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Correlations of synergistic effect of glucocorticoids on tacrolimus with CYP3A, MDR1 and PXR gene polymorphisms in pediatric patients receiving liver transplantation for malignancy and chronic liver disease

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Immune suppression after liver transplant for malignancy, such as hepatocellular carcinoma and end stage liver disease is done using immunosuppressant drugs like tacrolimus. As the immune system in children is not fully developed, combining immunosuppressants like glucocorticoids influence the efficacy and reduce the chances of transplant rejection, particularly in patients with gene polymorphism. However, whether interaction between glucocorticoid and tacrolimus is associated with CYP3A, MDR1, and PXR gene polymorphisms, remains unclear. Here, we explored correlations of synergistic effect of glucocorticoids on tacrolimus with CYP3A, MDR1 and PXR gene polymorphisms in pediatric patients receiving liver transplantation for malignancy and chronic liver disease. A total of 340 eligible children were divided into glucocorticoid (+) (n=148) and glucocorticoid (−) groups (n=192). They were given tacrolimus + mycophenolate mofetil, based on which glucocorticoid (+) group took prednisone acetate tablets for ≥12 months. The blood trough concentration of tacrolimus was detected by chemiluminescence microparticle immunoassay 1, 3, 6 and 12 months after medication, and blood drug concentration corrected by daily concentration/daily dose (C0/D) was calculated. CYP3A, MDR1 and PXR gene polymorphisms were analyzed using PCR-RFLP. GG, AG and AA genotypes of CYP3A were observed in 130, 166 and 44 cases, respectively. CC, CT and TT genotypes of MDR1 were found in 152, 142 and 46 cases, respectively. There were 252, 74 and 14 cases of WW, WM and MM genotypes of PXR, respectively. The distribution frequencies of GG and AG+AA genotypes had significant differences between the two groups. One month after medication, C0/D of tacrolimus of GG genotype in glucocorticoid (+) group significantly exceeded that of glucocorticoid (−) group. C0/D was significantly higher in glucocorticoid (+) group with AG+AA genotype1, 3, 6 and 12 months after medication (P <0.05).

Keywords: Immunosuppressant

Immunosuppressants can suppress the proliferation and function of cells related to the immune response, such as T cells, B cells and other macrophages, thereby weakening the antibody immune response[1,2]. At present, liver, kidney, skin and heart transplantation have been quite successful, among which kidney and liver transplantation are applied most widely in clinical practice[3]. In children, living donor liver transplantation si considered as one of the effective and safe therapeutic regimen for malignancy and end-stage liver disease[4].

Currently, tacrolimus is mainly used as the first-line immunosuppressant after liver transplantation in adults and children[5]. However, tacrolimus needs to be taken for a long time and has high costs, with a short therapeutic window and large individual differences. In existing reports of liver transplantation centers in China, the dosage of tacrolimus and effective range of blood drug concentration after liver transplantation in children are inconsistent[6]. Compared with adult recipients, the immune system of child liver transplant recipients has not fully developed. Gene polymorphisms have been shown to be responsible for large individual differences in tacrolimus pharmacokinetics[7,8]. Tacrolimus is mainly metabolized by cytochrome P450 3A (CYP3A) in the liver and intestine[9], and it is the substrate of P-glycoprotein (P-gp) encoded by multidrug resistance gene 1 (MDR1)[10]. Human pregnane X receptor (PXR), a fat-soluble ligand-dependent transcription factor, can affect the metabolic pathways of tacrolimus in the liver and intestine through regulating the gene expressions of key metabolic enzymes (CYP3A and MDR1).

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Glucocorticoid (GC) is an extremely important class of regulatory molecules dominantly regulating development, growth, metabolism and immune function. After liver transplantation for hepatic malignancy in children, double or triple anti-rejection therapy with tacrolimus + GC and mycophenolate mofetil is also an authoritative immunosuppressive therapy. GC is the inducer of P-gp and/or CYP3A[11]. The combination of GC and P-gp and/or CYP3A substrate is known to alter the pharmacokinetic characteristics of drugs in patients, thus changing the efficacy or side effects[12]. GC can significantly increase the blood concentration of tacrolimus after pediatric liver transplantation[5]. Nevertheless, whether the interaction between GC and tacrolimus is associated with the gene polymorphisms of CYP3A, MDR1 and PXR has not been reported yet. Here, we explored the correlations of synergistic effect of glucocorticoids on tacrolimus with CYP3A, MDR1 and PXR gene polymorphisms in pediatric patients receiving liver transplantation.

Materials and methods

Baseline clinical data

A total of 340 pediatric patients who underwent liver transplantation for malignancy and end stage liver disease in our hospital from June 2015 to June 2020 and received tacrolimus maintenance therapy after operation were selected. Inclusion criteria: (i) Patients undergoing anti-rejection therapy with tacrolimus + mycophenolate mofetil after organ transplantation; (ii) aged <14 years old; (iii) with stable liver function (level of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) <3 times that of the upper limit of normal range); (iv) without using other drugs affecting CYP3A enzyme and P-gp (e.g. diltiazem, ketoconazole, berberine and bifendate); (v) with complete clinical data; and (vi) those who took GC for ≥12 months. Exclusion criteria: (i) Patients complicated with fungal infection; (ii) with ALT or AST level>5 times that of the upper limit of normal range; (iii) with renal creatinine clearance rate <20 mL/min; (iv) complication with severe blood diseases or complications. The research protocol was approved by the Medical Ethics Committee of our hospital, and the guardians of all children were informed and signed the informed consent. They were divided into GC (+) and GC (−) groups according to whether GC prednisone acetate was combined.

Treatment methods

In GC (−) group, the patients were treated with routine symptomatic treatment: Tacrolimus Capsules (Astellas Pharma Inc., Ireland) (0.5-4.5 mg, po, bid) + Mycophenolate Mofetil Tablets (Shanghai Roche Pharmaceutical Co., Ltd., China) (0.5-1 g, po, bid). Based on the above treatment, the patients in GC (+) group were given prednisone acetate tablets (Zhejiang Xianju Pharmaceutical Co., Ltd., China) (5-30 mg, po, qd). The treatment lasted for ≥12 months in both groups.

Measurement of blood concentration of tacrolimus

Before medication and at least 1 week after the dose of tacrolimus was adjusted or prednisone acetate tablets were combined, 5 mL of fasting venous blood was drawn on the next day morning. Then the blood trough concentration of tacrolimus in patients was detected within 3 hours by chemiluminescence microparticle immunoassay using Architect i1000 automatic immunoassay analyzer (Abbott, USA) 0, 1, 3, 6 and 12 months after medication, and the blood drug concentration corrected by daily dose, i.e. blood trough concentration/daily dose [C0/D, (ng/mL)/(mg/d)], at each time point was calculated.

Analysis of YP3A, MDR1 and PXR gene polymorphisms by PCR-RFLP

The peripheral blood DNA was extracted using Wizard DNA extraction kit (Promega, USA) according to the manufacturer′s instructions. Upstream primer of CYP3A 6986: 5'-CATGACTTAGTAGAC AGATGAC-3', downstream primer: 5'-GGTCCAAA CAGGGAAGAAATA-3'.Upstream primer of MDR1 exon 21 G2677A: 5'-TACCCATCATTGCAATAGC AG-3', downstream primer: 5'-TTTAGTTTTGACTC ACCTTTCTAG-3'. Upstream primer of PXR rs3842689: 5'AATCACCACAGGAGAAGC-3', downstream primer: 5'-AAAAGGAATCAGGACTGAGT-3'. PCR was performed as follows: pre-denaturation at 98°C for 3 min, (denaturation at 98°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 40 s) × 34 cycles, and extension at 72°C for 5 min finally.The PCR products were subjected to 1.5% agarose gel electrophoresis. Then the gel was recycled and identified by enzyme digestion on water bath at 37°C for 2 h, and the results were determined.

Statistical analysis

SPSS 20.0 software was used for statistical analysis. Normality analysis was performed using the Kolmogorov-Smirnov test. The parametric

method was adopted for the data in line with normal distribution and homogeneity of variance; otherwise the non-parametric method was utilized. Quantitative data in line with normal distribution were expressed as (mean ± SD), and the independent-samples t test was conducted for intergroup comparisons. Quantitative data not conforming to normal distribution were expressed as median (P_{25}, P_{75}), and the Mann-Whitney U test was carried out for intergroup comparisons. Numerical data were expressed as rate, and the χ^2 test or Fisher's exact test was used for intergroup comparisons. Whether the distribution of CYP3A, MDR1 and PXR genotypes conformed to Hardy-Weinberg equilibrium was evaluated by the χ^2 test. The change trend of C0/D of tacrolimus with time and the effect of GC on C0/D of tacrolimus were explored using repeated measures analysis of variance. The differences in C0/D were detected through analysis of covariance among patients with different genotypes in the two groups. C0/D of tacrolimus and red blood cell (RBC) count 0 month after medication were used as covariates in repeated measures analysis of variance and analysis of covariance, and C0/D was converted into natural logarithm ln C0/D. Analysis of variance was performed after lnC0/D conformed to normal distribution and homogeneity of variance. P <0.05 was considered statistically significant.

Results

Baseline clinical data

Among the 340 patients, there were 148 cases in GC (+) group, including 102 boys and 46 girls with an average age of (10.41±2.91) years old. There were 192 cases in GC (–) group, including 130 boys and 62 girls with an average age of (10.62±2.94) years old. The two groups had similar gender, age, body mass, BMI, time of transplantation, serum creatinine, ALT, AST and hemoglobin levels(P >0.05), but significantly different RBC counts (P <0.001) (Table 1).

Distribution of CYP3A, MDR1 and PXR genotypes

GG, AG and AA genotypes of CYP3A were observed in 130, 166 and 44 cases, respectively. CC, CT and TT genotypes of MDR1 were found in 152, 142 and 46 cases, respectively. Moreover, there were 252, 74 and 14 cases of WW, WM and MM genotypes of PXR, respectively. They all conformed to Hardy-Weinberg equilibrium (P >0.05).

GC (+) and GC (–) groups had significantly different distributions of CYP3A GG and AG+AA genotypes (P <0.001), different distributions of MDR1 CC and CT+TT genotypes (P=0.005), but similar distribution of PXR WW and WM+MM genotypes (P >0.05) (Table 1).

Effects of GC on C0/D of tacrolimus

Concentration/daily dose (C0/D) of tacrolimus was significantly higher in GC (+) group than that of

Table 1 — Baseline clinical data of selected patients and Distribution of CYP3A, MDR1 and PXR genotypes

	GC (+) group (n=148)	GC (-) group (n=192)	χ^2/t/U	*P*
Clinical data				
Male/female (case)	102/46	130/62	0.057	0.812
Age (year)	10.41±2.91	10.62±2.94	0.656	0.512
Body mass (kg)	33.09±4.61	33.45±4.69	0.707	0.480
BMI (kg/m^2)	19.78±2.18	20.02±2.19	1.004	0.316
Serum creatinine [median (P_{25}, P_{75})] (μmol/L)	118.62 (89.23, 150.27)	107.26 (90.23, 143.89)	0.375	0.643
Transplantation time [median (P_{25}, P_{75})] (month)	18.00 (6.00, 72.00)	23.50 (12.00, 29.00)	0.984	0.243
ALT [median (P_{25}, P_{75})] (U/L)	19.50 (12.00, 29.50)	19.00 (12.00, 27.50)	0.253	0.784
AST [median (P_{25}, P_{75})] (U/L)	16.50 (12.00, 20.00)	17.00 (12.00, 21.00)	0.324	0.675
RBC ($\times 10^{12}L^{-1}$)	3.66±0.72	4.00±0.84	3.934	<0.001
Hemoglobin [median (P_{25}, P_{75})] (g/L)	113.50 (96.50, 128.50)	123.00 (100.00, 137.50)	1.927	0.074
Genotypes				
CYP3A			35.817	<0.001
GG	30 (20.3)	100 (52.1)		
AG+AA	118 (79.7)	92 (47.9)		
MDR1			7.716	0.005
CC	54 (36.5)	98 (51.0)		
CT+TT	96 (63.5)	94 (49.0)		
PXR			0.179	0.672
WW	108 (73.0)	144 (75.0)		
WM+MM	40 (27.0)	48 (25.0)		

GC (–) group 0 month after treatment (P <0.05), which were similar at other time points (P >0.05). Repeated measures analysis of variance showed that the main effect of grouping was significant (P <0.05), while the main effect of time and the interactive effect of time and grouping were not significant within 1-12 months (P >0.05). C0/D of tacrolimus was higher in GC (+) group than that in GC (–) group, the change trend of C0/D of tacrolimus with time was not obvious, and the difference between the two groups did not change with time (Table 2).

Effects of CYP3A, MDR1 and PXR gene polymorphisms on synergistic effect of GC

One month after medication, C0/D of tacrolimus was significantly higher in patients with GG genotype in GC (+) group than that in patients with the same genotype in GC (–) group (P <0.05). C0/D of patients with GG genotype had nosignificant difference between the two groups 3, 6 and 12 months after medication (P >0.05). C0/D was significantly higher in patients with AG+AA genotype in GC (+) group than that in patients with the same genotype in GC (–) group (P <0.05). No statistically significant differences were found in C0/D of patients with various genotypes of MDR1 and PXR at each time point (P >0.05) (Table 3).

Table 2 — Concentration/Dose (C0/D) values of tacrolimus after select periods of treatment in CYP3A group [median (P_{25}, P_{27}), (ng/mL)/(mg/d)]

	GC (+) group (n=148)	GC (–) group (n=192)	U*	$P^{\#}$
After 0 month	0.73 (0.54, 1.04)	1.59 (0.95, 2.43)	6.098	<0.001
After 1 month	1.72 (1.37, 2.40)	1.65 (1.00, 2.63)	0.894	0.113
After 3 months	1.86 (1.32, 2.33)	1.77 (1.09, 2.43)	0.802	0.258
After 6 months	1.92 (1.37, 2.55)	1.74 (1.24, 2.51)	1.203	0.232
After 12 months	1.89 (1.50, 2.54)	1.74 (1.30, 2.72)	0.903	0.386
$F^{\#}$	19.293			
$P^{\#}$	<0.001			

[*Mann-Whitney U test; #repeated measures analysis of variance]

Discussion

After entering the human body, 83.2% of tacrolimus binds RBCs[13]. RBC count in GC (+) group was lower, so fewer RBCs bound to tacrolimus, which may be responsible for the markedly lower C0/D of tacrolimus in GC (+) group than that in GC (–) group 0 month after medication.

In this study, GC effectively raised C0/D of tacrolimus, being consistent with a previous literature[14]. The change in trend of C0/D of tacrolimus with time was not obvious within 1-12 months after taking GC (main effect of time, P >0.05). CYP3A enzyme can metabolize midazolam (with similar metabolic characteristics to tacrolimus *in vivo*) into 1'-hydroxy midazolam. After GC is used, the bioavailability of midazolam is greatly improved,

Table 3 — Concentration/Dose (C0/D) values of tacrolimus in patients with different genotypes after select periods of treatment in MDR1 and PXR groups [median (P_{25}, P_{27}), (ng/mL)/(mg/d)]

Genotype	Group	After 0 month	After 1 month	After 3 months	After 6 months	After 12 months
GG	GC (+) group (n=30)	1.08 (0.86,2.36)	2.55 (1.44,3.83)	2.21 (1.34,3.23)	2.36 (1.59,4.17)	2.04 (1.88,3.11)
	GC (–) group (n=100)	2.21 (1.53,3.23)	2.30 (1.54,3.31)	2.26 (1.52,2.69)	2.37 (1.66,3.24)	2.35 (1.59,3.17)
	F		5.718	0.836	1.092	0.165
	P		0.019	0.403	0.301	0.718
AG+AA	GC (+) group (n=118)	0.67 (0.51,0.89)	1.67 (1.36,2.14)	1.86 (1.32,2.28)	1.89 (1.32,2.41)	1.79 (1.36,2.50)
	GC (–) group (n=92)	1.19 (0.75,1.60)	1.19 (0.84,1.89)	1.22 (0.91,1.78)	1.42 (0.97,1.69)	1.33 (0.89,1.79)
	F		13.487	20.084	17.265	16.598
	P		<0.001	<0.001	<0.001	<0.001
CC	GC (+) group (n=54)	1.23 (0.83,2.45)	2.45 (1.26,3.56)	2.19 (1.18,3.32)	2.37 (1.51,4.12)	2.27 (1.56,3.23)
	GC (–) group (n=98)	2.08 (1.46,3.34)	2.40 (1.18,3.56)	2.23 (1.43,2.64)	2.37 (1.61,3.29)	2.32 (1.45,3.45)
	F		0.876	0.756	0.567	0.892
	P		0.226	0.376	0.584	0.205
CT+TT	GC (+) group (n=96)	0.75 (0.49,0.92)	1.61 (1.31,2.18)	1.56 (1.19,2.34)	1.69 (1.17,2.21)	1.49 (1.31,2.60)
	GC (–) group (n=94)	1.06 (0.69,1.65)	1.56 (1.14,2.03)	1.53 (1.01,2.09)	1.62 (1.07,1.98)	1.53 (1.29,2.54)
	F		0.943	0.805	0.683	0.298
	P		0.122	0.336	0.435	0.847
MM	GC (+) group (n=108)	1.12 (0.82,2.39)	2.36 (1.14,2.84)	2.22 (1.24,2.73)	2.35 (1.41,3.38)	2.19 (1.18,2.91)
	GC (–) group (n=144)	2.15 (1.45,3.45)	2.33 (1.24,2.86)	2.25 (1.32,2.62)	2.38 (1.46,3.45)	2.22 (1.19,3.02)
	F		0.764	0.647	0.924	0.671
	P		0.432	0.354	0.112	0.329
WM+MM	GC (+) group (n=40)	0.69 (0.52,0.96)	1.54 (1.12,2.45)	1.56 (1.12,2.45)	1.59 (1.12,2.67)	1.48 (1.17,2.70)
	GC (–) group (n=48)	1.15 (0.78,1.74)	1.48 (0.89,1.99)	1.54 (0.99,2.38)	1.52 (1.07,2.71)	1.46 (1.12,2.83)
	F		0.764	0.786	0.928	1.023
	P		0.337	0.342	0.194	0.098

proving that GC can inhibit the metabolic activity of CYP3A enzyme[15]. Both GC and tacrolimus are substrates of CYP3A enzyme. GC has higher affinity for CYP3A enzyme than tacrolimus. As a result, the number of CYP3A enzymes metabolizing tacrolimus declines, and the blood concentration of tacrolimus rises[16].

CYP3A GG genotype can encode abnormal mRNAs, and make CYP3A5 enzymes unable to be expressed normally, leading to the decline in the metabolic rate of tacrolimus. The patients with GG genotype are low-dose tacrolimus group. On the contrary, normal mRNAs can be produced and a large number of functional CYP3A5 enzymes are expressed in patients with AG+AA genotype, thus increasing the metabolic rate of tacrolimus, so patients with AG+AA genotype are high-dose tacrolimus group[17]. Herein, when tacrolimus was combined with GC, the effects of different CYP3A genotypes on C0/D of tacrolimus differ greatly. Patients with CYP3A AG+AA genotype were more sensitive to the synergistic effect of GC (at 1-12 months after medication). GC markedly raised C0/D of tacrolimus in patients with CYP3A AG+AA genotype, being consistent with the results of a prospective study[18] in which, however, patients with CYP3A GG genotype were not studied. In another study, the dosage of tacrolimus in patients with CYP3A GA genotype in GC group declined by 36% compared with that in control group, but it was not compared in patients with CYP3A GG and AA genotypes[19]. The metabolism of CYP3A enzymes is affected by substrates, inhibitors and number of enzymes. The individual differences caused by the same inhibitor and substrate are largely determined by the number of metabolic enzymes[18]. More CYP3A5 enzymes can be expressed in the case of CYP3A5 AG+AA genotype[20]. Patients with AG+AA genotype are more sensitive to GC, while GC has no significant effect on patients with GG genotype, but its mechanism has not been clarified yet. Probably, the activity of CYP3A5 enzymes is high and can be competitively inhibited by GC in patients with CYP3A expressed genotype, thus obviously raising the blood concentration of tacrolimus. Notably, the effects of CYP3A genotypes on drug interaction of tacrolimus are inconsistent. For example, the tacrolimus clearance rate is higher in patients with CYP3A AG+AA genotype when amlodipine is combined with tacrolimus[21]. C0/D of tacrolimus is higher in patients with CYP3A AG+AA genotype when diltiazem is combined with tacrolimus, while it is higher in patients with CYP3A GG genotype in the case of combination of ketoconazole and tacrolimus[22]. The results of this study are inapplicable to other drugs affecting the metabolism of tacrolimus, and the mechanism still needs further research.

Conclusion

Results of the present study have demonstrated that glucocorticoids (GC) could singnificantly increase the concentration/daily dose (C0/D) of tacrolimus in patients reveiving liver transplantation for malignancy and chronic liver disease with CYP3A AG+AA genotype, but not in patients with GG genotype. Hence, it is recommended that when GC is used as the synergist for tacrolimus, CYP3A genotypes may be considered.

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Conflict of Interest

Authors declare no competing interests.

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