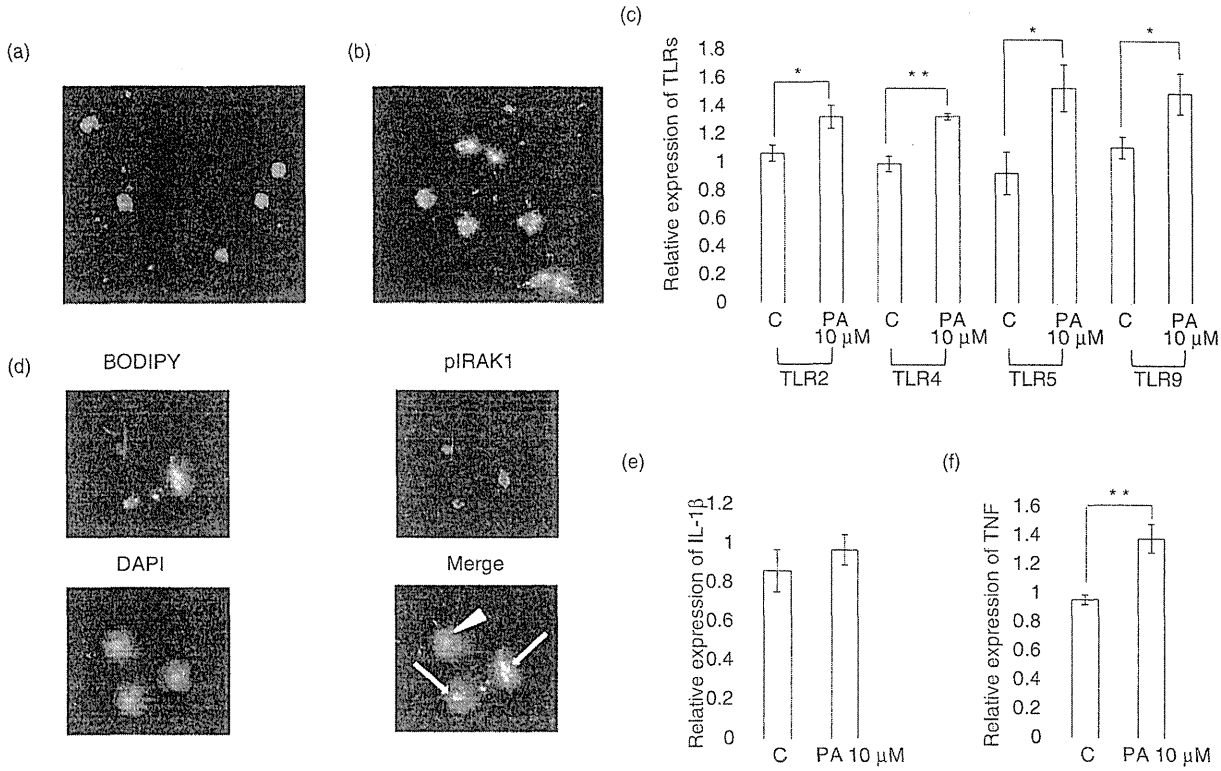

Figure 7 mRNA expression of Toll-like receptors and inflammatory cytokines in primary Kupffer cells treated with palmitic acid (PA). Treatment by PA for 24 h induced deposition of fat droplets in primary Kupffer cells (a: control, b: treatment with 10 μM PA, BODIPY (green), 4'6'-diamidino-2-phenylindole dihydrochloride (DAPI) [blue]). The expression of TLR2, TLR4, TLR5, and TLR9 was significantly higher in primary Kupffer cells treated with 10 μM PA for 24 h (C: control) (c). Immunocytochemistry demonstrated that the expression of phospho-interleukin-1 receptor-associated kinase1 (pIRAK) was strongly positive in fat deposited primary Kupffer cells treated with 10 μM PA for 24 h (d: BODIPY (green), pIRAK1 (red) and DAPI (blue). Arrow: Kupffer cells deposited in fat droplets. Arrow head: Kupffer cell deposited in absence of fat droplets). Tumor necrosis factor (TNF) expression was significantly higher in primary Kupffer cells treated with 10 μM PA for 24 h (IL-1β (e), TNF (f)) ($^{*}P < 0.05$, $^{**}P < 0.01$).

We used a mouse model gut-sterilized with antibiotics to confirm whether there is an association between gut microbiota and TLR expression in NAFLD. In our model, oral caloric intake was not significantly different between FA and FC mice, but, body weight, serum ALT levels, and serum free fatty acids levels were significantly suppressed in the FA mice. Furthermore, histopathological findings showed a marked decrease in steatosis in this group. Interestingly, the expression of TLRs and downstream cytokines was suppressed in FA mice compared with that in FC mice. In contrast, in an ethyl alcohol-induced liver injury model, liver TLR expression was found to be independent of gut microbiota despite a decrease in fatty liver and liver injury by antibiotic administration.[9] Our findings suggest that, directly or indirectly, gut microbiota contribute to TLR expression in the liver of the NAFLD model. Because antibiotics administration also decreased serum free fatty acids in FA mice, we focused on the association between fatty acid metabolism and gut microbiota. There are three reasons for the suppression of serum free fatty acids in FA mice. The first is a decrease in adipocytes accompanied by suppression of increasing body weight. This may be explained by the fact that obesity-associated microbiota, which increase the capacity to harvest energy from the diet, were eradicated by antibiotics.[18] We investigated 16 s rRNA based analysis of fecal microbiota by the terminal restriction fragment length polymorphism method and confirmed alteration in fecal microbiota of FA mice compared with that of FC

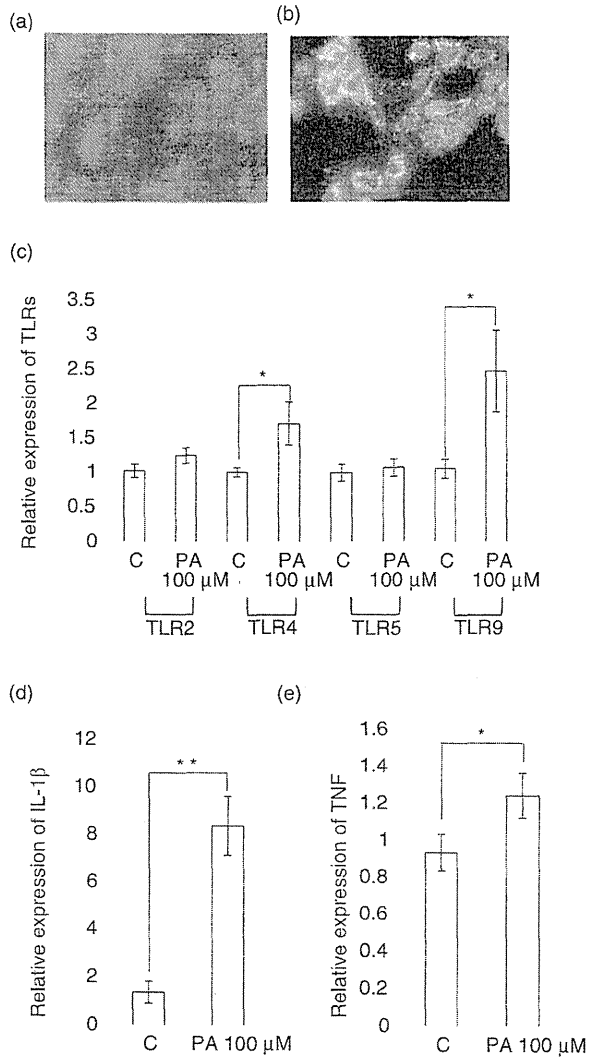

Figure 8 mRNA expression of Toll-like receptors and inflammatory cytokines in primary hetatocytes treated with palmitic acid (PA). Treatment by PA for 24 h induced the deposition of fat droplets in primary hepatocytes (a: control, b: treatment with 100 μM PA, BODIPY (green), 4'6'-diamidino-2-phenylindole dihydrochloride (DAPI) [blue]). The expression of TLR4 and TLR9 mRNA was significantly upregulated in primary hepatocytes treated with 100 μM PA for 24 h (C: control) (c). The expression of tumor necrosis factor (TNF) and interleukin (IL)-1β was significantly higher in primary hepatocytes treated with 100 μM PA for 24 h (IL-1β (d), TNF [e]) ($^{*}P < 0.05$, $^{**}P < 0.01$).

mice (data not shown). The second reason is a decrease in the release of free fatty acids from adipocytes. In obese individuals, it has been reported that gut microbiota can suppress the expression of fasting-induced adipose factor (Fiaf), which increases the activity of lipoprotein lipase, leading to the production of triglyceride storage in adipocytes and an increased supply of fatty acids.[32] The expression of intestinal Fiaf was significantly upregulated in FA mice compared with FC mice in our experiment (data not shown), indicating that gut-sterilization may contribute to a reduction in the release of serum free fatty acids from adipocytes through increased intestinal Fiaf expression. The third reason is a decrease in *de novo* liver lipogenesis. It is reported that microbiota can increase hepatic lipogenesis through the expression of sterol response element binding protein 1 (SREBP1) and carbohydrate response element binding protein (ChREBP).[33] The expression of both SREBP1 and ChREBP was significantly suppressed in FA mice compared with that in FC mice in our experiment (data not shown), indicating that the suppression of serum free fatty acids, at least in part, contributes to a decrease in hepatic *de novo* lipogenesis through the suppression of both SREBP1 and ChREBP. Our findings suggest both alteration in microflora and a decrease in *de novo* lipogenesis in the liver of the gut-sterilized model, but it is unknown whether the release of free fatty acids from adipocytes decreases.

Next, we hypothesized that fatty acids could alter TLRs expression in the liver. We showed that the expression of TLR2, TLR4, TLR5, TLR9, and TNF were significantly higher in primary Kupffer cells treated with PA than in control cells, while that of TLR4, TLR9, TNF, and IL-1β was upregulated in primary hepatocytes treated with PA. Although our *in vitro* study suggests that PA directly stimulates the induction of TLRs, others report that saturated fatty acids (SFA) including PA stimulate NFκB promoter activity through the activation of the TLR signal pathway. It is also reported that SFA plays a role as TLR4 ligand.[34,35] Therefore, the induction of downstream cytokines may have contributed to both the upregulation of TLRs and stimulation of TLR4 by PA. These findings suggest that Kupffer cells and hepatocytes are susceptible to bacterial components via upregulation of TLRs by PA in the pro-inflammatory state of NAFLD.

In contrast, although TLRs expression in the small intestine of HFD-fed mice was not altered by antibiotic treatment, it was downregulated in control mice. Therefore, we hypothesized that luminal HFD itself could attenuate TLRs expression in the small intestine. Caco2 cells that are human epithelial cell lines treated with PA or OA showed lower expression of TLR2, TLR5, and TLR9 compared with control cells (data not shown). These data suggest that fatty acids may partly contribute

to the attenuation of TLRs expression in the small intestine.

In conclusion, TLRs expression was downregulated in the NAFLD small intestine, and this may be contributed to an increase in free fatty acids through alteration of gut microbiota. In contrast, the hepatic TLR signal pathway was upregulated and susceptible to microbial components by increased free fatty acids in the pro-inflammatory state of NAFLD. Our findings suggest that discrepancy in TLR signals in the gut-liver axis may be associated with the pathogenesis of progression to NASH through an increase in free fatty acids. Because there is no specific treatment for human NASH, early intervention in the pro-inflammatory state may be important for the prevention of its development from simple steatosis. Because free fatty acids play an important role in the development of the pro-inflammatory state of NAFLD, we consider that both fatty acid metabolism and gut microbiota in the pro-inflammatory state may be useful targets for preventive treatment against NASH development.

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REFERENCES

1 Ludwig J, Viggiano TR, McGill DB, Brewer NS. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hithero unnamed disease. *Mayo Clin Proc* 1980; 55: 482–8.
2 Lazo M, Clark JM. The epidemiology of nonalcoholic fatty liver disease: a global perspective. *Semin Liver Dis* 2008; 28: 339–50.
3 Day CP, James OF. Steatohepatitis: a role of two "hit"? *Gastroenterology* 1998; 114: 842–5.
4 Marra F, Bertolani C. Adipokines in liver disease. *Hepatology* 2009; 50: 957–62.
5 George DK, Goldwurm S, MacDonald GA *et al.* Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. *Gastroenterology* 1988; 114: 311–18.
6 Szabo G, Velayudham A, Romics L Jr, Mandrekar P. Modulation of non-alcoholic steatohepatitis by pattern recognition receptors in mice: the role of toll-like receptor 2 and 4. *Alcohol Clin Exp Res* 2005; 29 (11 Suppl): 140S–5S.
7 Seki E, Brenner DA. Toll-like receptors and adaptor molecules in liver disease: update. *Gastroenterology* 2008; 48: 322–35.
8 Takeda K, Akira S. TLR signaling pathway. *Semin Immunol* 2004; 16: 3–9.
9 Gustot T, Lemmers A, Moreno C *et al.* Differential liver sensitization to Toll-like receptor pathways in mice with alcoholic fatty liver. *Hepatology* 2006; 43: 989–1000.
10 Rivera CA, Adegvoyage P, van Rooijen N, Tagalicud A, Allman M, Wallace M. Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. *J Hepatol* 2007; 47: 571–9.
11 Imajo K, Fujita K, Yoneda M *et al.* Hyperresponsivity to low-dose endotoxin during progression to nonalcoholic steatohepatitis is regulated by leptin mediated signaling. *Cell Metab* 2012; 16: 44–54.
12 Miura K, Kodama Y, Inokuchi S *et al.* Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1β in mice. *Gastroenterology* 2010; 139: 323–34.
13 Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; 444: 1027–31.
14 Shanab AA, Scully P, Crosbie O *et al.* Small intestinal bacterial overgrowth in nonalcoholic steatohepatitis: association with toll-like receptor 4 expression and plasma levels of interleukin 8. *Dig Dis Sci* 2011; 56: 1524–34.
15 Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Human gut microbes associated with obesity. *Nature* 2006; 444: 1022–3.
16 Cani PD, Bibiloni R, Knauf C *et al.* Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high fat diet induced obesity and diabetes in mice. *Diabetes* 2008; 57: 1470–81.
17 Bajzer M, Seeley RJ. Obesity and gut flora. *Nature* 2006; 444: 1009–10.
18 de La Serre CB, Ellis CL, Lee J, Hartman AL, Rutledge JC, Raybould EE. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota gut inflammation. *Am J Physiol Gastrointest Liver Physiol* 2010; 299: G440–8.
19 Vijay-Kumar M, Aitken JD, Carvalho A *et al.* Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science* 2010; 328: 228–31.
20 Santaolalla R, Fukata M, Abreu M. Innate immunity in the small intestine. *Curr Opin Gastroenterol* 2011, 27: 125–31.
21 Lodes MJ, Cong Y, Elson CO *et al.* Bacterial flagellin is a dominant antigen in Crohn disease. *J Clin Invest* 2004; 113: 1296–306.
22 Munthe-Kaas AC, Berq T, Seqlen PO, Seljelid R. Mass isolation and culture of rat Kupffer cells. *J Exp Med* 1975; 141: 1–10.
23 Gottipati S, Rao NL, Fung-Leung WP. IRAK1: a critical signaling mediator of innate immunity. *Cell Signal* 2008; 20: 269–76.
24 Seki E, Minicis SD, Osterreicher CH *et al.* TLR4 enhances TGF-β signaling and hepatic fibrosis. *Nat Med* 2007; 13: 1324–32.

25 Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumor necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2001; 48: 206–11.
26 Sabate JM, Jouet P, Harnois F *et al.* High prevalence of small intestinal bacterial overgrowth with morbid obesity: a contributor to severe hepatic steatosis. *Obes Surg* 2008; 18: 371–7
27 Pillotto A, Franceschi M, Del Favero G, Fabrello R, Di Mario F, Valerio G. The effect of aging on oro-cecal transit time in normal subjects and patients with gallstone disease. *Aging (Milano)* 1995; 7: 234–7
28 Elphick DA, Chew TS, Higham SE, Bird N, Ahmad A, Sanders DS. Small bacterial overgrowth in symptomatic older people: can it be diagnosed earlier? *Gerontology* 2005; 51: 396–401
29 Soza A, Riquelme A, Gonzalez R *et al.* Increased orocecal transit time in patients with nonalcoholic fatty liver disease. *Dig Dis Sci* 2005; 50: 1136–40.
30 Bertola A, Bonnafous S, Anty R *et al.* Hepatic expression patterns of inflammatory and immune response genes associated with obesity and NASH in morbidly obese patients. *PLoS ONE* 2010; 5: e13577
31 Rivera CA, Gaskin LT, Allman M *et al.* Toll-like receptor 2 deficiency enhances non-alcoholic steatohepatitis. *BMC Gastroenterol* 2010; 10: 52.
32 Backhed F, Ding H, Wang T *et al.* The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* 2004; 101: 15718–23.
33 Prakash S, Rodes L, Coussa-Charley M, Duchesneau CT. Gut microbiota: next frontier in understanding human health and development of biotherapeutics. *Biologics* 2011; 5: 71–86.
34 Lee JY, Plakidas A, Lee WH *et al.* Differential modulation of Toll-like receptors by fatty acids: preferential inhibition by n-3polyunsaturated fatty acids. *J Lipid Res* 2003; 44: 909–16.
35 Lee JY, Gao Z, Youn HS *et al.* Reciprocal modulation of Toll-like recptor-4 signaling pathways involving MyD88 and phosphatidylinositol 3-kinase/AKT by saturated and polyunsaturated fatty acids. *J Biol Chem* 2003; 278: 37041–51

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Original Article

Dose-finding trial of tolvaptan in liver cirrhosis patients with hepatic edema: A randomized, double-blind, placebo-controlled trial

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Aim: Liver cirrhosis represents the end stage of any chronic liver disease, and it is associated with hepatic edema such as ascites. Many patients with ascites do not respond to diuretic therapy or require administration of diuretics at high doses that can cause adverse events. This 7-day, multicenter, double-blind trial of tolvaptan was designed to determine the optimal dose of tolvaptan for producing the intended pharmacological effect in hepatic edema.

Methods: Liver cirrhosis patients with inadequate diuretic response despite having received a conventional diuretic therapy were enrolled in the trial. Participants were stratified randomly to four groups receiving tolvaptan at 7.5, 15 or 30 mg/day, or placebo as an add-on to conventional diuretics once daily for 7 days. Changes in bodyweight and abdominal circumference were analyzed. Serum sodium concentrations were measured. Safety assessment was performed.

Results: Tolvaptan at 7.5–30 mg/day reduced bodyweight and abdominal circumference compared with placebo. Serum sodium concentrations remained within the normal range in all tolvaptan groups. Serious adverse events were not observed, and most common adverse event was thirst. Tolvaptan at 7.5 mg/day showed the maximum change in bodyweight and abdominal circumference together with preferable tolerability.

Conclusion: Tolvaptan at 7.5 mg/day was considered the optimal dose in liver cirrhosis patients with hepatic edema who showed inadequate response to conventional diuretics.

Key words: aquaretic action, ascites, diuretics, hepatic edema, liver cirrhosis, tolvaptan

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Author contribution: Isao Sakaida, M.D. had full access to all the data in the trial and took responsibility for the integrity of the data and the accuracy of the data analyses and wrote the first draft of the manuscript. All authors contributed to trial concept and design and data analyses, drafted and reviewed the manuscript revisions, and approved the final draft for submission.

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INTRODUCTION

LIVER CIRRHOSIS REPRESENTS the end stage of any chronic liver disease.[1] Hepatic edema including ascites and lower limb edema is the most frequently observed complication in the disease, leading to deterioration in quality of life.[2,3] Therefore, improvement of hepatic edema is an important therapeutic strategy. Spironolactone, an aldosterone antagonist, either alone or in combination with the loop diuretic furosemide is prescribed as the first-line therapy for management of liver cirrhosis patients with persistent ascites.[4] Many patients with ascites do not respond to diuretic therapy or require administration of diuretics at high doses that can cause adverse events including activation of the rennin–angiotensin and sympathetic nervous systems, electrolyte disturbances such as dilutional hyponatremia, and worsening of renal function.[5–8] Thus, the development of effective drugs other than conventional diuretics is needed for the management of hepatic edema.

Because arginine vasopressin V_2 receptor antagonists promote electrolyte-free water excretion without disrupting electrolyte balance, they are expected to be clinically useful in the treatment of diseases associated with hyponatremia or fluid retention.[9,10] Tolvaptan, a novel aquaretic agent, is a non-peptide V_2 receptor antagonist.[10–14] By inhibiting reabsorption at the renal collecting tubules, tolvaptan increases electrolyte-free urine excretion without increasing electrolyte excretion.

In the USA,[13] tolvaptan at 15–60 mg/day has been approved for the treatment of hyponatremia, and in the EU,[15] tolvaptan within the same dosage range has been approved for the treatment of syndrome of inappropriate antidiuretic hormone. In Japan, tolvaptan at 15 mg/day has been approved for the treatment of heart failure-related edema.[16]

We initiated a program to obtain the additional indication for the treatment of hepatic edema. Therefore, we conducted the phase 2 study to determine an optimal dose of tolvaptan. In our previous, preliminary trial, tolvaptan at dose of 15 mg/day and higher exerted sufficient pharmacological response for improvement of hepatic edema including ascites in liver cirrhosis patients who showed resistance to furosemide.[16]

The aim of this trial was to determine the optimal dose of tolvaptan in hepatic edema showing inadequate response to conventional diuretics. The results of this trial will be used as the basis for a pivotal trial to be conducted to obtain an additional indication for tolvaptan in Japan.

METHODS

Trial setting

THE PRESENT TRIAL was a randomized, double-blind, placebo-controlled, multicenter trial conducted by Otsuka Pharmaceutical (the study sponsor). This trial was conducted in accordance with the Declaration of Helsinki and in compliance with Good Clinical Practice (GCP) guideline at all times.[17] The trial protocol was reviewed and approved by the institutional review board at each trial site, and written informed consent was obtained from all patients after being informed of the trial purpose and the nature of the procedures involved. All authors had access to the study data and had reviewed and approved the final manuscript. This trial is registered on Clinical Trials.Gov (NCT00479336).

Patients

This trial enrolled liver cirrhosis patients with ascites despite undergoing combination therapy with a loop diuretic and an anti-aldosterone drug at the doses from at least 7 days prior to commencement of trial drug administration, as described below. Prior treatment with diuretics needed to meet either one of the dose criteria being used in Japan. If the daily dose of furosemide and other loop diuretics were at least 40 mg and equivalent to 40 mg furosemide, respectively, then the daily dose of spironolactone was set at 25 mg. If the daily dose of spironolactone was at least 50 mg, then the daily dose of furosemide and other loop diuretics were set at 20 mg or equivalent to 20 mg furosemide, respectively.[18]

Patients who met the following criteria were randomized to one of the trial groups and allowed to advance to the treatment period to evaluate efficacy of the drug: patients with ascites during pretreatment observation

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period; patients orally treated with conventional diuretics without change in dose or mode of administration from 7 days before start of trial drug administration until final day of pretreatment observation period; patients with stable bodyweight (±1.0 kg) for 2 days before start of trial drug administration.

The patients were between 20 and 80 years of age and were required to be hospitalized from the start of a 3-day pretreatment observation period until completion of a 14-day post-treatment observation period. Major exclusion criteria were: (i) patients with hepatic encephalopathy (coma scale, ≥II);[12] (ii) patients with poorly controlled hepatocellular carcinoma; (iii) patients requiring new treatment for esophageal or gastric varices; (iv) patients with hemorrhoidal hemorrhage secondary to rectal varices; and (v) patients receiving blood products including albumin preparations.

Treatment protocol

This trial consisted of a 3-day pretreatment observation period (defined as baseline), a 7-day treatment period and a 14-day post-treatment observation period. Trial drugs were administrated to patients after breakfast. Day 1 was defined as the period from the first to the second administration. Days 2–7 were similarly defined. For all variables, data obtained immediately before the start of trial drug administration were used as baseline data. The day that each patient completed or discontinued the administration of trial drugs was defined as the final dosing day. The dose and mode of administration of conventional diuretics were to remain fixed from 7 days prior to the start of trial drug administration, and to be administrated p.o. in combination with tolvaptan throughout the trial period.

Patients were randomized to four groups receiving tolvaptan at 7.5, 15, 30 mg or placebo once daily for 7 consecutive days (defined as the 7.5-mg group, the 15-mg group, the 30-mg group and the placebo group). The registration center allocated the eligible patients to the treatment groups by stratifying them according to the presence/absence of lower limb edema. Random allocation was performed by a trial drug allocation manager from an independent contract research organization after confirmation that the packages containing tolvaptan tablets and matching placebo tablets were indistinguishable in appearance.

Evaluation

The primary end-point was change in bodyweight from baseline at the final dosing day in patients receiving trial drugs as a surrogate marker for improvement of hepatic edema.[13] The relationship of change in bodyweight and dose of tolvaptan was evaluated by using a linear regression model. Secondary end-points were change in abdominal circumference and increase in daily urine volume.[10]

Bodyweight was measured before breakfast following urination each day through the treatment period. Abdominal circumference was also measured before breakfast following urination on days 2–3 and on day 7. Changes in bodyweight and abdominal circumference from baseline to the final dosing day in the tolvaptan groups were compared with those in the placebo group.

Cumulative 24-h urine samples were collected after patients had urinated completely before drug administration (after breakfast) each day from the day before the start of trial drug administration until the end of the post-treatment period. Urinary sodium concentration was measured. Fluid intake was not restricted and measured values were recorded during the trial period. Mean differences between daily fluid intake and daily urine volume were calculated by the treatment groups. Serum sodium concentration was measured before breakfast (baseline), 4–8 h and 22–24 h post-dose on day 1, and before breakfast on days 2–3, on days 7 and 9, and on days 14–17. If patients discontinued, these values were measured at an appropriate time. Change in serum sodium concentration from baseline to the final dosing day was assessed.

Plasma concentration of tolvaptan was measured at baseline, on day 1 (22–24 h) and on day 7 (2–4, 8–16 and 22–24 h).

Safety assessment

Safety assessment was performed throughout the trial period. Safety variables included adverse events, adverse events that occurred before the start of trial drug administration, clinical laboratory tests, vital signs and electrocardiograms.

Statistical analysis

For the change from baseline to the final dosing day, ANOVA was performed for pair-wise comparison between each of the tolvaptan groups and the placebo group. The other continuous data were analyzed by ANOVA. The category data were analyzed by Fisher's exact test or Kruskal–Wallis test. None of the analyses were adjusted for multiple comparison. Linear regression analysis was performed by using the change in bodyweight from baseline at the final dosing day as the objective variable and the dose of tolvaptan as the explanatory variable.

Statistical significance was defined as $P < 0.05$. All statistical analyses were performed using SAS ver. 9.1.3 software (SAS Institute, Cary, NC, USA).

RESULTS

Patients

THIS TRIAL WAS conducted from June 2007 through July 2008 at 44 institutions. Of 104 patients who received at least one dose of the trial drug, two patients who were in deviation of GCP and one patient who received the trial drug at a dose higher than the specified daily dose at first dosing day were excluded from all analysis sets (Fig. 1).

A total of 101 patients were included in the safety analysis set, comprising 26 patients in the placebo group, 25 in the 7.5-mg group, 25 in the 15-mg group and 25 in the 30-mg group. One patient in the placebo group was not included in the efficacy analysis set because this patient underwent abdominal paracentesis on day 3. One missing data existed each in abdominal circumference analysis and urine volume analysis. Demographic and other baseline characteristics are shown in Table 1. No notable differences in background factors were observed among the four groups.

Efficacy of study drug

Change in bodyweight from baseline was −0.36 kg (standard deviation [SD], 2.06) in the placebo group, −2.31 kg (SD, 2.35) in the 7.5 mg group, −1.88 kg (SD, 2.45) in the 15 mg group and −1.67 kg (SD, 1.46) in the 30 mg group (Fig. 2). Change in bodyweight in all tolvaptan groups showed significant decreases compared with the placebo group ($P = 0.014$ for the 7.5-mg group, $P = 0.011$ for the 15-mg group and $P = 0.029$ for the 30-mg group). The regression coefficient of the dose was not statistically significant ($P = 0.3167$).

Change in abdominal circumference from baseline was −1.0 cm (SD, 2.8) in the placebo group, −3.0 cm (SD, 3.2) in the 7.5-mg group, −2.4 cm (SD, 2.5) in the 15-mg group and −2.6 cm (SD, 2.8) in the 30-mg group. Tolvaptan at 7.5 mg was significantly superior ($P = 0.030$) to the placebo in Figure 3.

Change in daily urine volume is shown in Figure 4. Increases in daily urine volume in all tolvaptan groups were observed in a dose-dependent manner. The differences in the change in urine volume between each tolvaptan group and the placebo group were statistically significant. All tolvaptan groups showed maximum increases in urine volume on day 1.

Serum sodium concentration in all tolvaptan groups increased, and further remained within the normal

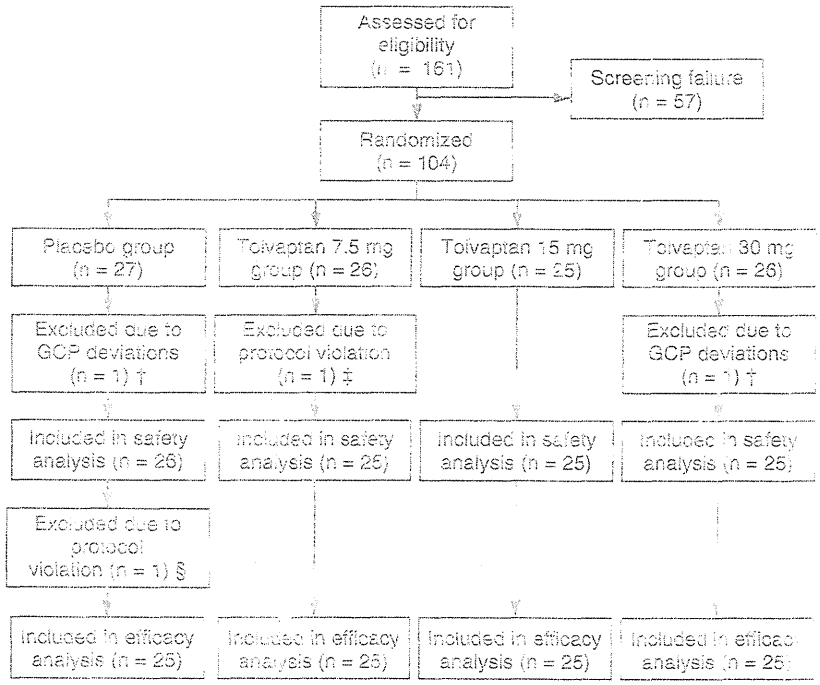

Figure 1 Patient flow chart. †Registration before the approval of the institutional review board. ‡Violation regarding specified daily dose. §Patient underwent abdominal paracentesis. GCP, Good Clinical Practice.

Table 1 Demographic and clinical characteristics at baseline

Characteristics	Placebo group ($n = 26$)	7.5-mg group ($n = 25$)	15-mg group ($n = 25$)	30-mg group ($n = 25$)	*P*-value
Age (years)	64 (10)	65 (9)	65 (10)	63 (10)	0.89†
Sex (male)	17 (65.4%)	18 (72.0%)	21 (84.0%)	15 (60.0%)	0.27‡
Bodyweight (kg)	57.8 (11.6)	61.9 (10.8)	59.1 (11.4)	60.0 (12.1)	0.62†
Abdominal circumference (cm)	86.6 (9.0)	89.8 (9.4)	86.6 (9.5)	88.6 (11.4)	0.58†
Cause of liver cirrhosis					
Hepatitis B	4 (15.4%)	4 (16.0%)	4 (16.0%)	0 (0.0%)	
Hepatitis C	13 (50.0%)	13 (52.0%)	16 (64.0%)	17 (68.0%)	
Alcoholic cirrhosis	6 (23.1%)	7 (28.0%)	7 (28.0%)	4 (16.0%)	
Other	3 (11.5%)	4 (16.0%)	0 (0.0%)	5 (20.0%)	
Child–Pugh classification					0.98§
A	0 (0.0%)	1 (4.0%)	0 (0.0%)	2 (8.0%)	
B	16 (61.5%)	13 (52.0%)	15 (60.0%)	13 (52.0%)	
C	10 (38.5%)	11 (44.0%)	10 (40.0%)	10 (40.0%)	
Complication of liver cance	10 (38.5%)	11 (44.0%)	9 (36.0%)	10 (40.0%)	0.97‡
Serum albumin concentration (g/dL)	2.8 (0.5)	2.7 (0.5)	2.8 (0.5)	2.9 (0.6)	0.70†
Serum creatinin concentration (mg/dL)	0.9 (0.3)	0.9 (0.3)	1.0 (0.4)	0.8 (0.2)	0.21†

Data are expressed as mean (standard deviation) or number of patients (%).
†ANOVA.
‡Fischer's exact test.
§Kruskal–Wallis test.

range. The placebo group showed no change in serum sodium concentration (Fig. 5). Changes in serum sodium concentration from baseline to the final dosing day were −0.7 mEq/L (SD, 2.0) in the placebo group, 1.2 mEq/L (SD, 3.0) in the 7.5-mg group, 2.8 mEq/L (SD, 3.1) in the 15-mg group and 3.2 mEq/L (SD, 3.9) in the 30-mg group. All tolvaptan groups showed significant differences compared with the placebo group (7.5-mg group, $P = 0.029$; 15-mg group, $P < 0.001$; 30-mg group, $P < 0.001$). Mean differences between daily fluid intake and daily urine volume were 601 mL in the placebo group, 1190 mL in the 7.5-mg group, 1245 mL in the 15-mg group and 1494 mL in the 30-mg group.

Time-courses of plasma tolvaptan concentrations are shown in Figure 6. Plasma tolvaptan concentration at 2–4 h post-dose on day 7 was 55 ng/mL (SD, 44) in the 7.5-mg group, 164 ng/mL (SD, 137) in the 15-mg group and 300 ng/mL (SD, 226) in the 30-mg group.

Safety assessment

Adverse events that occurred at an incidence of at least 5% are shown in Table 2. The most commonly reported adverse event was thirst in all tolvaptan groups, showing a dose-dependent increase. Thirst was also observed in one patient in the placebo group. Other adverse events that occurred frequently in the tolvaptan groups were pollakiuria, insomnia, and increased blood uric acid, blood urea and blood alkaline phosphatase.

Serious adverse events were observed as follows: anemia, abdominal distension, chronic hepatitis, hepatic failure, hepatitis B, dyspnea and hepatorenal syndrome in the placebo group; renal impairment and hemorrhagic shock in the 15-mg group; and gastrointestinal hemorrhage, hepatic failure and hepatic encephalopathy in the 30-mg group.

Changes in creatinine from baseline at the final dosing day were −0.001 mg/dL in the placebo group, 0.041 mg/dL in the 7.5-mg group, 0.057 mg/dL in the 15-mg group and 0.072 mg/dL in the 30-mg group.

DISCUSSION

IN THE PRESENT trial, tolvaptan at daily doses of 7.5, 15 and 30 mg demonstrated notable pharmacological effect including improvement of hepatic edema in liver cirrhosis patients in comparison with placebo. Tolvaptan at 7.5 mg/day showed the maximum change in bodyweight and abdominal circumference. Preferable tolerability was shown at 7.5 mg of tolvaptan. Therefore, tolvaptan at 7.5 mg/day was considered the optimal dose in the treatment of hepatic edema.

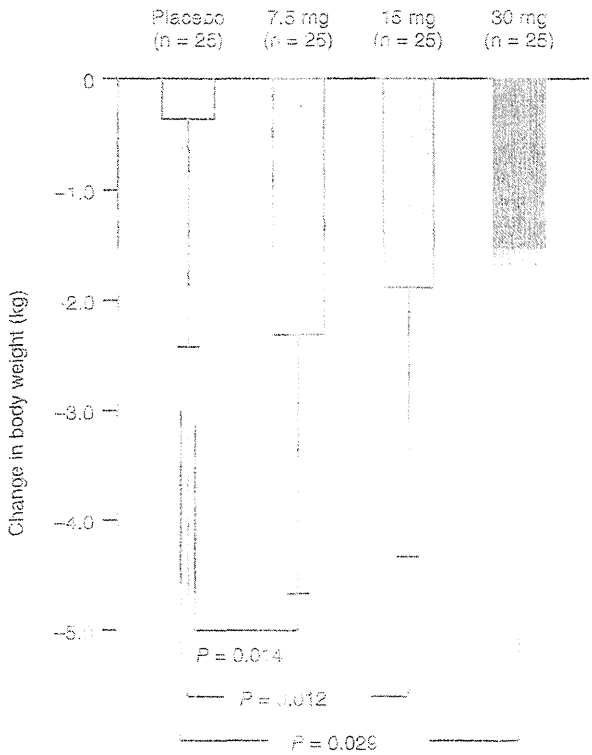

Figure 2 Changes in bodyweight from baseline at the final dosing day. Data are expressed as mean ± standard deviation. Contrast tests between each tolvaptan group and the placebo group were performed by using multiplicity-unadjusted ANOVA.

The present trial was conducted to determine the optimal dose of tolvaptan based on the results of our previous trial.[16] In that previous trial, the targeted pharmacological action as reduction in bodyweight was confirmed at tolvaptan doses of 15 mg and higher. In the present trial, in addition to that same tolvaptan dose of 15 mg, a half dose (7.5 mg) and a double dose (30 mg) were also evaluated. No linear dose response to change in bodyweight was observed, while urine volume and fluid intake showed a dose-dependent manner. However, the largest reduction in bodyweight was observed in the 7.5-mg group. Investigation at doses of tolvaptan less than 7.5 mg/day may be required. Various factors can be considered, water restriction was not included as a rule and regulation in this trial. Thirst was observed in a dose-dependent manner, therefore, it may be one of the major factors.

Hyponatremia is one of the problems in loop diuretic therapy. Serum sodium concentration was significantly increased in the tolvaptan groups, but not in the placebo group, indicating that tolvaptan produced improvement of electrolyte imbalance. This is caused by tolvaptan's aquaretic action.[11–15] This result demonstrated that tolvaptan in combination with conventional diuretics contributes to treating cirrhotic patients with hepatic edema.[16]

Plasma tolvaptan concentration at 2–4 h post-dose on day 7 was 55 ng/mL (SD, 44) in the 7.5-mg group, 164 ng/mL (SD, 137) in the 15-mg group and 300 ng/mL (SD, 226) in the 30-mg group. Kim *et al.* reported that following administration of tolvaptan at 30 mg in healthy subjects for 7 days, plasma tolvaptan concentration reached a maximum of 198 ng/mL (SD, 32) within 2–4 h post-dose.[21] Plasma tolvaptan concentration in liver cirrhosis patients with hepatic edema are considered to be higher than in healthy subjects. Tolvaptan is metabolized exclusively in the liver, primarily by cytochrome P450 3A4.[24] Therefore, plasma

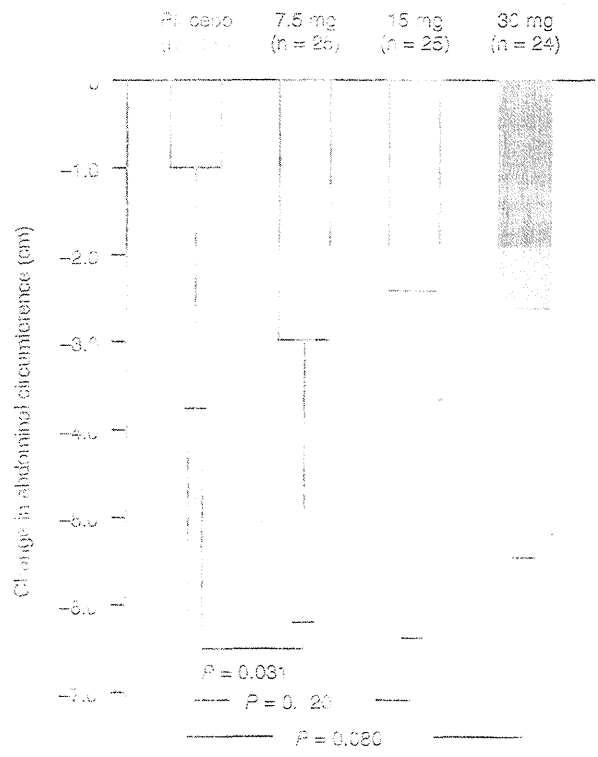

Figure 3 Changes in abdominal circumference from baseline at the final dosing day. Data are expressed as mean ± standard deviation. Contrast tests between each tolvaptan group and the placebo group were performed by using multiplicity-unadjusted ANOVA.

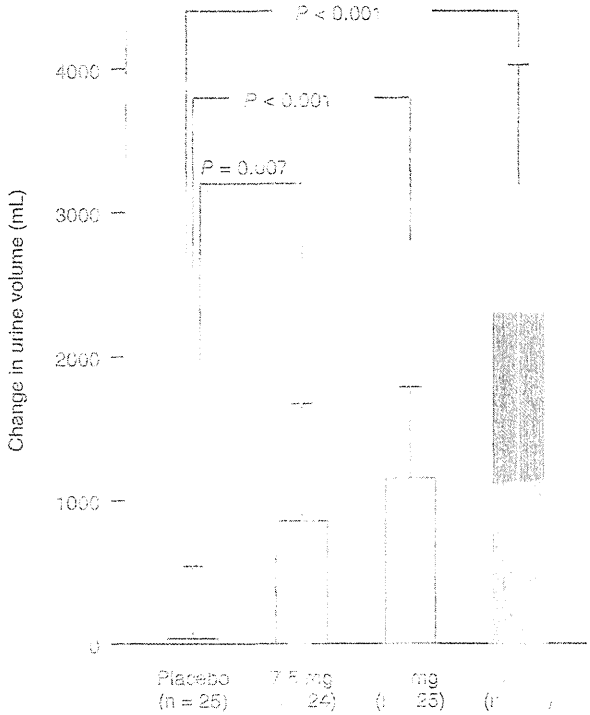

Figure 4 Changes in daily urine volume from baseline at the final dosing day. Data are expressed as mean ± standard deviation. Contrast tests between each tolvaptan group and the placebo group were performed by using multiplicity-unadjusted ANOVA.

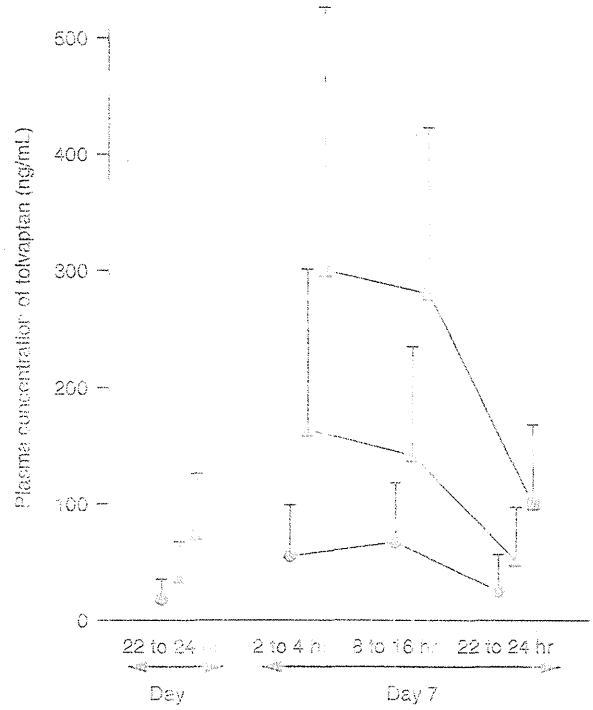

Figure 6 Time courses of plasma tolvaptan concentration. Data are expressed as mean ± standard deviation. —○—, 7.5 mg; —▲—, 15 mg; —●—, 30 mg.

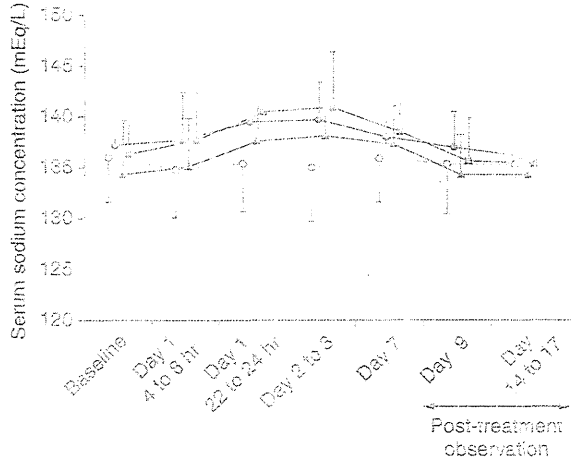

Figure 5 Time courses of serum sodium concentration. Data are expressed as mean ± standard deviation. —○—, placebo; —▲—, 7.5 mg; —△— 15 mg; —●— 30 mg.

concentration of tolvaptan in patients with hepatic dysfunction at dialysis may be higher than that in patients with normal hepatic function.

Although the satisfactory results were obtained, the present trial was limited in that it did not include an evaluation of tolvaptan's potential for improving ascites volume and symptoms related to hepatic edema. Therefore, the next trial should be designed to evaluate tolvaptan's effect on these outcome variables in liver cirrhosis patients.

In conclusion, tolvaptan at 7.5 mg/day showed the maximum change in bodyweight and abdominal circumference together with preferable tolerability. Therefore tolvaptan at 7.5 mg/day was considered the optimal dose in the treatment of hepatic edema.

ACKNOWLEDGMENT

OTSUKA PHARMACEUTICAL FUNDED this trial and provided tolvaptan.

Table 2 Incidence of adverse events

Adverse events	Placebo ($n = 26$)	Tolvaptan			
		7.5 mg ($n = 25$)	15 mg ($n = 25$)	30 mg ($n = 25$)	Total ($n = 75$)
Total	22 (84.6)	22 (88.0)	22 (88.[illegible]	25 (100.0)	69 (92.0)
Observed in ≥5% of patients					
Thirst	1 (3.8)	6 (24.0)	14 (5[illegible]	15 (60.0)	35 (46.7)
Pollakiuria	0 (0.0)	8 (32.0)	5 (20.0)	12 (48.0)	25 (33.3)
Insomnia	1 (3.8)	1 (4.0)	6 (24.0)	6 (24.0)	13 (17.3)
Blood uric acid increased	1 (3.8)	3 (12.0)	4 (1[illegible]	5 (20.0)	12 (16.0)
Blood alkaline phosphatase increased	1 (3.8)	6 (24.0)	3 ([illegible]	2 (8.0)	11 (14.7)
Blood urea increased	4 (15.4)	4 (16.0)	4 (16.0)	3 (12.0)	11 (14.7)
Constipation	2 (7.7)	1 (4.0)	5 (2[illegible]	[illegible]	7 (9.3)
Diarrhea	2 (7.7)	5 (20.0)	2 (8.0)	0 (0.0)	7 (9.3)
Blood creatinine increased	3 (11.5)	2 (8.0)	3 (12.[illegible]	2 (8.0)	7 (9.3)
Malaise	1 (3.8)	1 (4.0)	3 ([illegible]	2 (8.0)	6 (8.0)
Pyrexia	3 (11.5)	[illegible] (8.0)	[illegible]	[illegible]	6 (8.0)
Blood bilirubin increased	5 (19.2)	[illegible] (4.0)	[illegible] (4.0)	[illegible]	5 (6.7)
Blood osmolarity increased	1 (3.8)	0 (0.0)	0 (0.0)	4 [illegible]	4 (5.3)
Decreased blood pressure	0 (0.0)	2 (8.0)	1 (4[illegible]	[illegible]	4 (5.3)

Data are expressed as number of patients (%).

REFERENCES

1 Schuppan D, Afdhal N[illegible] Liver cirrhosis. *Lancet* 2008; 371: 838–[illegible]
2 Kotoh K, Nakamuta M, [illegible]ashima N *et al.* High relative fat-free mass is important for maintaining serum albumin [illegible] patients with decompensated liver cirrhosis. *World J Gastroenterol* 2005; 11 [illegible]56–60.
3 [illegible] Management of [illegible] and ascites. *N Engl J Med* 2004; 350: 1646–54.
4 European Association for the Study of the Liver (EASL) clinical practice guidelines [illegible] the management of ascites, spon[illegible] peritonitis, and hepatorenal syndrome in [illegible]. *J Hepatol* 2010; 53: 397–417.
5 [illegible] management of ascites in cirrhosis. *J Gastroenterol* [illegible] 2012; 27: [illegible]
6 [illegible] *et al.* Diuretic strategies in patients [illegible] decompensated heart failure. *N Engl J* [illegible] 364: 797–805.
7 [illegible] Treatment of [illegible] *Intern Med* 2008; 47: 885–9[illegible]
8 [illegible] Abraham WT *et al.* Relationship between [illegible] development of worsening renal function among hospitalized patients. *Am* [illegible] 200[illegible] 47: 35[illegible]
9 [illegible] prolonged use of the vasopressin antagonist tolvaptan is safe and effective treatment for hyponatremia[illegible] *Res Nephrol* 201[illegible]; 6: 315.
10 [illegible] G, Schrier[illegible] Non-peptide arginine-vasopressin antagonists: the vaptans. *Lancet* 2008; 371: [illegible]
11 Yamamura [illegible] Nakamura [illegible] *et al.* OPC-41061, a highly potent human vasopressin V2-receptor antagonist: pharmacological profile and aquaretic effect by single and multiple oral dosing in rats. *J Pharmacol Exp Ther* 1998; 287: 860–7
12 Gassanov N, [illegible] N, Semmo M, Nia AM, Fuhr U, Er F. Arginine vasopressin (AVP) and treatment with arginine vasopressin receptor antagonists (vaptans) in congestive heart failure, [illegible] cirrhosis and syndrome of inappropriate antidiuretic hormone secretion (SIADH). *Eur J Clin Pharmacol* 2011; 67: 333–46.
13 Schrier RW, [illegible] P, Ghali[illegible] M *et al.* Tolvaptan, a selective oral vasopressin V2-receptor antagonist, for hyponatremia. *N Engl J Med* 2006; 355: 2099–112.
14 [illegible] Shin[illegible] V2 receptor antagonist; tolvaptan. [illegible] 201[illegible]; 9: 30–4
15 Zmily HD, D[illegible], Ghali JK. Tolvaptan, hyponatremia, and heart failure. *Int J Nephrol Renovasc Dis* 2011; 4: 57–71.
16 Okita K, S[illegible] Okada M *et al.* A multicenter, open-label, dose[illegible] study to [illegible] evaluate the efficacy, [illegible] dose-response of tolvaptan in patients with decompensated liver cirrhosis. *J Gastroenterol* 2010; 45: 979–87
17 Giordano [illegible] 2008 Declaration of Helsinki: some reflections. *J Med* [illegible] 2010; 36: 598–603.
18 Uemura M, [illegible]moto M [illegible] Effect of "body compression" [illegible] parameters related to ascites formation: therapeutic [illegible] cirrhotic patients. *J Gastroenterol* 1999; 34: 73–82.
19 Mochida S, [illegible] Y, N[illegible]ma Y *et al.* Diagnostic criteria of acute liver failure: [illegible] report by the Intractable

Hepato-Biliary Diseases Study Group of Japan. *Hepatol Res* 201[illegible]; 41: 805[illegible]2.

20 Yang YY, [illegible] HC, Lee WP *et al.* Association of the G-protein and α2-adrenergic receptor gene and plasma norepinephrine level with clonidine improvement of the effects of diuretics in patients with cirrhosis with refractory ascites: a randomised clinical trial. *Gut* 2010; 59: 1545–53.

21 S[illegible] BL, D[illegible] PV. Evaluation and management of [illegible] *World J Gastroenterol* [illegible]

22 [illegible] Phase III clinical pharmacology study of [illegible]ptan. *Cardiovasc Drugs Ther* 201[illegible]; 2[illegible] (Suppl [illegible]): S[illegible]

23 Kim SR, Hasunuma T, Sato O *et al.* Pharmacokinetics, pharmacodynamics and safety of tolvaptan, a novel, oral, selective nonpeptide AVP V2-receptor antagonist: results of single- and multiple-dose studies in healthy Japanese male volunteers. *Cardiovasc Drugs Ther* 2011; 25 (Suppl 1): S5–[illegible]7.

24 Rangasetty U[illegible], Gheorghiade M, Uretsky BF *et al.* Tolvaptan: [illegible] selective [illegible] pressin type 2 receptor antagonist in congestive [illegible]ure. *Expert Opin Investig Drugs* 2006; 15: 53[illegible]40.

Intraluminal duodenal diverticulum with refractory pancreatitis successfully treated by endoscopic diverticulectomy

The current report presents a rare case of intraluminal duodenal diverticulum (IDD), which is a congenital anomaly whereby the formation of a pocket-like diverticulum causes intestinal obstruction and/or refractory pancreatitis.[1–3] The present case was cured by endoscopic resection with a two-channel procedure.

A 33-year-old woman was admitted to Asahikawa Red Cross Hospital due to frequent epigastralgia. Blood examination revealed high levels of serum amylase (2278 IU/L) and lipase (7227 U/L). Computed tomography showed swelling of the pancreas and an intussusception-like change of the duodenum. She was diagnosed to have acute pancreatitis. After improving the pancreatitis with gabexate mesilate, gastroduodenoscopy detected a pocket-like lesion beside the true lumen in the duodenum. The major Vater's papilla was observed at the septum separating the pocket-like lesion. The pocket-like lesion was easily observed by pulling the bottom of the lesion (Fig. 1A,B). Barium duodenography revealed a 4-cm stagnation framed by a clear and smooth translucent line (Fig. S1). The patient was diagnosed with IDD.

Magnetic resonance cholangiopancreatography and endoscopic retrograde cholangiopancreatography revealed normal bile and pancreatic duct structures without ectopic ducts in the pocket-like lesion. The patient provided written informed consent and the lesion was endoscopically reversed and removed using a two-channel endoscope with no complications (Fig. 2A,B). This procedure allows one to maintain the reverse position of the pocket-like lesion and to clearly observe the cutting site during the resection (Video S1). Histological findings showed a submucosal layer between two mucosal layers, showing a mirror image (Fig. S2). There have been no abdominal symptoms 3 months after treatment, and at the following endoscopy the lesion had disappeared. While endoscopic treatment has been used for removing IDD in several cases,[4,5] this is the first report to show the efficacy of the two-channel procedure for removing IDD (Table S1).

Written informed consent was obtained from the patient for publication of this report and any accompanying images.

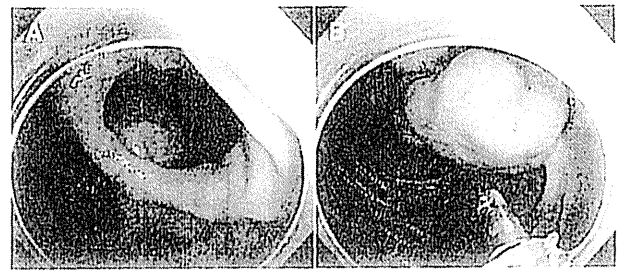

Figure 1 Endoscopic findings of intraluminal duodenal diverticulum (IDD). Gastroduodenal endoscopy revealed a pocket-like lesion beside the true lumen in the duodenum. (A) The major Vater's papilla was observed at the septum separating the pocket-like lesion from the true lumen. (B) The pocket-like lesion was easily observed by pulling the bottom of the lesion, suggesting the lesion contained a mucosal and a submucosal layer, but not a muscle layer or serosa.

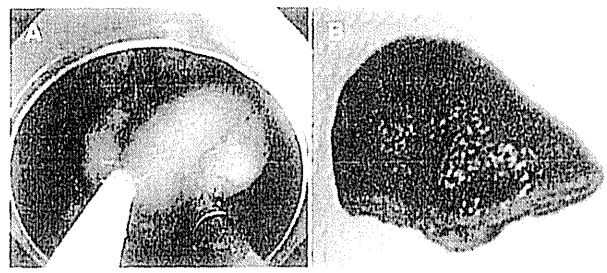

Figure 2 Endoscopic diverticulectomy. (A) The lesion was endoscopically reversed and removed by endoscopic diverticulectomy using a two-channel endoscope. (B) A pocket-like lesion with a smooth surface was removed without any complications.

Authors declare no conflict of interests for this article.

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T.I. and M.F provided major input into the conceptual development of the present report, carried out the endoscopic examinations and treatment and wrote the manuscript. Y.K. prepared/reviewed the manuscript. C.H. found the lesion and took care of the patient.

1 Silcock AQ. Epithelioma of ascending colon: congenital duodenal septum with internal diverticulum. *Tr. Path. Soc. London* 1885; 36: 207.

特　　集

肝疾患に対する外科治療
肝細胞癌治療の最近の話題

旭川医科大学　外科学講座消化器病態外科学分野[1)]
同　内科学講座病態代謝内科学分野[2)]
同　内科学講座消化器・血液腫瘍制御内科学分野[3)]
同　放射線医学講座[4)]
旭川赤十字病院消化器内科[5)]
旭川厚生病院消化器内科[6)]
札幌東徳洲会病院放射線科[7)]

宮本　正之[1)]　谷口　雅彦[1)]　内田浩一郎[1)]　渡邊　賢二[1)]
今井　浩二[1)]　麻生　和信[2)]　大竹　孝明[3)]　山田　有則[4)]
長谷部千登美[5)]　斎藤　義徳[6)]　齋藤　博哉[7)]　古川　博之[1)]

北海道外科雑誌　第58巻　第1号　別刷

特　　集

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要　　約

肝細胞癌に対して，1980年代より手術治療の進歩とともに，ラジオ波凝固療法，経皮的エタノール注入療法，肝動脈化学塞栓療法（TACE）を組み合わせた集学的治療法が発展し，予後の改善に繋がってきた。近年では従来の治療に加えて，腹腔鏡やロボット（da Vinci）による肝切除術，肝移植，分子標的薬，陽子線療法などの新しい治療法が加わり，更に幅広い集学的治療が可能となってきている。しかしながら，個々の治療に関しては未だ試験段階のものも多い。我々は，新たな知見の集積による治療法・治療戦略の開発はもちろん，定期的なフォローと各科横断的な診療体制の構築が，肝細胞癌の予後向上につながるものと考えている。

Key Words：肝細胞癌，肝切除術，集学的治療

はじめに

肝細胞癌に対して，1980年代より手術治療の進歩とともに，ラジオ波凝固療法，経皮的エタノール注入療法，TACEを組み合わせた集学的治療法が発展し，予後の改善に繋がってきた。近年ではこれらの治療法に加え，腹腔鏡やロボット（da Vinci）による肝切除術，肝移植，分子標的薬，陽子線療法などの新しい治療法が加わり，更に幅広い集学的治療が可能となってきている。

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札幌東徳洲会病院放射線科[7)]

本稿では肝細胞癌における最近の治療法について概説する。

腹腔鏡下肝切除術

2010年より我が国で腹腔鏡下肝外側区域切除術，腹腔鏡下肝部分切除術が保険収載となり，肝細胞癌に対しても腹腔鏡下肝切除術を導入する施設が増加している。外側区域以外の腹腔鏡下肝切除についても，先進的治療として，種々のアプローチでの導入が報告されている。

非肝硬変患者における腹腔鏡下肝切除（major）の検討では，患者予後と医療コストについては，開腹手術と同様の成績であったが[1)]，腫瘍の局在によっては，腹腔鏡下肝切除術の方が安全性が高いことが判明した。Slakey, D. Pらは，単施設での腹腔鏡下肝切除45例と開腹肝切除17例の比較において，腹腔鏡下肝切除の方

が合併症（15.5％ vs 52.9％，p＝.007），術中出血（95.0±89ml vs 988.0±1450ml，p＝.0001）が少なく，ICU 在室日数５日以上（腹腔鏡３例 vs 開腹５例，p＝.05），在院日数10日以上（腹腔鏡３例 vs 開腹６例，p＝.01）についても腹腔鏡手術群の方が良好と報告している。特に，後上区域切除の患者で，開腹に比して，腹腔鏡下の方が合併症率が低いのが特徴的であった[2]。腹腔鏡下肝切除と開腹肝切除を比較した meta-analysis では，腹腔鏡下肝切除は，手術時間，胆汁漏，術後出血，肺合併症，腹腔内膿瘍，死亡率，断端陽性率，再発率には差がなかったものの，術中出血が129ml少なく（95％ C.I.：224-36ml，p＝.008），輸血の頻度も少なく（オッズ比0.49，95％ C.I.：0.26-0.91，p＝0.02），在院日数が3.19日短く（95％ C.I.＝4.09-2.28，p＜0.00001），術後腹水の発生頻度も少なかった（オッズ比0.32，95％ C.I.：0.16-0.61，p＝0.006）と結論づけている[3]。一方，腹腔鏡を用いた肝切除術（葉切除）の長期予後に関して述べた論文は少なく，肝細胞癌に対する腹腔鏡下肝葉切除術後の５年生存率は50-64.9％と報告されており[4][5]，開腹肝切除術に比して遜色ない結果と考えられる。

以上より，背景肝，合併症などの患者因子と，解剖学的局在や大きさ，脈管侵襲などの腫瘍因子を揃えて患者群を比較した場合，腹腔鏡下肝切除術の周術期合併症の発生率は開腹手術と比して遜色なく，腹腔鏡下手術では，さらに創が小さいことによる整容性向上や術後疼痛軽減などの利点が得られると思われる。腹腔鏡下肝葉切除術の長期予後に関しては，今後さらなる症例の蓄積が待たれる。

ロボット（da Vinci）肝切除術

近年，ロボット手術の導入が積極的に行われるようになり，肝切除においても施行例が報告されている。単一の術者による30例のロボット肝切除の報告では，右葉切除６例，左葉切除14例，その他10例を行い，平均手術時間は右葉切除724分，左葉切除518分，平均出血量は右葉切除629ml，左葉切除328mlであった。開腹手術移行率は全体で6.7％，葉切除で10.0％であった。合併症は43％（13例）に認められたが，肝切除に直接起因する合併症は，胆汁漏を２例に認めたのみであった。11ヶ月（中央値）の追跡期間で肝癌の再発は認めていない。この結果を他の腹腔鏡下肝切除の論文[7]と比較・参照することにより，ロボット手術を施行可能で安全な手技であると結論づけている。手術時間の長さが問題であるが，今後，経験の蓄積に伴って短縮されることが期待される。

また，meta-analysis による217例のロボット肝切除術のまとめによると，ロボット肝切除で最も行われている手技は楔状切除と区域切除であった。開腹手術への移行率は4.6％，移行の理由は，腫瘍マージンが不明瞭であったとするものが最も多かった。ロボット肝切除手術は腹腔鏡手技に長けた外科医にとっては，安全に施行可能であり，周術期合併症などを含めた短期成績は，従来の腹腔鏡下肝切除術と遜色なかった[8]。

ロボット手術は費用対効果や長期成績がまだ不明であり，低侵襲性に関する利点と比較検討した今後の研究が待たれる。

陽子線療法

これまで HCC に対する放射線療法は，正常肝への影響が大きいことから，その役割は限定的であった。３次元原体照射や定位照射の進歩によって，腫瘍への放射線照射を集中できることから，照射線量の増加が可能となったが，依然として正常肝への影響は無視できない。陽子線は体内の照射経路においてほとんど放射線を放出しない生理的特質を持ち，目的とする腫瘍領域へより多量の放射線を照射することが可能である[9]。

本邦での肝細胞癌手術非施行患者への陽子線治療の治療成績は，162患者の５年生存率が23.5％，162患者192腫瘍の５年局所制御率が86.9％であった。全死亡例145例の53.1％（77例）は癌死ではなく背景の肝硬変に起因する死亡であり，局所療法としては十分な成績をあげたものと考えられる[10]。また，10cm以上の肝細胞癌患者22例に対して陽子線療法を行った報告では，追跡期間13.4ヶ月（中央値）で，２年腫瘍制御率は87％，２年全生存率と無増悪生存率は，それぞれ36％，24％であった。Grade 3 以上の合併症は認めなかった。このことから著者は，陽子線療法は効果的で安全な治療法であると結論づけている[11]。

問題として，我が国で陽子線治療を受けられる施設は数施設に限られていること，保険適用外となるため治療費が高額（約300万円）となることがあげられる。

ラジオ波焼灼術（RFA）

RFA は肝細胞癌に対する局所治療として，内科的，

外科的に広く行われている。我が国の肝癌診療ガイドラインでは，穿刺局所療法の適応は，Child-Pugh 分類AまたはBの肝機能，腫瘍径3 cm以下かつ腫瘍数3個以下のHCCとされる[12]。さらに，経皮的エタノール注入療法（PEIT）に比してRFAが局所制御能にすぐれ，生存率を向上した[12][13]。RFAは一般的に，局所の腫瘍制御，再発抑制，症状緩和，肝切除不可能な患者の予後改善に用いられる。現在，RFAは，限局しているが切除不能なHCC，肝転移に対する原発または補助治療として評されている[14]。Cucchettiらのレビューでは，2 cm以下のHCCで解剖学的に穿刺可能なものに対してRFAを行うことが，完全壊死とsafety marginの点から推奨されている。2 cm以上または3 cm以上で，焼灼が安全で効果的でない腫瘍に関しては，外科的切除が好ましいようである[15]。

Duanらの2編のRCTと10編のnon-RCTを含む論文のMeta-analysisによると，Milan基準（HCCが単発5 cm以下，多発3個以下でおのおの3 cm以下，遠隔転移，リンパ節転移，Vp2以上の肉眼的脈管浸潤なし）内のHCCに対する肝切除とRFAの比較では，1年全生存率では有意差はなかったが，1年無増悪生存率，3年，5年の全生存率，無増悪生存率に関しては，肝切除の方がRFAよりも有意に良好であった。合併症率，在院期間についてはRFAの方が有意に良好な結果であった。以上の成績から，肝細胞癌の治療として肝切除が第一選択と考えられた[16]。現在我が国において，初発肝癌患者に対するRFAと肝切除のRCTが施行中である（SURF trial）[17]。肝細胞癌治療の新たな一歩となるエビデンスが得られることが期待されており，症例の集積が進められている。

全身化学療法/分子標的薬/TACE

進行・切除不能肝細胞癌に対する全身療法としては，2007年のASCOで発表されたSorafenib[18]が2009年より我が国でも使用可能となり，広く臨床で使用されている。しかし，Sorafenib以降の治療ラインについては，様々な報告がなされているものの，未だ標準的な治療法はない[19]。

肝細胞癌の発癌に関する分子経路としては，Sorafenibの標的であるVEGFR，PDGFR，FGFなどに代表される血管新生関連経路の他，EGFRなどの成長因子，HGF/c-Met，PI3K/Akt/mTORなどの多様な経路が見つかっており，それぞれに対する阻害剤が開発され，臨床試験が行われている[20][21]。具体的には，表1に示すような分子標的薬に対して，Phase IからIIIの試験が行われているが[21]，これら以外にエピジェネティックなDNAの修飾に関連した発癌を阻害する薬剤（Belinostat）も開発されている。切除不能肝細胞癌に対してBelinostatを投与したPhase II試験では，無増悪生存期間と全生存期間はそれぞれ2.64か月と6.60か月，Stable disease rateは45.2%であり[22]，許容範囲の毒性で腫瘍の進行を阻止できる薬剤として期待されている。

HCCの発癌経路は非常に多様であり，主となる経路の存在は確認されていない。肝細胞癌の全遺伝子解析において，同一患者の多中心性発生と思われる腫瘍のペアからでも，共通の体細胞変異は同定されなかった[23]。Sorafenib以外の新規薬剤に関して，単剤での有効性が明らかであるものは未だ存在せず，個々の腫瘍において活性化している経路に応じた分子標的薬剤の複合治療が必要となる可能性がある。TACEにより腫瘍内が低酸素環境に置かれることから，VEGFなどの血管新生関連因子が増加することが示されており[24]，TACEと血管新生阻害剤の組み合わせによる治療の可能性が検討されている[21]。また，Doxorubicin溶解ビーズを用いたTACEとSorafenibの複合治療もPhase II trialが行われており，その効果が期待されている[25]。

また，近年様々な固形腫瘍において癌幹細胞（CSCs）の存在が報告されている。最近の研究では上皮－間葉移行（EMT）とCSCsが密接に関連していることが考えられている[26]。HCCにおいても，CD133，CD90などの分子がCSC markerとして報告されており，新たなbiomarkerとしての臨床応用が期待される。

肝　移　植

肝硬変を背景とした肝細胞癌に対する最も根治的な治療は，肝移植である。我が国では，海外に比して脳死肝移植が圧倒的に少なく，慢性的なドナー不足の問題がある。今後，生体肝を含むドナープールの効率的な利用と，移植後の再発抑制と免疫抑制を両立させるレジメンの開発が課題とされる[27]。

肝細胞癌に対する肝移植適応に関しては，長年Milan基準が世界標準であった。しかし，Milan基準外の進行した腫瘍を持つ患者について，肝移植後，4分の3近くが癌を再発していないことから，これらの患者をいかにして肝移植適応患者として拾い上げるか

表1 肝細胞癌に対する新規標的薬剤の早期治療成績（文献(20)より引用）

Agent	Phase	N	Efficacy
Tivantinib[45]	Randomized Phase II Tivantinib vs placebo ITT population c-Met high	 71 vs 36 22 vs 15	 Median TTP: 6.9 weeks vs 6.0 weeks Median OS: 6.6 months vs 6.2 weeks Median TTP: 11.7 weeks vs 6.1 weeks Median OS: 7.2 months vs 3.8 weeks
Sunitinib	Phase II[40]	34	Median PFS: 3.9 months Median OS: 9.8 months
	Phase III[41] Sunitinib vs sorafenib	1073	Median OS: 7.9 vs 10.2 months;
Brivanib	Phase II[42]	55	Median PFS: 2.7 months Median OS: 10 months
	Phase III (BRISK-PS)[43] Brivanib vs placebo	395	Median OS: 9.4 months vs 8.3 months TTP: 4.2 months vs 2.7 months RR: 12% vs 2%
Linifanib[44]	Phase II	44	TTP: 5.4 months Median OS: 10.4 months
Erlotinib	Phase II[45]	38	Median OS: 13.0 months
	Phase II[46]	40	Median OS: 10.8 months
	Phase III (SEARCH)[47] Sorafenib/erlotinib vs sorafenib/placebo	362	Median OS: 9.5 months vs 8.5 months TTP: 3.2 months vs 4.0 months
Bevacizumab[48]	Phase II	46	Median PFS: 6.9 months Median OS: 12.4 months
Ramucirumab[49]	Phase II	42	Median PFS: 3.9 months Median OS: 14.9 months
Everolimus[50]	Phase I/II	20	Median PFS: 3.8 months Median OS: 8.4 months
Pegylated arginine deiminase[51]	II	80	Mean OS: 15.8 months

Abbreviations: ITT, intent to treat; OS, overall survival; PFS, progression-free survival.

が大きな課題であった。そのため，従来のMilan基準よりも多くの患者に移植の機会をもたらし，かつ再発率を抑え生存率を担保した基準の策定が模索されている。術前画像診断，術後病理診断において，Milan基準を超える腫瘍条件でも，術前のAFPが200ng/ml以下かつPIVKA-IIが100mAU/ml以下であれば，Milan基準内でAFPとPIVKA-IIが高値である症例とほぼ同等の予後が得られることが示されている[28]。表2は現在提唱されている移植基準と，それぞれの5年生存率を示した表である（文献29)より抜粋)。各拡大基準とも，Milan基準と概ね同等の75%前後の5年生存率が得られており，適応外症例ではMilan基準よりも低い生存率となっている。すなわち，移植機会の増加に伴い生存率を低下させることなく，Milan基準では移植適応外となっていた患者の中で，移植により良好な予後が得られる患者群と，予後不良となる患者群の，より鋭敏な選別に成功していると言える。近年では，腫瘍の大きさと数のみでなく，腫瘍の分化度や微小脈管浸潤が肝移植後の予後に影響を与えることが明らかとなり，画像上認識できない微小脈管浸潤の存在を拾い上げるbiomarkerの探索や，これらの因子の移植適応基準への応用が模索されている[29]。

表2 肝移植適応基準ごとの成績（文献(28)より抜粋）

Criteria			5yr PS(%)
Milan;	Single, <5cm	within	77.5
	n<3, s<3cm	beyond	61.6
Up to 7;	n + size(cm) <7	within	76.8
		beyond	56.8
Asan;	n<6, s<5cm	within	75.5
	Vp0 or Vp1	beyond	54.7
Kyoto;	n<10, s<5cm	within	75.2
	PIVKA-II <400	beyond	55.6
Tokyo;	5 x 5 rule	within	75.9
		beyond	55.9
Kyushu;	s <5cm	within	73.7
	PIVKA-II <300	beyond	57.8