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MDR1(ABCB1) gene polymorphisms associated with steroid-induced osteonecrosis of femoral head in systemic lupus erythematosus

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Received March 28, 2007, accepted April 23, 2007

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Pharmazie 62: 930–932 (2007) *doi: 10.1691/ph.2007.12.7583*

This study investigated the relationship between genetic polymorphism in the MDR1 (C3435T, G2677T) and the development of steroid-induced osteonecrosis of femoral head (ONF) in Chinese systemic lupus erythematosus (SLE) patients. 127 patients with active SLE, receiving 40 mg/day or more prednisolone were included. Patients were observed for the development of ONF by magnetic resonance imaging (MRI) and plain radiography first at three months after the beginning of steroid treatment and subsequently every year for five years. Genomic DNA was obtained from peripheral blood lymphocytes. The MDR1 2677G > T and 3435C > T genotypes were determined by the PCR-RFLP assay. 21 patients developed steroid-induced ONF. The incidence of ONF was significantly higher in steroid pulse therapy. The MDR1 3435 TT genotypes were significantly lower in the incidence of ONF (adjusted odds ratio = 0.14, 95% CI 0.017–1.153, p = 0.038). The MDR1 2677 TT was also lower in the incidence of ONF (adjusted odds ratio = 0.21, 95% CI 0.018–1.301, p = 0.05). Our findings suggest that MDR1 (ABCB1) gene polymorphisms can be used for predicting the development of ONF.

1. Introduction

Osteonecrosis of femoral head (ONF) is one of the major complication of systemic lupus erythematosus (SLE) and badly affects daily life of patients. Around 10% of patients are developing clinically symptomatic ONF but the prevalence reaches 30–40% when asymptomatic ONF is included (Gladman et al. 2001), and the development of ONF is closely related to corticosteroid administration.

Although it is well known that corticosteroid therapy is a cause of ONF in SLE, previous clinical studies have provided conflicting results about the possible relationship of corticosteroid therapy with the development of ONF. The total treatment period, the highest daily dose, a continuous high dose and the cumulative dose of corticosteroid have been reported as the risk factors of ONF in SLE (Zonana-Nacach et al. 2005; Migliaresi et al. 1994; Zizic et al. 1985; Rascu et al. 1996). These conflicting results seem to be mainly due to the presence of individual difference in steroid sensitivity (Asano et al. 2003a).

It was recently suggested thad this individual difference in drug sensitivity is mediated by genetic polymorphisms in drug-metabolizing enzymes or drug target molecules (Ambudkar et al. 1999). The multidrug resistance gene 1 (MDR1, ABCB1) encodes P-glycoprotein (P-gp), which acts as an energy-dependent membrane efflux pump for a wide spectrum of therapeutic agents including steroids and plays an important role in absorption and distribution of drugs (Sakaeda et al. 2002; Dean et al. 2001). The genetic polymorphism of MDR1 may have an impact on the expression and function of P-gp, thereby influencing the intracellular and extracellular distribution and pharmacokinetics of steroids and their metabolites and consequently, this may lead to individual differences in steroid sensitivity. So far many single nucleotide polymorphisms (SNPs) of MDR1 have been identified, of which mutations in exon 21 (G2677T) and exon 26 (C3435T) are associated with alteration of P-gp expression and/or function, as recently reviewed by Sakaeda et al. (2003) and Fromm et al. (2003). There were several clinical studies on the effects of the C3435T polymorphism and drug treatment with cyclosporine, tacrolimus, digoxin and tricyclic antidepressants (Hauser et al. 2005; Hesselink et al. 2003; Greiner et al. 1999; Roberts et al. 2002).

We have hypothesized that C3435T or G2677T of MDR1 gene could be involved in the development of steroid-induced ONF in SLE patients by affecting the expression and function of P-gp. To test this hypothesis, we evaluated the relationship between MDR1 C3435T, G2677T and the development of ONF in SLE patients treated with steroids.

2. Investigations and results

2.1. Characteristics of patients with or without osteonecrotic lesions

We diagnosed ONF as described below. ONF developed in 21 patients, 27 hips were affected. Bilaterality was found in 7 patients. Table 1 shows the clinical characteristics of in the ONF group and non-ONF group. There were no significant differences in sex, age, SLEDAI, initial dose of prednisolone between the ONF and non-ONF groups. In a comparison of the two groups, the incidence of ONF was significantly higher under steroid pulse therapy.

Table 1: Characteristics of study patients

	ONF group	Non-ONF group
Age (years)		
mean	30.67	34.13
range	17–51	11–67
Sex		
Female	19	97
Male	2	9
Initial corticosteroid dose	46.67 (4.93)	44.32 (6.32)
Mppt#	9 (42.8%)*	11 (10.4%)

#: intravenous 1000 mg methylprednisolone for three days
* $p < 0.001$ by step-wise multi regression analysis as well as by univariate chi-square analysis

2.2. Relationship between MDR1 genotypes/haplotypes and ONF development

The frequencies of the MDR1 3435 CC, CT and TT genotypes among the patients were 39.4%, 42.5% and 18.1%, respectively. The frequencies of the MDR1 2677GG, GT and TT genotypes among the patients were 31.5%, 52.8% and 15.7%. The genotype distributions of both polymorphisms among the patients were in Hardy-Weinberg equilibrium. Relationships between MDR1 genotypes/haplotypes and ONF development are shown in Table 2.

3. Discussion

Steroids are widely administered to patients with SLE. Among the known adverse events, the development of ONF is frequently observed and often requires surgical treatment. By far, no means can predict the development of ONF because of the individual differences in steroid sensitivity. The individual differences may be correlated to steroid metabolism, corticosteroid receptors or drug transport. It has been demonstrated that the oral steroid most frequently administered to SLE patients is prednisolone (PSL), a substrate for P-gp which might influence the steroid metabolism and result in the development of ONF.

In this paper we shown that the risk of ONF development in the homozygous wild-type 3435TT was significantly lower than that of the homozygous variant type 3435CC in the multivariate analysis, which indicates that the TT genotype decreases the incidence of corticosteroid-induced ONF in SLE patients. The result suggest that the development of steroid-induced ONF might be correlated with P-gp expression and function.

A strong relationship between the two polymorphisms G2677T in exon 21 and C3435T in exon 26 has been found (Tanabe et al. 2001). The $G \rightarrow T$ mutation at position 2677 results in change of the amino acid Ala893 into Ser. As described in previous studies (Tang et al. 2002; Kroetz et al. 2003), we also found the linkage disequilibrium between C3435T and G2677T. As well as with C3435T, the risk of ONF development decreased in the homozygous variant type G2677T. The result suggest the relationship between MDR1 polymorphism and the development of steroid-induced ONF in SLE patients.

Several studies have stated the relationship of the polymorphisms G2677T in exon 21 and C3435T in exon 26 with P-gp expression and function, but there is still controversy. Hoffmeyer et al. (2003) reported a significant correlation of a polymorphism in C3435T in exon 26 with the expression level and function of P-gp. Patients with 3435TT genotype had higher plasma digoxin levels than patients with 3435CC genotype. Kim et al. (2001) found that the 2677TT was associated with a 2-fold enhanced efflux of digoxin compared with the 2677CC. In contrast, Drescher et al. (2002) examined the plasma levels of fexofenadine, another substrate for P-gp, and revealed no significant differences between individuals with different C3435T and G2677T genotypes. In the present study, the 2677TT and 3435 TT genotype decreases the risk of ONF development. These findings are in agreement with the result reported by Asano et al. (2003b). The result indicated that the 2677TT and 3435 TT genotype can increase the pump activity of P-gp, which may affect distribution of steroids and their metabolites. The increase in P-gp activity may decrease the accumulation of steroids in specific tissues, which may induce the development of ONF. Therefore, MDR1 C3435T and G2677T polymorphisms might decrease the risk for ONF.

In our study, one patient with the 3435TT genotype and two patients with the 2677TT genotype developed ONF. The three patients all received steroid pulse therapy. And we also found steroid pulse therapy apparently contributed to enhancing the development of ONF. The result is unclear. It may be because these patients, despite the genotype of 3435TT or 2677TT, had not enough activity of P-gp to deal with the highest dose of steroid, which may result in an increase in intracellular steroid concentrations and finally damaged the specific tissue contributing to the development of ONF. In addition to genetics, non-genetic factors play an important role in modifying expression and function of P-gp. It should also be considered that steroid pulse therapy could result in ONF by other mechanisms (Nagasawa et al. 2005; Oinuma et al. 2001).

In conclusion, we found that the MDR1 polymorphism may have an influence on the risk of ONF. Although many factors may be involved in the pathogenesis of ONF, including hyperocagulation, altered lipid metabolism, vasculitis, primary cell death and others (Assouline-Dayan et al. 2002; Nagasawa et al. 2006), we suggest

Table 2: Relationship between MDRI G2677T and C3435T genotypes and the development of ONF

	On group	Non-on group	Crude or (95% ci)	p	Adjusted or (95% ci)	p
3435cc	11(52.3)	39(36.8)	1		1	
3435ct	9(42.9)	45(42.4)	0.709 0.266–1.889	0.491	0.687 0.253–1.781	0.450
3435tt	1(4.8)	22(20.8)	0.161 0.019–1.333	0.059	0.14 0.017–1.153	0.038
2677gg	10(47.6)	30(28.3)	1			
2677gt	10(47.6)	57(53.7)	0.526 0.197–1.405	0.196	0.478 0.183–1.254	0.129
2677tt	1(4.8)	19(17.9)	0.158 0.019–1.335	0.059	0.21 0.018–1.301	0.05

Adjusted for age, sex, SLEDAI and MPPT

that these polymorphisms contribute to the development of steroid-induced ONF in SLE patients.

4. Experimental

4.1. Patients

We screened 127 new patients with active SLE who were treated with a high dose (40 mg/day or more) prednisone as the initial treatment including pulse therapy with 1000 mg/day methylprednisolone for three days. All the patients fulfilled the 1982-revised classification of ARA criteria for SLE (Tan et al. 1982). The study group consisted of 11 males and 116 females ranging from 11–63 years of age. The disease activity of SLE was evaluated using the SLE Disease Activity Index (SLEDAI) (Bombardier et al. 1992). To make a diagnosis of ONF, all the patients underwent plain radiography and MRI first at three months after the beginning of steroid treatment and subsequently every year for five years. Plain radiography consisted of antero-posterior pelvic and lateral projections of individual hips. The radiographic changes consisted of radiolucency with marginal sclerosis, crescent sign beneath the articular cartilage or distinct collapse. T1 weighted MRI was obtained with a spin-echo pulse sequence and coronal and axial views were analysed. ONF development was diagnosed according to the criteria of the Research Committee, Ministry of Health and Welfare of Japan. At the end, patients were divided into the ONF group and the non-ONF group based on the presence or absence of abnormal MRI.

We excluded patients with the following conditions: (a) those with a history of steroid therapy; (b) those who had been confirmed to have ONF by MRI before starting steroid therapy; (c) those who did not complete the study.

This study was approved by the Ethics Committee of College of Medicine Zhejiang University and written informed consent was obtained from all patients before enrollment.

4.2. MDR1 Genotyping

Genomic DNA was obtained from peripheral blood lymphocytes MagNA Pure LC (Roche, Mannheim, Germany). The MDR1 2677G > T and 3435C > T genotypes were determined by the PCR-RFLP assay. PCR primers were designed based on the GenBank reference sequence (Accession number: M29440 for G2677T; M29445 for C3435T). The PCR primers for 2677G > T and 3435C > T polymorphisms were 5′-GCAGGAGTTGTTGAAATGAAAATG (forward) and 5′-CGCCTGCTTTAGTTTGACTCA (reverse), and 5′-TGTTTTCAGCTGCTTGATGG (forward) and 5′-AAGGCATGTATGTTGGCCTC (reverse), respectively. The PCR amplification conditions consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 30 s at 94 °C; 30s at 58 °C for 2677G > T and at 56 °C for 3435C > T; 30 s at 72 °C; and a final elongation step at 72 °C for 10 min.

4.3. Statistical analysis

A chi-square test was used to compare the clinical variable between the ONF and the non-ONF group. The crude and adjusted odds ratios (OR) and their 95% confidence interval (CI) were calculated by the logistic regression model. P-values ≤ 0.05 were considered to be of statistical significance.

Acknowledgement: This project was supported by the Zhejiang Province Nature Science Foundation of China, No. Y204431.

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