

Original Article

Efficacy of sirolimus on ischemic-type biliary lesions after liver transplantation

Lu Wang, Yunjin Zang, Shichun Lu, Chuanyun Li, Dongdong Lin

Department of General Surgery Center, Affiliated You'an Hospital to Capital Medical University, Beijing, China

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Abstract: *Objective:* To explore and summarize the effect of sirolimus on ischemic-type biliary lesions (ITBL) after liver transplantation. *Methods:* A retrospective study was carried out on 52 patients using sirolimus for biliary complications after liver transplantation from 2004 to 2016 in our hospital. Serum alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin, glutamyl endopeptidase, and alkaline phosphatase (ALP) after the surgery, and six months later were recorded and analyzed, the 1- and 3-year graft survival rates were analyzed. *Results:* Serum alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin, glutamyl endopeptidase, and alkaline phosphatase (ALP) significantly increased in 52 patients with ITBL. Among the patients with ITBL, 30 patients were treated with sirolimus after the surgery, and six months later, the above indices significantly improved in the treatment group compared with the control group. The patients in control group were treated by traditional plan, tacrolimus based treatment without sirolimus. The 1- and 3-year graft survival rates in the sirolimus group were higher than those in the control group. Moreover, the second transplantation rate was lower in the treated group. The blood lipid level was higher in the sirolimus group compared with the control group. *Conclusion:* Sirolimus is clinically effective for management of ITBL. Until a better treatment is found, sirolimus is worth attempting, and secondary transplantation may be avoided.

Keywords: Sirolimus, ischemic-type biliary lesion, glutamyl endopeptidase, hyperlipidemia

Introduction

Ischemic-type biliary lesion (ITBL) is the most common and refractory complication of liver transplantation. Moreover, it is one of the major causes of early and middle-stage mortality in patients who received a liver transplant [1]. In recent years, liver transplants using donated organs after cardiac death increased gradually. Meanwhile, the incidence of ITBL also increases, which has become a critical factor affecting the long-term survival of grafts and recipients [2]. Therefore, it is of great importance to explore the underlying mechanism of ITBL and to find an effective prevention and treatment for it. Throughout our long-term clinical practice, we found that sirolimus can improve the recovery of liver function in patients with ITBL, and can even result in clinical cure.

Materials and methods

General data

From May 2004 to April 2016, a total of 52 patients were diagnosed with ITBL in Beijing

You'an Hospital, Capital Medical University, including 35 males and 17 females, aged 24-54 years, with a mean age of 41.6 ± 7.7 years. The primary diseases included primary liver cancer (18 cases), hepatitis B-related cirrhosis (24 cases), hepatitis C-related cirrhosis (6 cases), cirrhosis caused by autoimmune disease (2 cases), and alcoholic liver cirrhosis (2 cases). The warm ischemic time of grafts was 1~11 min, with an average of 3.9 min, and the cold ischemic time was 32~1120 min, with an average of 572 min. ITBL occurred 4-10 months after the surgery, with an average of 6.3 months.

Diagnostic criteria of ITBL

The diagnostic criteria of ITBL are as follows: (1) postoperative jaundice 3 to 12 months after the surgery and (2) non-anastomotic bile duct stricture, dilatation, damage, and biliary cast on imaging examinations including magnetic resonance cholangiopancreatography (MRCP) and cholangiography. Anastomotic strictures and bile duct stenosis caused by an excessively

The effect of sirolimus on ITBL after liver transplantation

Table 1. The effect of sirolimus on ITBL after liver transplantation

Item	Case	Pre-injury	During injury	Post-treatment	P-value 1	P-value 2
ALT (U/L)	Control group	84±32	331±192	352±186		0.003
	Treatment group	88±42	357±111	136±101	0.000	
AST (U/L)	Control group	85±36	398±119	262±152		0.002
	Treatment group	82±26	402±166	188±42	0.000	
Total bilirubin (µmol/L)	Control group	26±8	39±68	35±74		0.006
	Treatment group	21±5	43±75	43±55	0.000	
R- Glutamyl endopeptidase (U/L)	Control group	132±76	336±202	428±358		0.000
	Treatment group	120±68	352±198	148±71	0.000	
Alkaline phosphatase (U/L)	Control group	124±85	324±182	386±346		0.000
	Treatment group	132±87	337±245	132±102	0.000	

P1: comparison between during injury and post-injury in the treatment group, P2: comparison between groups.

long bile duct or bending of the bile duct must be excluded [3].

Grouping

After the surgery, patients were routinely treated with a triple immunosuppressive regimen including tacrolimus, mycophenolate mofetil, and corticosteroids. Moreover, ursodeoxycholic acid was added in all patients. The average initial dosage of tacrolimus (Prograf®, Astellas) was 4 mg/day. The dosage regimen was adjusted according to the clinical condition of the patient, adverse effects, plasma concentration of the drugs, and concomitant medications, to maintain the drug concentration at 3-8 ng/mL. One-thousand milligram of glucocorticoid was administered intravenously during the surgery and the dosage was gradually decreased after the surgery to 240 mg/d until being withdrawn in three months. One-thousand milligram of mycophenolate mofetil was added per day according to the patient's condition. The patients were followed up regularly at intervals of 1-3 months. Clinical symptoms, liver and kidney functions, plasma drug concentration, and if necessary liver biopsy, were included in the follow-up.

After being diagnosed with ITBL, patients in the treatment group (30 cases) received sirolimus and the plasma concentration was maintained at 4-8 µg/L. Small doses of tacrolimus, myco-

phenolate mofetil, and corticosteroids were combined to prevent rejection, and ursodeoxycholic acid was used to promote the flow of the bile. On the other hand, patients in the control group (21 cases) continued to receive tacrolimus, mycophenolate mofetil, and corticosteroids to prevent rejection in addition to the cholegogue drug, ursodeoxycholic acid.

Analyzed data

Ordinary Liver function tests were conducted, we collected the test results before injury (2 months before the occurrence of ITBL), during injury (when ITBL was diagnosed), and after treatment (6 months after the diagnosis of ITBL). The one- and three-year graft survival rates (death or repeated transplantation were considered as graft loss) in the two groups of patients were analyzed.

Statistical analysis

SPSS 14.0 statistical software was used for data analysis. Quantitative data were presented as mean ± SD. The *t*-test was used for comparisons between the two groups. The degree of bile duct injury was presented as semi-quantitative scores, and analyzed by Wilcoxon rank-sum test. The Chi-squared test was used to analyze the graft survival rates. A *p*-value < 0.05 was considered statistically significant.

The effect of sirolimus on ITBL after liver transplantation

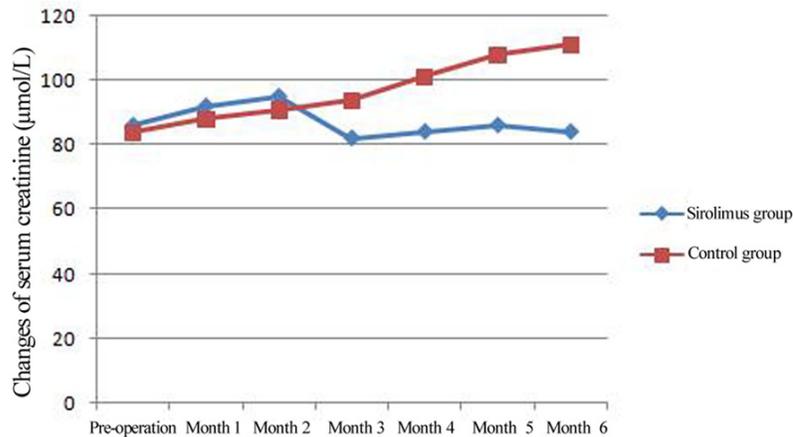


Figure 1. Changes of serum creatinine in patients of the two groups with ITBL after liver transplantation at different time points after treatment (µmol/L).

Table 2. Changes of serum creatinine in patients of the two groups with ITBL after liver transplantation at different time points after treatment (µmol/L)

	Pre-operation	One month	Two months	Three months	Four months	Five months	Six months
Sirolimus group	86	92	95	82	84	86	84
Control group	84	88	91	94	101	108	111
P-Value				0.066	0.002	0.001	0.001

Results

Changes in the liver function after the occurrence of ITBL

After the occurrence of ITBL, serum alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin, glutamyl endopeptidase, and alkaline phosphatase (ALP) significantly increased (**Table 1**). Six months later, the above indicators were not significantly changed in the control group, whereas in the treatment group, the liver function tests significantly improved.

Effect of sirolimus on the prognosis

Of the 21 patients in the control group, 9 received repeated liver transplantation (2 deaths), 7 died, 3 achieved long-term survivals with the presence of jaundice, and 2 were cured using conventional therapies and their biochemical liver function tests and histopathological examinations showed normal results. Of the 30 patients in the treatment group, four cases received repeated liver transplantation (2 deaths), 4 cases died, 18 cases were cured

with conventional therapies, and their biochemical liver function tests and histological examinations became normal, and four cases achieved a long-term survival with the presence of jaundice. The 1- and 3-year graft survival rates were 14/21 and 12/21, respectively in the control group, and 25/30 and 24/30, respectively in the treatment group. The difference between the two groups was statistically significant.

Kidney function

The creatinine levels in patients of the two groups during injury and 6 months after treatment are shown in **Figure 1** and **Table 2**. Preoperative creatinine and blood lipid levels showed no significant difference between the

two groups ($P < 0.05$). Creatinine levels in the sirolimus treatment group exhibited a slight increase trend at the beginning and then decreased, with a pre-injury level of 86 ± 18 µmol/L and a highest value of 95 ± 24 µmol/L, which occurred 1-3 months after treatment and decreased gradually later. In the control group, creatinine showed a slow upward trend, from 84 ± 18 µmol/L to 111 ± 28 µmol/L, and then showed no statistically significant difference at 6 months after the surgery compared to the previous months.

Blood lipid levels

The blood lipid levels of patients in the two groups during injury and 6 months after treatment are shown in **Figure 2** and **Table 3**. Before the surgery, serum levels of total cholesterol, triglycerides, and high-density lipoprotein showed no statistically significant difference between the two groups. The differences in the triglyceride level and serum total cholesterol level 6 months after the surgery were statistically significant between the two groups ($P < 0.05$), whereas no significant differences were found in HDL and LDL.

The effect of sirolimus on ITBL after liver transplantation

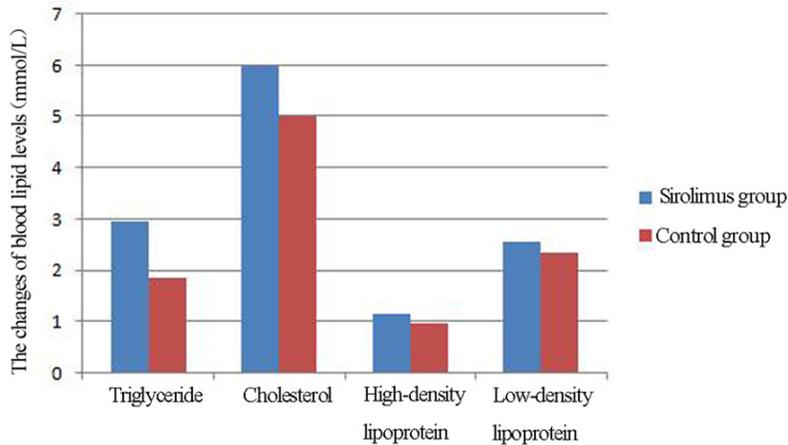


Figure 2. The changes of blood lipid levels 6 months after treatment in patients with post-liver transplantation ITBL in the two groups (mmol/L).

Table 3. The changes of blood lipid levels 6 months after treatment in patients with post-liver transplantation ITBL in the two groups (mmol/L)

	Triglyceride	Cholesterol	High-density lipoprotein	Low-density lipoprotein
Sirolimus group	2.97	5.98	1.16	2.58
Control group	1.85	5.02	0.98	2.36
P value	0.01	0.01	0.32	0.55

Discussion

Post-liver transplantation ITBL is characterized by non-anastomotic intrahepatic biliary tree strictures and dilation of the transplant after liver transplantation, causing intrahepatic biliary tree damage, and eventually leading to mechanical biliary obstruction and secondary infection [4]. The incidence of ITBL is about 3% to 18% [5, 6]. The common causes of bile duct injury after liver transplantation include the following four causes: 1. biliary ischemia caused by the technical factors of surgery, hemodynamic disorders, and hypercoagulation; 2. cold and warm ischemia times of the grafts are too long and ischemia-reperfusion causes injury in the bile duct epithelial cells and vascular endothelial cells; 3. the bronchoalveolar lavage in the grafts is insufficient and the intrahepatic bile remnants, mainly bile salts, can damage the biliary epithelium; and 4. repeated rejection responses can damage the endothelial cells and bile ducts [7]. Bile duct injuries are compensated by fibrosis. In addition, in patients with ischemic biliary injury, fibrosis is enhanced

and degradation processes are diminished, resulting in bile duct fibrosis [8].

Rapamycin is a common immunosuppressant used after organ transplantation. After long-term clinical practice, we have found that rapamycin can delay or reduce ITBL. We conducted a retrospective analysis. Serum ALT, AST, total bilirubin, glutamyl endopeptidase, and ALP in the patients with ITBL in the rapamycin treatment group were improved compared with the patients in the sirolimus treatment group. For patients with mild ITBL, treatment with rapamycin at early stage can significantly improve the liver function tests.

Sirolimus is a specific inhibitor of mTOR. It affects cell proliferation and differentiation by inhibiting

the kinase activity of mTOR, thus blocking the cell cycle in the G1 phase, and exerting an anti-proliferative activity. Its effect on the compensatory intrahepatic bile duct hyperplasia has been confirmed in animal experiments. Tian-tian Wu *et al.* [9] have established a rat biliary ischemia model by complete transection of rat hepatic artery. Some rats were given rapamycin treatment and it was found that in the ischemia group, the mean number of the interlobular bile ducts was significantly increased after the surgery, whereas in the rapamycin treatment group, the mean number of the interlobular bile ducts was significantly decreased, demonstrating that rapamycin can inhibit the compensatory hyperplasia of intrahepatic bile ducts.

The specific pathogenesis of hyperplasia after biliary lesions is very complex, wherein immunopathological damage may be an important factor [10]. Sirolimus can, to some extent, alleviate the immunopathological damage of the bile ducts and indirectly promote biliary epithelial repair and recovery, which allows some patients with mild ITBL to avoid repeated transplantation.

The effect of sirolimus on ITBL after liver transplantation

The main adverse effect of sirolimus is reported to be hyperlipidemia [11, 12]. In this study, we also found that the serum total cholesterol and triglycerides of patients in the sirolimus treatment group increased. Thus, during treatment with sirolimus, blood lipid levels should be closely monitored.

Currently, the specific studies on therapies for ITBL are few. Ursodeoxycholic acid exerts a preventive effect on ischemic cholangitis caused by non-anastomotic biliary stricture after liver transplantation, thus, the prophylactic use of ursodeoxycholic acid should be continued in such patients. Drugs that improve the microcirculation can improve the bile duct ischemia, and have certain efficacy in preventing and treating ITBL. Although antibiotic prophylaxis may not reduce the recurrence of cholangitis upon ITBL, it is still necessary for the prevention of biliary tract infections [13, 14]. Clinically, sirolimus is effective in treating ITBL and is worth attempting until more effective treatments are identified, by which a second transplantation may be avoided.

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Disclosure of conflict of interest

None.

Address correspondence to: Yunjin Zang, Department of General Surgery Center, Affiliated You'an Hospital to Capital Medical University, No. 8, Xi Tou Tiao, You Wai Street, Fengtai District, Beijing 100069, China. E-mail: zang129w000@sina.com

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