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ORIGINAL ARTICLE

Apelin is a marker of the progression of liver fibrosis and portal hypertension in patients with biliary atresia

Wei Chen · Takaharu Oue · Takehisa Ueno · Shuichiro Uehara · Noriaki Usui · Masahiro Fukuzawa

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Abstract
Purpose Apelin, the endogenous ligand of the angiotensin-like-receptor 1 (APJ), is thought to play an important role in liver disease. This study investigated the apelin expression in different stages of biliary atresia (BA) and investigated whether it is associated with the progression of disease.
Methods Liver tissues were obtained from patients at Kasai's procedure (KP), the follow-up stage after KP (Post-KP) and at liver transplantation (LT). Immunohistochemistry for apelin and its receptor APJ and real-time quantitative reverse transcriptase polymerase chain reaction for *apelin* mRNA expression were conducted.
Results The immunohistochemical study revealed that apelin was mainly localized in the perivenular areas of control liver tissue, and slightly detected in the hepatic stellate cells (HSC) and hepatocytes, whereas intense apelin immunoreactivity was detected in perivenular areas, HSC and hepatocytes of LT liver tissue. The *apelin* mRNA expression level was significantly higher in the LT group than in the KP and Post-KP group. Significant linear correlations were observed between the *apelin* mRNA level and liver fibrosis, serum total bilirubin and the grade of esophageal varices.
Conclusions The hepatic apelin–APJ system is markedly activated in the progression of BA, especially in end-stage cirrhosis. The apelin expression level accurately reflects the severity of hepatic fibrosis and esophageal varices and therefore could be used as a prognostic factor in BA patients.

Keywords Apelin · APJ · Biliary atresia · Liver fibrosis · Esophageal varices · Liver transplantation

W. Chen · T. Oue (✉) · T. Ueno · S. Uehara · N. Usui · M. Fukuzawa
Department of Pediatric Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
e-mail: ooue@pedsurg.med.osaka-u.ac.jp

W. Chen
Department of Pediatric Surgery, Beijing Children's Hospital, Xicheng, Beijing 100045, China

Introduction

Apelin, initially isolated by Tatemoto [1] and his coworkers from bovine stomach homogenates in 1998, is recognized as the endogenous ligand of angiotensin-like-receptor 1 (APJ), and the human orphan G-protein-coupled receptor, which has a close identity with the angiotensin II receptor, but does not bind angiotensin-II [2].

Apelin and its receptor are highly expressed in the central nervous system and in peripheral tissues, where it is involved in the regulation of the cardiovascular tone [3], cardiac contractility [4], glucose metabolism [5], gastrointestinal track physiology [6], and water homeostasis [7].

Recent studies have demonstrated that apelin is overexpressed in hepatic stellate cell (HSC) from both cirrhotic human and rats [8, 9] and the expression is enhanced in proliferative hepatic arterial capillaries in human cirrhotic liver [10].

Biliary atresia (BA) is characterized by complete obliteration of extrahepatic bile duct. Although bile flow can be established by a Kasai portoenterostomy (KP), progressive liver fibrosis and portal hypertension continue to develop in most patients with BA [11]. Therefore, apelin is thought to

be over-expressed in the liver of BA patients. However, no information is available regarding the apelin expression in liver tissue of BA patients. The present study was undertaken to investigate the apelin expression in different stages of BA patients, and especially focused on the correlation between apelin expression and the clinical features such as liver function, hepatic fibrosis, and esophageal varices.

Materials and methods

Patients and samples

Fifty-five BA patients including 19 males and 36 females with a mean age of 8.4 ± 8.2 years (range 37 days–29.6 years) that were treated and followed at Osaka University Hospital between June 2009 and June 2012 were included in this study. A total of 72 liver tissue samples were taken from them, including 4 wedge or needle biopsy samples taken at the time of KP, 29 needle biopsy samples from the follow-up stage after KP (Post-KP) patients, 9 liver tissues taken at time of LT, and 30 needle biopsy samples from Post liver transplantation (Post-LT) patients. Control liver samples included non-tumor containing parts of surgically removed liver tissues from three children with hepatoblastoma and two normal tissues from patients with choledochal cysts. All liver tissues in the current study were obtained after acquiring written informed consent from the parents or healthy adult donors. The protocol for this study was approved by the Ethics Committee of the Institutional Review Board of Osaka University Hospital.

Immunohistochemistry

Liver tissues were fixed in formalin and embedded in paraffin. 4 μm sections were cut, deparaffinized, and dehydrated using graded ethanol. They were incubated overnight at 4 °C with 1:800 dilution of anti-apelin rabbit antibody (Phoenix Pharmaceuticals, INC., Burlingame, CA, USA.) or 1:500 dilution of apelin receptor rabbit antibody (MBL International, Woburn, MA, USA). The sections were washed with Dako Wash Buffer (Dako, Tokyo, Japan), incubated with peroxidase labeled polymer conjugate (Envision® system) (Dako) at room temperature for 30 min and then reacted with the DAB chromogen. The sections were finally counter-stained with hematoxylin for light microscopic study. The intensity of immunostaining was scored as: 0, none; 1+, weak; 2+, moderate; 3+, intense.

Evaluation of *apelin* mRNA expression using quantitative real-time PCR

Total RNA was extracted from frozen samples using TRIzol RNA isolation reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations. Template cDNA was obtained by reverse transcription of 1 μg of total RNA using a cDNA synthesis Kit (Prime Script™ RT-PCR Kit, TaKaRa, Japan). The reaction mixtures were incubated at 30 °C for 10 min, 42 °C for 30 min and 95 °C for 5 min. The cDNA was diluted five-fold for real-time PCR.

The sequences of the primers in this study were: sense primer 5′-GGCCATCACCAGCCATTCCTTG-3′ and anti-sense primer 5′-GGGCATCAGGCTCTTGTCTTCTCT-3′. The quantification of gene-expression levels for apelin was carried out by real-time quantitative PCR on an ABI ViiA™ 7 System (TaqMan, Perkin-Elmer Applied Biosystems). The SYBR Premix Ex TaqT II kit (TaKaRa) was used for real-time monitoring of amplification (45 cycles: 95 °C/15 s, 60 °C/1 min). The study used the comparative cycle threshold (Ct) method to calculate relative mRNA expression. All quantifications were normalized by the corresponding expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression (forward primer: 5′-GAAGGTGAAGGTCGGAGTCA-3′; reverse primer: 5′-GAAGATGGTGATGGGATTTC-3′).

Clinical presentation of BA patients following clinical features collected from the patients charts

Serum total bilirubin TB (mg/dl), aspirate amino-transferase AST (IU/L), alanine amino-transferase ALT (IU/L), and gamma glutamyl transpeptidase GGTP (IU/L) were measured in the Osaka University Hospital laboratory and analyzed.

Classification of esophageal and liver fibrosis

Esophagogastroduodenoscopy (EGD) and a liver biopsy were performed at the same time in Post-KP and Post-LT patients, as a routine examination to evaluate the progression of esophageal varices and liver fibrosis, respectively. The grade of an esophageal varix was classified according to Japan Society for Portal Hypertension classification, as: F0, no varicose appearance; F1, straight, small-caliber varices; F2, moderately enlarged, beady varices; F3, markedly enlarged, nodular or tumor-shaped varices [12], and the grade of liver fibrosis was classified

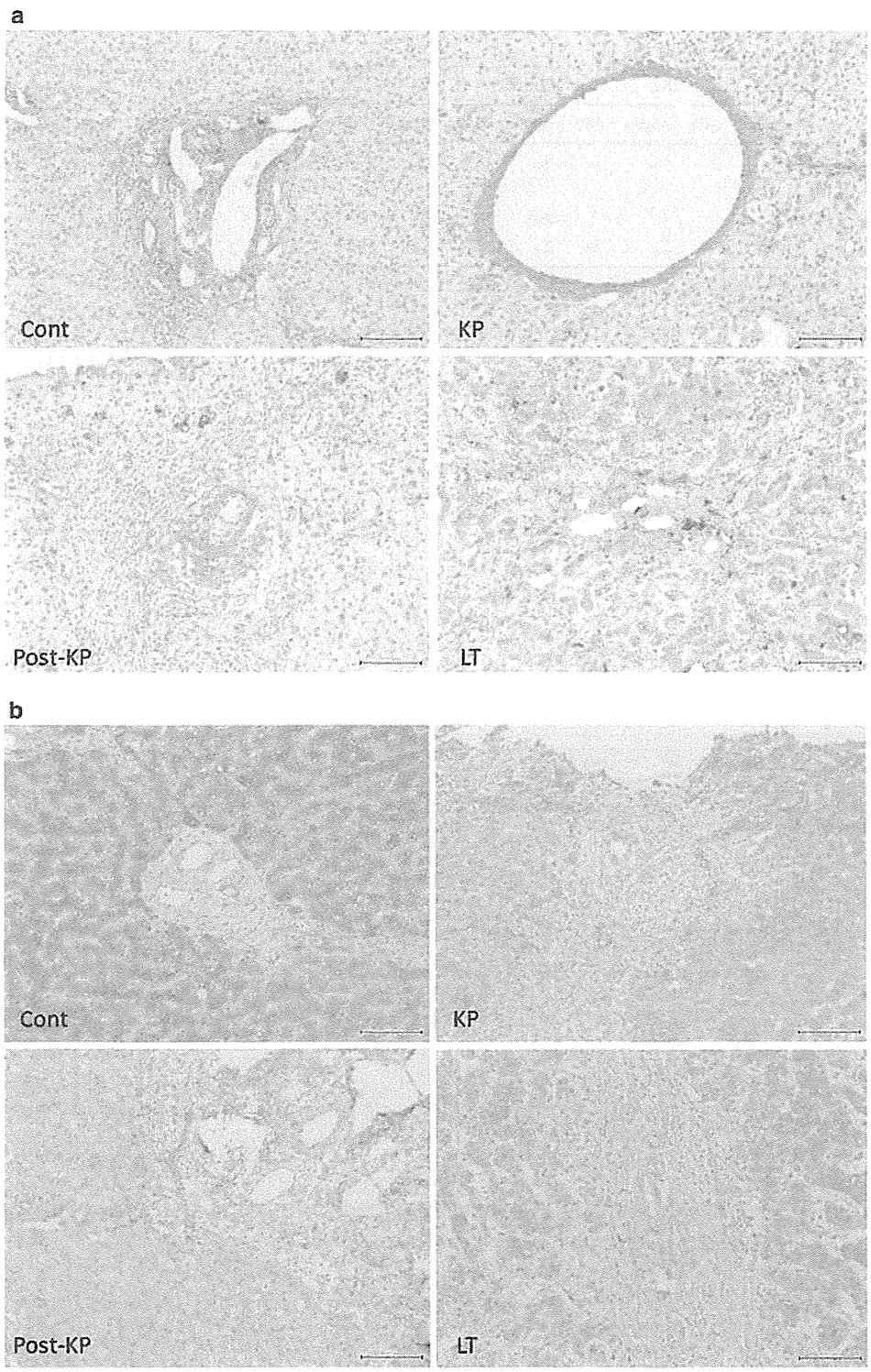

Fig. 1 The protein expression of apelin (**a**) and apelin receptor APJ (**b**) was immunolocalized in liver sections of control (*Cont*) and the stages of BA, including Kasai procedure (*KP*), *Post-KP* and liver transplantation (*LT*). **a** The control liver tissue showed that apelin was mainly localized in the perivenular areas (large portal vein and ventral vein; white arrows), and slightly detected in the HSC and hepatocytes. Apelin was observed mainly in perivenular areas and capillaries in *KP* and *Post-KP* liver tissue, and slight to moderately in HSC. Intense apelin immunoreactivity was detected mainly in perivenular areas, HSCs and hepatocytes in *LT* liver tissue. **b** APJ was almost undetected in perivenular areas but detected mainly in the hepatocytes in controls and all stages of BA

according to New Inuyama classification, as: F0, no fibrosis; F1, fibrous portal expansion; F2, bridging fibrosis; F3, bridging fibrosis with architectural distortion; and F4, liver cirrhosis [13].

Statistical analysis

Data were entered into the SPSS (Chicago IL) 16.0 software package. Mann–Whitney *U* test, Kruskal–Wallis test,

Table 1 Intensity of apelin and APJ immunostaining in different stages of BA

	CO		KP		Post-KP		LT	
	Apelin	APJ	Apelin	APJ	Apelin	APJ	Apelin	APJ
Perivenular area	2+	–	2+	+	2+	+	3+	+
HSC	+	–	+	–	2+	–	3+	–
Hepatocytes	+	2+	2+	2+	2+	2+	3+	3+

The intensity of immunostaining was scored as: –, none; 1+, weak; 2+, moderate; 3+, intense

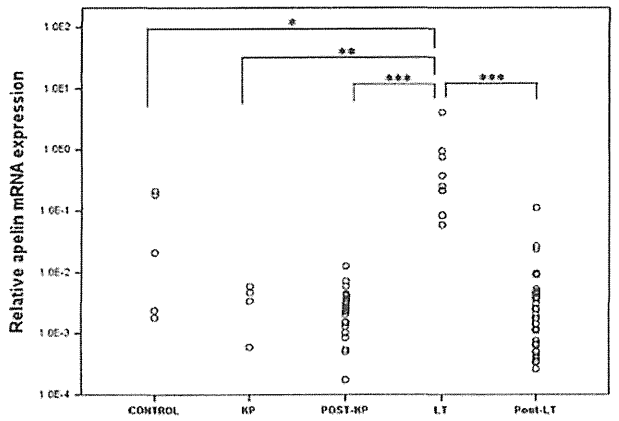

Fig. 2 Relative mRNA expression in the livers of BA patients. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

Wilcoxon signed rank test, and Spearman's correlation coefficient were used. Significance levels were set at $p < 0.05$.

Results

Immunohistochemical expression of apelin and its receptor APJ (Fig. 1a, b)

Apelin was mainly localized in the perivenular areas (large portal vein and ventral vein), in control liver tissue and slightly detected in the HSC and hepatocytes. Apelin was observed mainly in perivenular areas in KP and Post-KP liver tissue, and slightly to moderately expressed in HSC and hepatocytes. Intense apelin immunoreactivity was detected in perivenular areas, HSC and hepatocytes in LT liver tissue (Fig. 1a). APJ was mainly and markedly detected in the hepatocytes, but almost undetected in the perivenular area and HSC. Slight APJ expression was also observed in perivenular areas at the KP, Post-KP and LT stage (Fig. 1b) (details summarized in Table 1).

Table 2 Details of BA patients that provided specimens in several stages

Patients	Sex	Age when the operation was performed		
		KP	Post-KP	LT
1	M	67 days	191/331 days[a]	518 days
2	F	74 days	383 days	–
3	M	88 days	–	242 days
4	F	–[b]	4.2/4.6 years[a]	–
5	F	–[b]	3.1/4.1 years[a]	–
6	F	–[b]	183 days	265 days
7	F	–[b]	408 days	977 days

[a] Patient underwent a needle liver biopsy after KP twice

[b] Patient underwent KP in other institution

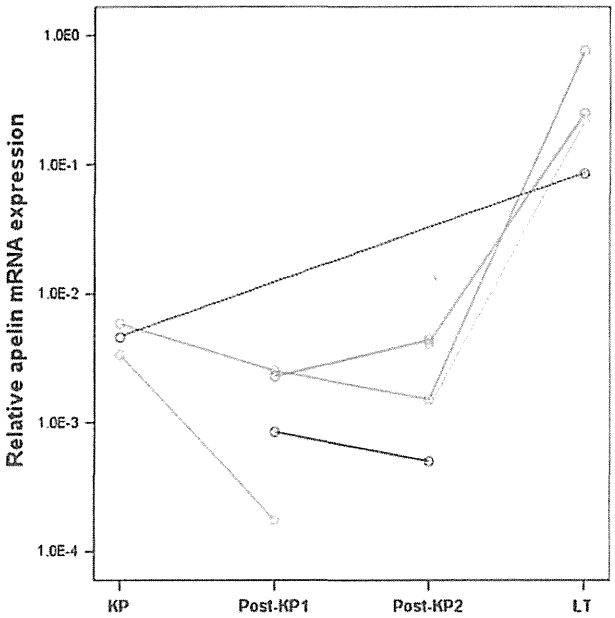

Fig. 3 Changes in the relative *apelin* mRNA expression in the same patients during the progression of BA

mRNA expression of apelin in liver tissue in BA

Apelin mRNA expression was significantly higher in the LT group than in the control, KP, Post-KP and Post-LT groups (Fig. 2: LT versus CO, $p < 0.05$; LT versus KP, $p < 0.01$; LT versus Post-KP, $p < 0.001$, and LT versus Post-LT, $p < 0.001$).

The alteration of apelin expression during the progression of BA was also analyzed in the same patients (see the details in Table 2). The result showed that *apelin* mRNA expression levels in the samples from the patients that underwent LT (patient 1, 3, 6 and 7, Fig. 3) were increased nearly ten-fold in comparison to the samples taken from them in the KP and Post-KP stages ($p = 0.068$).

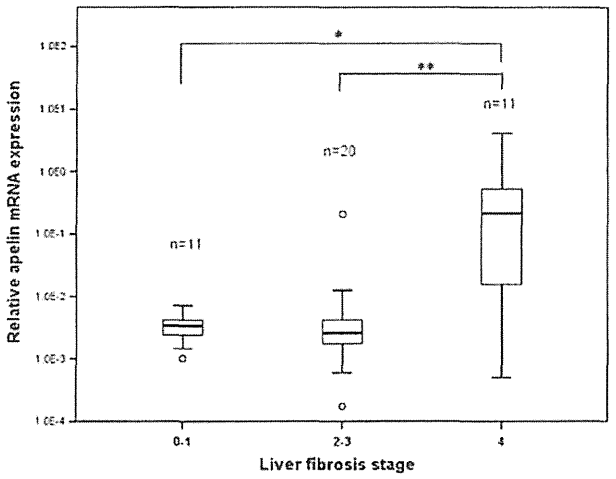

Fig. 4 Apelin increases with the progression of fibrosis in BA patients. The number of patients per group is shown above each bar. *Boxes* encompass the 25th to 75th percentile, *horizontal lines* represent the median (50th percentile), and *whiskers* extend to the smallest and highest values. (*$p < 0.05$, **$p < 0.01$)

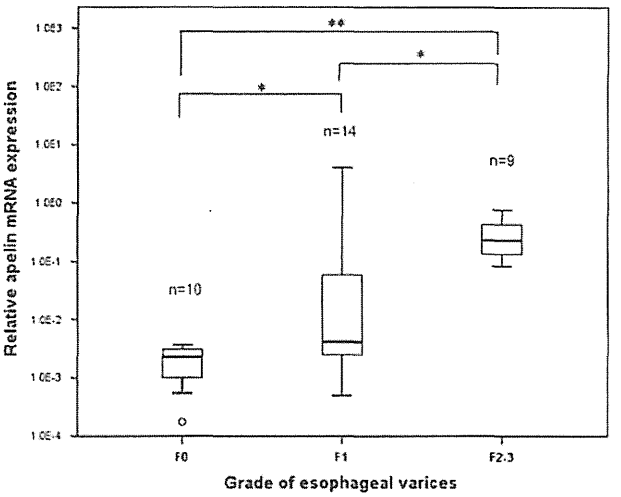

Fig. 5 *Apelin* mRNA expression with the progression of esophageal varices in BA patients. (*$p < 0.05$, **$p < 0.01$)

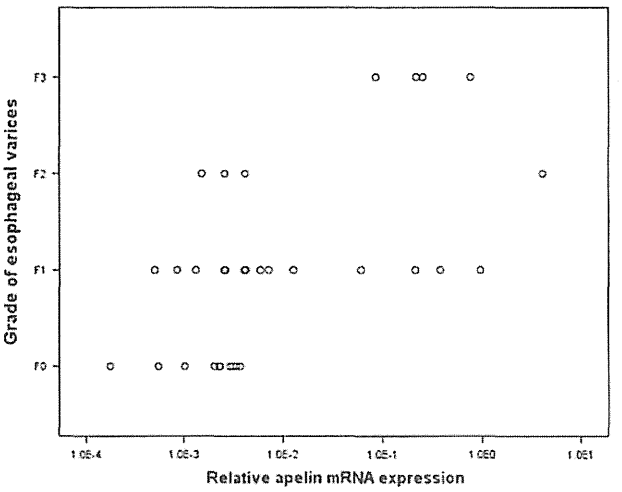

Fig. 6 Correlation between the relative *apelin* mRNA expression and the grade of esophageal varices in BA. (Spearman's $r_s = 0.522$, $p < 0.01$)

Forty-two liver samples from BA patients of KP, Post-KP and LT were subjected to real-time PCR in order to assess *apelin* mRNA expression during the progression liver fibrosis. *Apelin* mRNA expression was significantly higher at fibrosis stage 4 than at stage 0–1 and stage 2–3 (stage 4 versus stage 0–1, $p < 0.05$; stage 4 versus stage 2–3, $p < 0.01$: Fig. 4).

Endoscopy was performed in 32 patients to evaluate the grade of esophageal varices. *Apelin* mRNA expression was significantly correlated with grade of esophageal varices, a grade-dependent upregulation of *apelin* mRNA expression increased significantly during the progression of esophageal varices (grade 2–3 (F2–3) versus grade 0 (F0), $p < 0.01$; grade 1 (F1) versus grade 0 (F0) and grade 2–3 (F2–3), $p < 0.05$; Fig. 5). A significant correlation was found between the *apelin* mRNA expression in the liver tissues and the grade of esophageal varix ($r_s = 0.522$, $p < 0.01$; Fig. 6).

The relative apelin RNA expression level was significantly correlated with the serum TB level ($r_s = 0.520$ $p < 0.001$), but did not correlate with the value of AST, ALT or GGP (Table 3).

Discussion

Apelin and its receptor system have attracted widespread research interest and their pathophysiological roles are gradually emerging [14, 15]. Studies assessing the cell distribution of apelin and APJ in normal and cirrhotic human and rat liver tissues demonstrated a strong positive signal for apelin and APJ in the liver of cirrhotic animals [9] and humans [16]. Therefore, an immunohistochemical study was performed to establish whether apelin or APJ were expressed in different stages of BA liver. The results show for the first time that apelin was expressed in liver tissue of BA patients. The finding that apelin was mainly identified in HSCs and perivenular areas, especially strongly in cirrhotic liver and that APJ was mainly identified in hepatocytes is consistent with previous studies [9, 16]. However, the strong detection of apelin in hepatocytes in a cirrhotic liver has not been reported. These results indicate that apelin and the APJ system in liver tissue are also active in pediatric patients with BA, and beyond its hepatic paracrine presence in the cirrhotic liver, apelin may

Table 3 Correlation between the expression of *apelin* mRNA and laboratory data in BA patients

	Correlation coefficent	Statistical significance
TB	0.527	<0.0001
AST	0.095	n.s.
ALT	0.109	n.s.
GGP	−0.238	n.s.

Spearman correlation test

n.s. not significant

also behave as an autocrine substance in the BA liver. Hepatocytes themselves, therefore, may play an important role in the progression of liver fibrosis in BA.

Furthermore, the current study extracted total mRNA from BA liver and performed real-time PCR to assess *apelin* mRNA expression during the progression of liver fibrosis. The results showed that the *apelin* mRNA expression was significant higher in end-stage (LT) than controls, early-stage (KP, Post-KP) BA, and decreased to normal in comparison to the controls after liver transplantation. These results of mRNA expression were consistent with the findings of apelin protein expression detected by immunohistochemistry. These findings suggest that apelin may be a significant predictor of poor prognosis (need liver transplantation) in BA patients. Although the statistical analysis showed no significant alternations of *apelin* mRNA expression in the same patients during the progression of BA that may be due to the limited number of cases, because the expression in those four patients was much higher at the end stage than the early stage. In addition, the current study showed that *apelin* mRNA expression was significantly correlated with the level of serum TB; however, there was no association with liver function (AST, ALT, and GGP). This finding may indicate that apelin is associated with cholestasis, but is not affected by liver inflammation.

The development of the KP improved the prognosis for children with biliary atresia [17–20]. Despite the increasing number of patients who survive jaundice-free for an indefinite period after KP, liver fibrosis progresses in many patients [21, 22] and the risk of gastrointestinal (GI) bleeding due to HP is why BA continues to be the leading indication for pediatric liver transplantation. The current study showed a grade-dependent upregulation of *apelin* mRNA expression in liver tissue with the progression of cirrhosis and esophageal varices in BA, which demonstrated that apelin can accurately reflect the severity of cirrhosis and esophageal varices in BA and therefore could be used as a prognostic factor to estimate the timing of liver transplantation in BA patients.

These results suggest that apelin could be used as a clinical parameter in evaluating the severity of cirrhosis and esophageal varices.

Principe et al. [9] reported that rats with cirrhosis treated with the apelin receptor antagonist showed diminished hepatic fibrosis and vessel density, improved cardiovascular performance, and renal function and lost ascites. These findings suggest that the apelin–APJ system could be a candidate for a therapeutic target of anti-fibrosis and anti-portal-hypertension treatment. Further investigation is needed to establish the clinical application of the apelin–APJ system in patients with BA.

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References

1. Tatemoto K, Hosoya M, Habata Y et al (1998) Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem Biophys Res Commun 25:471–476
2. O'Dowd BF, Heiber M, Chan A et al (1993) A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. Gene 136(1–2):355–360
3. Ishida J, Hashimoto T, Hashimoto Y et al (2004) Regulatory roles for APJ, a seven-transmembrane receptor related to angiotensin-type 1 receptor in blood pressure in vivo. J Biol Chem 279:26274–26279
4. Szokodi I, Tavi P, Foldes G et al (2002) Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cadiac contractility. Circ Res 91:434–440
5. Boucher J, Masri B, Daviaud D et al (2005) Apelin, a newly identified adipokine up-regulated by insulin and obesity. Endocrinology 146:1764–1771
6. Wang G, Anini Y, Wei W et al (2004) Apelin, a new enteric peptide: localization in the gastrointestinal tract, ontogeny, and stimulation of gastric cell proliferation and of cholecystokinin secretion. Endocrinology 145:1342–1348
7. De Mota N, Reaux-Le Goazigo A, El Messari S et al (2004) Apelin, a potent diuretic neuropeptide counteracting vasopressin actions through inhibition of vasopressin neuron activity and vasopressin release. Proc Natl Acad Sci 101:10464–10469
8. Hiroaki Y, Masaya O, Kazunori Y et al (2011) Overexpression of apelin receptor (APJ/AGTRL1) on hepative stellate cells and sinusoidal angiogenesis in human cirrhotic liver. J Gastroenterol 46:222–231
9. Principe A, Melgar-Lesmes P, Ferna'ndez-Varo G et al (2008) The hepatic apelin system: a new therapeutic target for liver disease. Hepatology 48:1193–1201
10. Yokomori H, Oda M, Yoshimura K et al (2012) Enhanced expressions of apelin on proliferative hepatic arterial capillaries in human cirrhotic liver. Hepatol Res 42(5):508–514
11. Okazaki T, Kobayashi H, Yamataka A et al (1999) Long-term postsurgical outcome of biliary atresia. J Pediatr Surg 34:312–315
12. Tajiri T, Yoshida H, Obara K et al (2010) General rules for recording endoscopic findings of esophagogastric varices (2nd edn). Dig Endosc 22:1–9

13. Ichida F, Tsuji T, Omata M et al (1996) New Inuyama classification for histological assessment of chronic hepatitis. Hepatol Commun 6:112–119
14. Lee DK, Cheng R, Nguyen T et al (2000) Characterization of apelin, the ligand for the APJ receptor. J Neurochem 74:34–41
15. Hosoya M, Kawamata Y, Fukusumi S et al (2000) Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin. J Biol Chem 275:21061–21067
16. Hiroaki Y, Masaya O, Kazunori Y et al (2012) Enhanced expression of apelin on proliferative hepatic arterial capillaries in human cirrhotic liver. Hepatol Res 42:508–514
17. Kasai M (1974) Treatment of biliary atresia with special reference to hepatic portoenterostomy and its modifications. Prog Pediatr Surg 6:5–52
18. Karrer FM, Price MR, Bensard DD, Sokol RJ, Narkewicz MR, Smith DJ et al (1996) Long-term results with the Kasai operation for biliary atresia. Arch Surg 131:493–496
19. Ohi R, Nio M, Chiba T, Endo N, Goto M, Ibrahim M (1990) Long-term follow-up after surgery for patients with biliary atresia. J Pediatr Surg 25:442–445
20. Tagge DU, Tagge EP, Drongowski RA, Oldham KT, Coran AGA (1991) Long term experience with biliary atresia. Ann Surg 214:590–598
21. Gautier M, Valayer J, Odièvre M et al (1984) Histological liver evaluation 5 years after surgery for extrahepatic biliary atresia: a study of 20 cases. J Pediatr Surg 19:263–268
22. Lykavieris P, Chardot C, Sokhn M et al (2005) Outcome in adulthood of biliary atresia: a study of 63 patients who survived for over 20 years with their native liver. Hepatology 41:366–371

ELSEVIER

Conversion to Prolonged-Release Tacrolimus for Pediatric Living Related Donor Liver Transplant Recipients

T. Ueno, Y. Takama, K. Masahata, S. Uehara, S. Ibuka, H. Kondou, Y. Hasegawa, and M. Fukuzawa

ABSTRACT

Prolonged-release tacrolimus allows for once-daily dosing. Although many adult recipients have been switched from standard tacrolimus, prolonged-release tacrolimus has not been popular for pediatric patients despite the potential benefits for medication compliance. We report on prolonged-release tacrolimus for 11 pediatric living related donor liver transplant (LRDLT) recipients. Patients under 18 years of age who were receiving standard tacrolimus-based immunosuppression and steroid taper underwent conversion from standard to prolonged-release tacrolimus. We monitored tacrolimus trough levels and liver function tests (LFTs). We also assessed adverse effects and satisfaction levels for prolonged-release tacrolimus. Mean age at transplantation was 4.3 years. The mean duration of follow-up was 12 months. The ratios of trough levels with prolonged-release vs standard tacrolimus were 0.97, 0.95, and 0.92 at 1, 2, and 4 weeks post conversion, respectively. Two patients discontinued prolonged-release tacrolimus owing to abnormal LFTs and neurological abnormalities, respectively; but symptoms resolved after reconversion. One patient returned to standard tacrolimus and the other was converted to cyclosporine. Once-daily administration satisfied 89% of patients. In the overall assessment, conversion to prolonged-release tacrolimus satisfied all patients. Prolonged-release tacrolimus was useful for pediatric patients after LRDLT. Trough levels after conversion were compatible with those before conversion. Most patients were satisfied with prolonged-release tacrolimus. However, some patients failed conversion because of unexpected responses. Close observation after conversion is required even if patients have previously had an uneventful course on standard tacrolimus.

PROLONGED-RELEASE tacrolimus, which is now commercially available, allows for once-daily dosing. It is widely used in adults; however, the new formulation is currently not popular for pediatric patients. Immunosuppression can be withdrawn in some pediatric patients after living related donor liver transplantation (LRDLT), but most subjects require life-long immunosuppression. Once-daily administration is potentially beneficial in terms of medication compliance for pediatric patients. Herein we have reported on prolonged-release tacrolimus in pediatric patients after LRDLT.

MATERIALS AND METHODS

Patients who were less than 18 years old at the time of conversion and who underwent LRDLT at our institution were included in this study. They had originally received the standard tacrolimus formulation with a steroid taper. Our tacrolimus taper was a target tacrolimus trough level of 10–15 ng/mL for the first month after transplantation; 5–10 ng/mL for 1 year, and 3–5 ng/mL thereafter. Steroids were administered to all patients. Patients received a bolus dose of methylprednisolone (20 mg/kg) at the time of transplantation, and were tapered off by 4 months thereafter. Prednisolone was continued for patients who had an episode of biopsy-proven acute cellular rejection or posttransplant hepatitis. Mycophenolate mofetil was administered to selected patients who experienced a steroid-resistant acute

From the Departments of Pediatric Surgery (T.U., Y.T., K.M., S.U., S.I., M.F.), Osaka University Graduate School of Medicine, and Pediatrics (H.K., Y.H.), Osaka University Graduate School of Medicine, Suita, Japan.

Address reprint requests to Takehisa Ueno, Pediatric Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail: ueno@pedsurg.med.osaka-u.ac.jp

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cellular rejection episode proven by another biopsy following steroid therapy. No patients were withdrawn from immunosuppressive therapy.

Prolonged-release tacrolimus at twice the dose of standard tacrolimus was given in the morning immediately before the switch. All subsequent prolonged-release doses were taken daily in the morning. Because once-daily prolonged-release tacrolimus was only available in capsule form, capsules were prescribed even if patients had taken the granule form of standard tacrolimus.

Blood samples to measure tacrolimus trough levels were taken just before the morning dose of prolonged-release or standard tacrolimus. Trough levels were measured 12 hours after the previous standard tacrolimus dose; prolonged-release tacrolimus levels were measured 24 hours after the previous dose. Samples were collected in the clinic at 1, 2, and 4 weeks following conversion. Whole blood samples were placed in tubes containing EDTA and stored at 4°C. Concentrations were measured within 4 hours using the Architect i2000 (Abbott Laboratories).

Serum aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (GGTP), and total bilirubin levels were measured concurrently with the tacrolimus trough levels. We assessed adverse effects of prolonged-release tacrolimus and levels of patient and parent satisfaction.

Data were analyzed using the JMP Ver. 8.0 software package (SAS, Cary, NC). Continuous variables presented as median values with ranges were compared using nonparametric tests or Student *t* test if the data were normally distributed; categorical variables, as numbers with percentages were evaluated with Pearson's χ^2 test or Fisher Exact Test. A *p* value less than .05 was considered to be statistically significant.

RESULTS

The characteristics of the 11 patients switched from standard to prolonged-release tacrolimus are shown in Table 1 Mean age at transplantation was 4.3 years (range, 1.1–8.2). Mean age at conversion was 11.3 years (range, 6.9–16.3). The mean duration of follow-up was 12.0 months (range, 2.4–20.4). Underlying diseases included biliary atresia (n = 9), Wilson's disease (n = 1), and ornithine transcarbamylase deficiency (n = 1). Tacrolimus trough levels are shown in Fig 1. Trough levels just before conversion did not correlate with prolonged-release values at 4 weeks thereafter (P = .21). The average ratios of standard to prolonged-release tacrolimus trough levels at 1, 2, and 4 weeks after conversion are shown in Fig 2. Mean tacrolimus trough levels after conversion decreased gradually. The trough level 4 weeks post conversion was 0.92 of standard tacrolimus, a difference that was not significant (P = .26). The outcomes of conversion are shown in Fig 3. Two patients discontinued prolonged-release tacrolimus owing to abnormal liver function tests and neurological abnormalities, respectively (Fig 4). One patient returned to standard tacrolimus and the other was switched to cyclosporine, with resolution of symptoms soon after discontinuation.

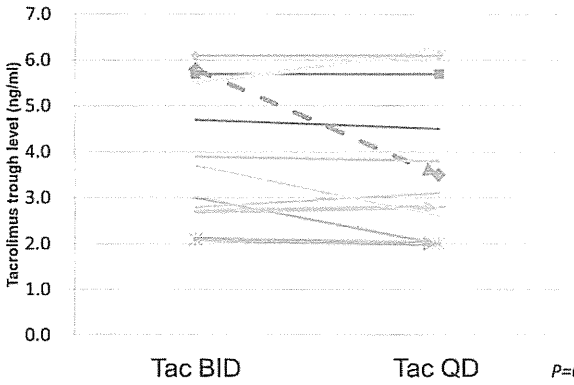

Fig 1. Prolonged-release tacrolimus trough levels 4 weeks after conversion. Each line indicates the change in the tacrolimus trough level for an individual patient. Standard tacrolimus (Tac BID) trough levels were measured just prior to conversion, and prolonged-release tacrolimus (Tac QD) trough levels were measured 4 weeks after conversion (P = .21; paired t test).

Of the 9 children who continued with prolonged-release tacrolimus, 89% of patients were satisfied with once-daily administration and the capsule formulation, although younger patients needed training on how to swallow capsules. In the overall assessment, all patients were satisfied with conversion to prolonged-release tacrolimus.

Table 1. Patient Characteristics

Patient	Gender	Age at Tx	Original Disease	Age at CV	BW (kg)	Observation (mo)	Tac BID Dose (mg)	Other IS
1	Male	4.1	Biliary Atresia	16.3	53	14.3	1.5	PSL
2	Male	1.3	Biliary Atresia	11.4	38	20.4	2.5	PSL
3	Female	1.1	Biliary Atresia	10.3	28	14.3	1.5	MMF
4	Female	8.2	Wilson's disease	15.8	49	14.3	2	None
5	Male	5.9	Biliary Atresia	13.3	45	10.1	3	PSL
6	Female	1.6	Biliary Atresia	8.3	23	3.6	0.5	PSL
7	Female	1.4	Biliary Atresia	7.6	21	2.4	0.8	PSL
8	Female	7.2	Biliary Atresia	13.2	39	2.4	2	PSL
9	Female	6.0	Biliary Atresia	8.8	26	20.0	3	PSL + MMF
10	Male	4.7	Biliary Atresia	7.7	29	18.3	1	PSL
11	Female	5.6	OTCD	6.9	20	3.1	1.2	None

Tx, transplant; CV, conversion; BW, body weight; IS, immunosuppression; PSL, prednisolone; MMF, mycophenolate mofetil; OTCD, ornithine transcarbamylase deficiency.

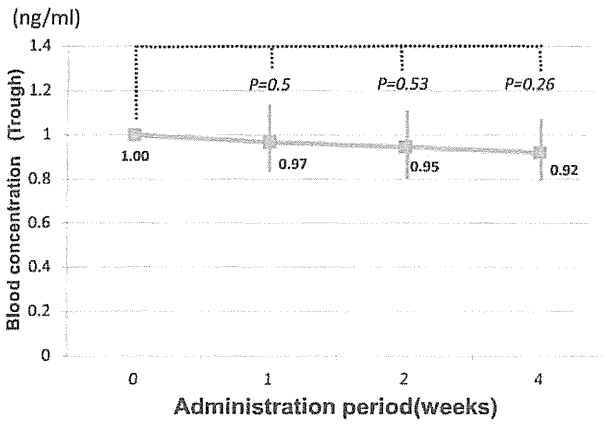

Fig 2. Mean prolonged-release tacrolimus trough levels over time. Tacrolimus trough level ratios were plotted for 1, 2, and 4 weeks postconversion. Week 0: standard tacrolimus trough level = 1. Each number corresponds to (prolonged-release tacrolimus trough level)/(standard tacrolimus trough level). Each *P* value represents a comparison with the standard tacrolimus trough level (week 0).

DISCUSSION

Prograf, an immediate-release formulation, is administered twice daily to prevent and treat allograft rejection in liver transplant patients. A prolonged-release formulation (Graceptor) has been developed to provide once-daily dosing; however, it is only available in capsule form.

Liver transplant patients are generally monitored with routine blood tests that include liver function tests (LFTs). Some patients may no longer require immunosuppression. However, pathological changes are sometimes detected even when blood test results are within the normal range.[1] Therefore, patients require life-long immunosuppression. Once-daily administration is also beneficial for drug compliance in pediatric patients who require continued immunosuppression.

This study was designed to determine the efficacy, safety, and level of patient satisfaction from a switch from the standard tacrolimus to the prolonged-release formulation. Trunečka et al studied the safety and efficacy of dual-therapy regimens of twice-daily tacrolimus and once-daily tacrolimus (Advagraf) administered with steroids among 475 adult primary liver transplant recipients who did not receive antibody induction.[2] The rate of biopsy-proven acute rejection episodes at 24 weeks was 33.7% for standard vs 36.3% for the prolonged-release tacrolimus group. At 12 months, the number of episodes requiring treatment was similar for patients on both standard and prolonged-release forms (28.1% and 24.7%, respectively). Twelve-month patient and graft survivals were 90.8% and 85.6% vs 89.2% and 85.3% for the standard vs prolonged-release tacrolimus groups, respectively. Adverse event profiles were similar. Prolonged-release tacrolimus was well tolerated with similar efficacy and safety profiles as standard tacrolimus. In our study, 2 patients experienced unexpected reactions. Close observation after conversion is required even if patients have had an uneventful course on standard tacrolimus.

Beckebaum et al reported that switching of adult liver transplant recipients from twice-daily to once-daily tacrolimus on a 1:1 mg basis was associated with lower tacrolimus trough levels in nearly two-thirds of patients (>25% lower in 28.8% of patients) at 1 week postconversion. Tacrolimus concentrations were approximately 10% lower than baseline at week 1 without any dose changes, remaining significantly lower at week 2 and prompting us to increase the dosage of tacrolimus with once-daily dosing in the corresponding patients. These observations suggested that close monitoring of tacrolimus trough levels is essential during the early postconversion period. In our study, pediatric liver transplant recipients displayed lower tacrolimus trough levels after conversion, consistent with results among adults.[3]

Satisfaction levels were excellent among both patients and their parents. In general, medication adherence tends to decrease during adolescence. So far only a capsule form is available on the market. Therefore, only patients who can take capsules can benefit from once-

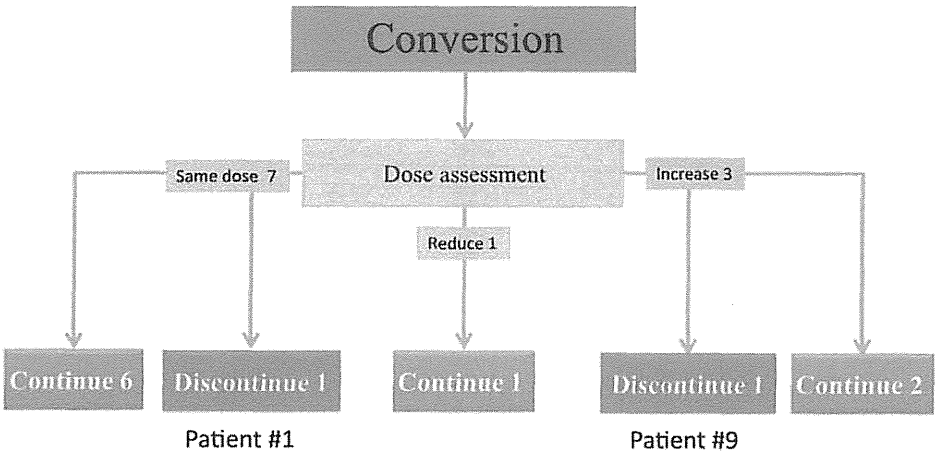

Fig 3. Summary of outcomes after conversion. The flowchart shows patient outcomes after conversion. The numbers at each step represents the number of patients. Patients 1 and 9 discontinued tacrolimus.