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Effect of Bicyclol tablets on drug induced liver injuries after kidney transplantation

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Abstract: Liver injury is one of the most common complications in patients after kidney transplantation. Bicyclol tablets possess obvious anti-inflammatory and liver-protective functions. This study aimed to explore the clinical effect of preventive application of Bicyclol on drug induced liver injuries at an early stage after kidney transplantation.

A total of 1600 patients who accepted kidney transplantations at our hospital from January 2009 to May 2015 were enrolled in this study, and divided into the prevention group (Bicyclol) and the control group (no hepatic protectors) based on whether or not hepatic protectors were regularly administered after the operation. The occurrence of liver injuries at an early stage after the operation and their influencing factors were analyzed.

Total of 745 cases were included in the final analysis of which 82 developed liver injuries post-operation, with 22 in the prevention group (4.82%) as compared to 60 in the control group (20.76%) (P= 0.001). As compared to the control group, OR (95% CI) of the prevention group was 0.197 (0.116, 0.334) after revising HBsAg status, age and maintenance immunosuppression.

Prophylactic application of Bicyclol as liver-protective treatment was a protective factor against drug induced liver injuries at an early stage after kidney transplantation.

Keywords: Kidney Transplantation; Drug-Induced Liver Injury; Immunosuppressive Agents; Retrospective Studies; Bicyclol

1 Introduction

Liver injury is one of the most common complications in patients after kidney transplantation. The incidence of liver injuries soon after kidney transplantation is 20%~50%, and is the second most common postoperative complication post transplantation [1]. It has a serious impact on patients' life expectancy and life quality. It is recommended to comprehensively assess the status of the liver before transplantation [2]. Liver function examinations and relevant morphological examinations should be performed regularly after the transplantation. Once liver function abnormalities occur, drug-drug interaction must be avoided when liver-protective medicines are applied in order to maintain the concentrations of tacrolimus (Tac) and cyclosporine (CsA). Liver function abnormalities occurring after kidney transplantation are difficult to treat [3], so their prevention is very important. Another study [4] has shown that monitoring liver functions regularly after kidney transplantation, discovering possible liver injuries induced by medicines early, adjusting the immunosuppressant treatment scheme and strengthening liver-protective treatment are beneficial for achieving satisfactory outcomes. Bicyclol(4,40-dimethoxy-5,6,50,60-dimethylene-dioxy-2,20-dicarboxylate biphenyl) is a liver protectant for patients with various liver diseases used in many countries. Bicyclol tablets possess obvious anti-inflammatory and liver-protective functions [5], can prevent and control liver injuries at an early stage after kidney transplantation, and improve prognosis of kidney transplantation without affecting the pharmacokinetics of Tac [6]. In order to assess the preventive and protective function of Bicyclol tablets on drug induced liver injuries (DILIs) after kidney transplantation, this retrospective analysis was performed with 1600 patients who accepted kidney transplantations at our hospital from January 2009 to May 2015.

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2 Materials and methods

2.1 Kidney transplant cases

Source(s) of the transplanted kidneys: In this study, the source(s) of kidneys used in transplant came from 2 groups. 1. Immediate family member donors: 480 cases. These were living donor from relatives who are immediate family members of the acceptors of kidney transplant.2. Heart and brain death donors: 265 cases. The source of heart and brain death donors was the Red Cross Organ Donation Management Center in China.

The medical records of 1600 patients who accepted allogenic kidney transplantations at our hospital from Janauary 2009 to May 2015 were collected and investigated. The age of patients ranged from 5 to 60 years, including both males and females. A total of 615 cases with serum alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) abnormalities, HBV DNA quantity > 102 IU/ml, taking other liver-protective medicines and patients with Hepatitis C infection were initially excluded. The remaining 985 cases were divided into the prevention group and the control group according to whether Bicyclol tablets were regularly administered or not after the operation. Cases with missing data, rejection reaction, non-alcoholic and alcoholic liver diseases, autoimmune hepatitis and serious infections were also excluded. Finally, data statistics and analysis of occurrence of liver injuries in the early postoperative period in all included patients were performed (As shown in Figure 1). Data was collected and quality control was performed according to the scheme in the survey forms.

2.2 Ethics statement

This study was approved by the ethics committees of our hospital. No conflict of interest exists in this study, and none of the transplant donors were from a vulnerable population and all donors or next of kin provided written informed consent that was freely given.

In this study, kidney donors' informed consent process is as follows: (1) Immediate family member donors: a.Fill in living organ donation letter of intent, agreed and signed by immediate family members, and acknowledged and agreed by acceptor family members; b.Pass donor comprehensive inspection, in line with kidney donation conditions, no contraindication for kidney surgery; c. the local police station issues acceptor immediate family member certificates, reviewed and dis-

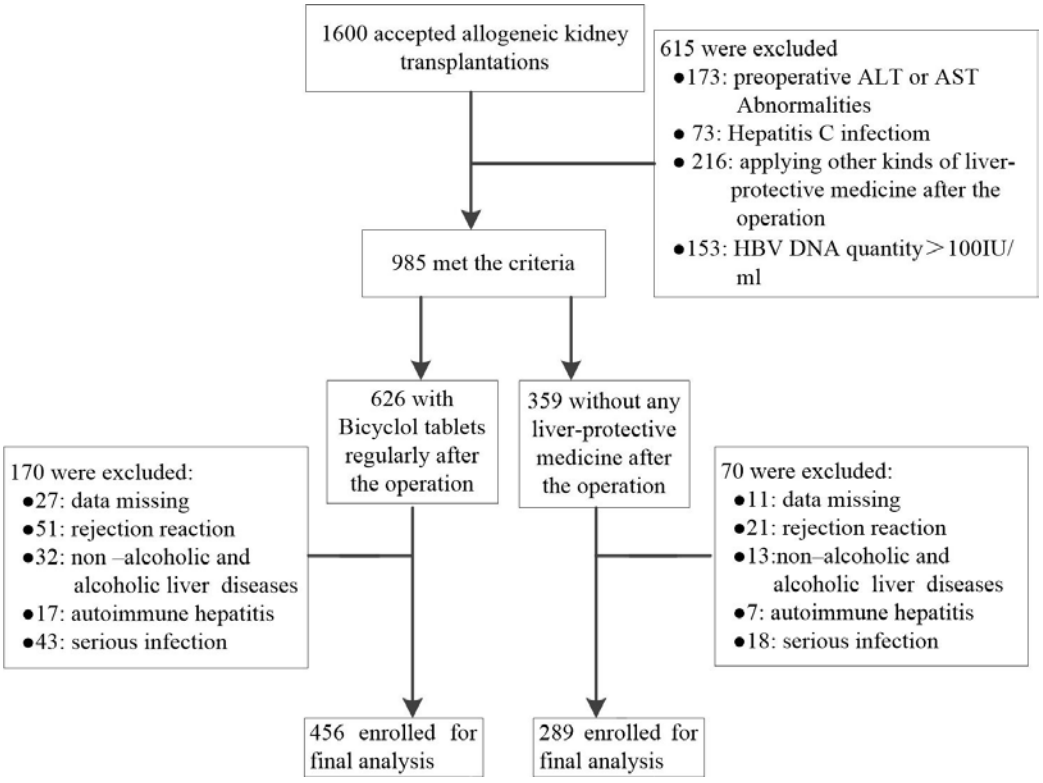

Figure 1: Case selection flow chart

cussed by "Human Organ Transplant Clinical Application and Ethics committee" of our hospital, and approved by the Provincial Health Department to implement a kidney transplant for the acceptors. (2) Heart and brain death donors: Donors assigned by China's human organ distribution and computer system and approved by the Human Organ Transplant Clinical Application and Ethics Commission of our hospital to implement a kidney transplant for the acceptors.

2.3 Study method

2.3.1 Grouping method

This was a retrospective cohort study. Patients who met the criteria were divided into two groups: (1) the prevention group: patients taking Bicyclol tablets after kidney transplantation (manufactured by Beijing Union Pharmaceutical Factory, 25 mg/tablet, 25 mg three times daily,12 weeks); Criteria for determining whether patients would receive Bicyclol tablets include: medication dispensed by nurses with dosage as prescribed under direct observationfor inpatients; Telephone follow-up of patients' medications and checking the amount of medicine distributed in pharmacy to outpatients. The control groupconsisted of patients not taking any liver-protective medicine after kidney transplantation.

2.3.2 Data collected

Demographics of patient cases were collected, including gender, age, height, weight, smoking, drinking, etc.. (2) Medical histories of patients was recorded, including primary diseases requiring kidney transplantation, past diseases and treatment history, coexistent diseases or symptoms, drug combinations before kidney transplantation, drug allergy history, etc.. Patient lab results were collected including symptoms and signs examinations and liver function indexes (ALT and AST) before kidney transplantation and at 4, 8 and 12 weeks after kidney transplantation, preoperative HBV DNA quantitative determination and HBsAg qualitative determination. The immunosuppressant scheme and terminal point of the investigation (DILI occurred within three months after the operation) were also recorded.

2.4 Investigation methods and quality control

In-hospital record data was interrogated,survey forms were created and a database was set up to track data. Double-person recording for contrasting error-checking was adopted. Up to 10% samples were selected randomly. When the qualified rate was above 90% and the core indexes were above 95%, the data was regarded as qualified. In order to guarantee authenticity of the data and for reducing errors, quality control was performed throughout the investigation process including personnel training, pre-survey (By questionaire and on-the-spot statistics, the survey forms is examined, which is designed in advance), on-site investigation, data processing and analysis.

2.5 Main observation indexes

Main observation indexes included ALT and AST. When ALT and/or AST levels were more than 2 times the upper limit of normal values (ULN), it was suggestive of liver injuries. The occurrence of liver injuries in both groups were counted and their causative factors analyzed.

2.6 Statistical analysis

Data was summarized using EPIDATA 3.1 software, sample capacity was calculated using PASS 11.0 software, and statistical analysis was performed using SPSS 17.0 statistical software. The incidence of liver injuries after kidney transplantation is about 30% [7-8]. The incidence of liver injuries is about 18% in patients who took Bicyclol tablets as preventive liver-protective treatment [9]. Using PASS 11.0 software (α= 0.05, β= 0.10, *two-sided test*), every group needed at least 265 patients. Inter-group differences in continuous variables were analyzed using independent-samples t-test. Inter-group differences in categorical data were analyzed using χ^2-test. Inter-group differences in ordinal categorical data were assessed using Wilcoxon test; non-normal distribution data were analyzed using non-parametric test. The causative factors of liver injuries were determined using monofactorial and multifactorial logistic regression analyses. All statistical tests were two-sided, and $P< 0.05$ was considered to be statistically significant.

3 Results

3.1 Selected cases

A total of 745 cases were included in this analysis, of which 456 were in the prevention group and 289 in the control group. Both groups had no significant differences in age, gender, BMI, HBsAg, maintenance immunosuppression, ALT and AST at baseline (P> 0.05). (Table 1).

3.2 Analysis of the occurrence of liver injuries and their influencing factors

Monofactorial analysis of the occurrence of liver injuries revealed 82 cases in both groups at early stage liver

Table 1: Comparison of baseline characteristics of the two groups

General condition	Prevention group (n= 456)	Control group (n= 289)	statistical quantity	P
Age			χ^2= 1.306	0.253
≥ 40	91	48		
< 40	365	241		
Gender			χ^2= 0.794	0.373
male	298	198		
female	158	91		
BMI			χ^2= 2.062	0.151
≥ 25	102	78		
< 25	354	211		
HBsAg			χ^2= 0.587	0.444
positive	31	24		
negative	425	265		
Primary diseases			χ^2= 10.511	0.015
chronic renal failure	304	175		
hypertensive nephropathy	85	45		
diabetic nephropathy	56	55		
other	11	14		
maintenance immunosuppression			χ^2= 0.118	0.732
MMF+ CsA + Pred	204	133		
MMF+ Tac + Pred	252	156		
baseline transaminases				
ALT(U/L)	19.13 ± 13.14	17.70 ± 11.27	*t*= 1.529	0.127
AST(U/L)	21.71 ± 8.84	22.04 ± 7.96	*t*= 0.525	0.600

MMF: mycophenolate mofetil, Tac: tacrolimus,CsA: cyclosporine,Pred: prednisone. The initial dosages of CsA and Tac were 6-8 mg·kg-1·d-1 and 0.08-0.15 mg·kg-1·d-1, respectively, the dosage should be adjusted according to blood concentration and clinical symptoms during the treatment. Trough levels were maintained at 180-220ng/ml and 5-10ng/ml, respectively)

injury after the operation. Group (prevention and control group), HBsAg status, age and maintenance of immunosuppression significantly affected the occurrence of liver injuries at an early stage after the operation (P< 0.05). Of the 82 cases, 22 were in the prevention group (4.82%) and 60 in the control group (20.76%), and the difference of liver injury incidence between the groups was statistically significant (P= 0.001) (Table 2). After controlling for age, HBsAg status and maintenance of immunosuppression, the OR (95% CI) of the prevention group was 0.197 (0.116, 0.334) as compared to the control group, and the difference was statistically significant (P= 0.001) (Table 3).

4 Discussion

The factors causing liver injuries after kidney transplantation are very complicated, including medications, chronic hepatitis B infectio (CHB), and biliary duct infection. Many of the liver injuries occurring at an early stage after kidney transplantation are drug-induced [10]. The pathogenic mechanism of liver injuries caused by CHB involves suppression of the patient's immunity by immunosuppressants and active HBV replication [11]. Prognosis of kidney transplantation can be improved if the clinical treatment

Table 2: Monofactorial analysis of the occurrence of liver injuries

Factor	Number of patients with liver injuries	Number of patients without liver injuries	P	Unadjusted OR (95% CI)
Groups			0.0001	0.193(0.116,0.323)
Prevention group	22	434		
Control group	60	229		
Age			0.046	1.711(1.009,2.900)
≥ 40	22	117		
< 40	60	546		
Gender			0.095	1.558(0.925,2.625)
male	61	425		
female	21	228		
BMI			0.824	0.940(0.546,1.618)
≥ 25	19	161		
< 25	63	502		
HBsAg			0.000	5.253(2.845,9.698)
positive	19	36		
negative	63	627		
Primary diseases			0.443	
Chronic renal failure	47	432		
Hypertensive nephropathy	18	112	0.189	1.477(0.826,2.643)
Diabetic nephropathy	15	96	0.254	1.436(0.771,2.675)
Other	2	23	0.766	0.799(0.183,3.497)
maintenance immunosuppression			0.021	1.727(1.086,2.746)
MMF+ CsA + Pred	47	287		
MMF+ Tac + Pred	35	368		

Table 3: Multifactorial analysis of the occurrence of liver injuries

Factor	Wald Chi-square	P	Adjusted OR (95% CI)
Groups (prevention or control)	36.459	0.001	0.197(0.116,0.334)
Age (≥ 40 or < 40 years)	0.126	0.722	1.116(0.610,2.042)
HBsAg (positive or negative)	19.947	0.001	4.817(2.416,9.603)
Maintenance immunosuppression (MMF+ CsA+ Pred or MMF+ Tac + Pred)	4.097	0.043	1.659(1.016,2.710)

can effectively prevent or control liver injuries occurring at an early stage after the transplantation.

In this study, it is proposed that the liver injuries occurring at an early stage after kidney transplantation are mainly DILIs. First, it is thought that liver injuries are mainly caused by commonly used immunosuppressants such as CsA, Tac, Sirolimu (SRL), azathioprin (Aza), mycophenolate mofeti (MMF) and glucocorticoid. Moreover, hepatotoxicity of immunosuppressants negatively impacts the clinical effect after organ transplantations, and can even lead to hepatic failure or death [12]. DILIs often occur between two weeks and three months after kidney transplantation, and the main symptoms are transaminase and bilirubin elevation [13]. Liver injuries induced by immunosuppressants may be related to the administration of large-dosage medicine, especially CNI, which can influence the stability and permeability of liver cell membranes by disturbing the membrane proteins' function of microbody. Simultaneously, it disturbs mRNA production and impacts the synthesis of DNA and ribosomes, thereby causing synthesis and secretion dysfunctions of protein and bile, finally impacting detoxification and other metabolic functions of the liver. Cases with liver injuries caused by autoimmune liver diseases,non-alcoholic or alcoholic liver diseases after the operation were excluded from this study. Cases with preoperative abnormal liver functions and HBV DNA > 102 IU/ml were also excluded from this study. Patients with chronic HBV infection are more likely to develop DILI. This may be due to the fact that HBV-infected persons have poor drug tolerance and suffer from defects in drug metabolism; as a consequence hepatitis B virus may be reactivated with elevated ALT/AST, especially at reduced dose or withdrawal of immunosuppressive therapy.

The factors influencing liver injuries after kidney transplantation included groups (prevention and control group) (P= 0.001), HBsAg status (P= 0.000) and different maintenance immunosuppression regimes (P= 0.043). The risk of occurrence of liver injuries in the prevention group was 0.197 (95% CI:0.116, 0.334) times that of the control group, while that of patients with positive HBsAg was 4.817 (95% CI:2.416, 9.603) times that of patients with negative HBsAg. The risk of occurrence of liver injuries after application of MMF + CsA + prednisone (Pred) maintenance immunosuppression was 1.659 (95% CI:1.016, 2.710) times that of application of MMF + Tac + Pred maintenance immunosuppression. When liver injuries occur after kidney transplantation, the dosage of immunosuppressants should be reduced and liver-protective medicines should be administered. Rejection reaction or the necessity of a long-term liver protection treatment often occur because of excessive dosage reduction of immunosuppressants. This study revealed that the preventive application of Bicyclol tablets combined with maintenance immunosuppression treatment after kidney transplantation were protective factors against liver injuries and ensure successful treatment for the primary disease.

Bicyclol tablets possess intensive anti-inflammatory and liver-protective functions against liver injuries induced by different factors. They can improve impaired liver cell functions and relieve pathological injuries of the liver, and are widely administered abroad as in widely geographically. Bicyclol tablets can inhibit the expression and activity of nuclear transcription factor-κB (NF-κB) induced by inflammation, reduce proinflammatory factors including tumor necrosis factor α (TNF-α), IL-18 and inducible nitric oxide synthase (iNOS), inhibit inflammatory injuries and oxidative damage induced by the synthesis of oxygen radicals (ROS) and nitric oxide (NO) [14-15], prevent apoptosis of liver cells, stabilize liver cell membranes, improve mitochondrial function and protect the structure and functions of liver cell DNA [16-24], and to relieve liver cell injuries. Hence, Bicyclol tablets possess functions of anti-oxidation and protection of liver cell membranes, chondriosome and nucleus. Bicyclol tablets can protect mice from liver energy metabolism

dysfunction and chondriosome dysfunction induced by paracetamol [17]. Theses tablets could prevent hepatotoxicity by enhancing the metabolism of Aflatoxin B1 [18] and prevent ConA liver injuries by inhibiting Fas/FasL mRNA expression and TNF-α release [19]. Chu and collegues [20] proved that Bicyclol tablets for liver-protective treatment combined with other basic liver-protective treatments can prevent the occurrence of liver injuries induced by antituberculosis drugs in patients with basic liver diseases, and guarantee successful antituberculosis treatment. Li et al [21] showed that Bicyclol tablets for liver-protective treatment combined with chemotherapy can reduce the occurrence and severity of DILIs in elderly cancer patients above 60 years, and guarantee the safety and tolerance of chemotherapy. Li Bing et al [22] showed that therapeutic dosage (25 mg, 3 times/d) of Bicyclol tablets does not affect the Tac blood concentration of patients who have accepted kidney transplantation. The liver-protective and enzyme lowering effects of BiCyclol are clear; it is safe for those with abnormal liver functions and normal Tac blood concentration. Laskow et al [23] reported that CsA's impact on the liver is greater than that of Tac, due to the following possible reasons: (1) FK506 possesses more intensive immunosuppressive action, which is about 100 times more than CsA, so the oral dosage is much less than CsA; (2) threshold concentration of liver injuries induced by Tac is much higher than the therapeutic window concentration range. In our study, the dosage of CsA and Tac should be adjusted according to blood concentration and clinical symptoms during treatment. We monitored trough levels of CsA and Tac once every 3 to 4 days during the perioperation period and once a week during the first three months post kidney transplantation in order to maintain a certain level (180-220ng/ml and 5-10ng/ml, respectively)., The results revealed that the risk of occurrence of liver injuries by MMF + CsA + Pred maintenance immunosuppression is higher than that of MMF + Tac + Pred maintenance immunosuppression, perhaps because the impact of CsA on liver functions is greater than Tac. Also, the risk of occurrence of liver injuries in patients with positive HBsAg is higher than those with negative HBsAg, because DILI can easily occur when kidney transplantation patients with viral hepatitis take immunosuppressants, due to the lower enzymatic activity of cytochrome CYP 3A4 and expression of mRNA in liver cells, and lower ALT as compared to healthy subjects, suggesting that drug metabolic defects exist in patients with HBV [24].

5 Conclusions

The preventive administration of Bicyclol tablets for liver-protective treatment was a protective factor against liver injuries at an early stage after kidney transplantation. Since this was a retrospective study with limited observation indexes, a large sample randomized control clinical study of DILI induced by anti-rejection medicines, especially a control study of hepatic histology qualitative and quantitative analyses should be performed. Interdisciplinary academic and experience exchange should be strengthened in order to assess the clinical value of Bicyclol tablets in preventive and liver-protective treatment of patients who have accepted maintenance immunosuppression.

Conflict of interest statement: Authors state no conflict of interest.

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