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Correlation of single-nucleotide polymorphisms of the *1C3435T* and *ECE-1* genes with the efficacy of irbesartan in primary hypertension.

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Abstract

Objective: To investigate the correlation of single-nucleotide polymorphisms of the Multidrug Resistance Gene (MDR) *1C3435T* and the human Endothelin Converting Enzyme (*ECE-1*) gene with the efficacy of irbesartan in primary hypertension.

Methods: A total of 186 patients with primary hypertension in our hospital from May 2015 to December 2016 received irbesartan oral medication. The *1C3435T* and *ECE-1* gene polymorphic sites were detected. Meanwhile, the plasma concentration was measured every hour after taking medicine for 8 h, and the plasma concentration peak was recorded. The systolic and diastolic blood pressures of the patients were recorded before and after treatment. The relationships between the different genotypes and plasma concentrations, as well as the drop of blood pressure, were compared. The antihypertensive effects of different genotypes were also investigated.

Results: The distribution frequencies of the *1C3435T* CT, TT, and CC genotypes were 37.63%, 34.95%, and 27.42%, respectively, whereas those of the *C-338A* CA, AA, and CC genotypes were 32.80%, 30.65%, and 36.56%, respectively. The plasma concentration peak, drop of blood pressure, and total effective rate of the *1C3435T* TT genotype were significantly higher than those of CT and CC (P<0.05). The corresponding data of CT were significantly also higher than those of CC (P<0.05). Moreover, the plasma drug concentration, drop of blood pressure, and total effective rate of the *C-338A* CC genotype were significantly higher than those of CA and AA (P<0.05), and the corresponding data of CA were significantly higher than those of AA (P<0.05).

Conclusion: During the oral treatment of primary hypertension by irbesartan tablets, the *MDR1 1C3435T* TT and *ECE-1 C-338A* CC genotypes can significantly increase the plasma concentration peak and significantly improve the curative effect.

Keywords: Multidrug resistance gene *1C3435T*, Endothelin converting enzyme-1, Primary hypertension, Irbesartan.

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Introduction

Hypertension is characterized by adult Systolic Blood Pressure (SBP) ≥ 140 mmHg or Diastolic Blood Pressure (DBP) ≥ 90 mmHg in the resting state. Primary hypertension is a condition where the cause of blood pressure elevation is unclear. This disease features slow progression, insidious onset, and long course. Early symptoms of primary hypertension include headache, distention in the head, tinnitus, dizziness, amnesia, insomnia, dreaminess, and fatigue [1,2]. At present, the prevalence rate of hypertension is high in the Chinese population, and patients with this disease are becoming younger. Irbesartan is an effective antihypertensive drug, but its clinical antihypertensive effects vary because some patients are resistant to this drug. Studies have reported that the Multidrug Resistance gene (MDR) polymorphic site *1C3435T* and the human endothelin converting enzyme (ECE) polymorphic site *C-338A* are closely correlated with the antihypertensive effect of irbesartan [3,4]. In the present study, the effects of *1C3435T* and *C-338A* gene polymorphisms on the antihypertensive effect of irbesartan were further explored. A total of 186 patients with primary hypertension received irbesartan oral medication, and the Single-Nucleotide Polymorphisms (SNPs) of the gene sites mentioned above were detected and compared.

Materials and Methods

General data

A total of 186 patients with primary hypertension in our hospital from May 2015 to December 2016 were selected. In accordance with the diagnostic criteria of primary hypertension, the inclusion criteria for patient selection were arterial SBP ≥ 140 mmHg or DBP ≥ 90 mmHg for 3 consecutive days (different days) (in sitting position), hypertension grade of 1 or 2, without any oral antihypertensive drugs for ≥ 2 w, and provision of informed consent letter. Meanwhile, the exclusion criteria for patient selection were isolated systolic hypertension, secondary hypertension, cerebrovascular disease, cancer, diabetes, severe liver and kidney dysfunction, cardiomyopathy, heart failure, coronary heart disease, severe heart valve disease, and stenocardia. Of the 186 patients enrolled in this study, 96 were males and 90 were females, with an age range of 40-73 y and a mean age of 56.7 ± 2.4 y.

Methods

Reagents and materials: Irbesartan tablets (0.15 g) were purchased from Xiuzheng Pharmaceutical Co., Ltd. QIAamp DNA Blood Mini Kit was obtained from Qiagen Company (Germany), DNA Marker from Biyuntian Biological Technology Co. Ltd., and agarose from Beijing RuiDaHengHui Science and Technology Development Co., Ltd. Pure chromatograph was purchased from Merck (Germany), a multi-purpose gel imaging analysis system from BIO-RAD Co. (U.S), a high-speed centrifuge from BIOBASE Boke, a NanoDrop2000c ultraviolet spectrophotometer from Thermo Company (American), and a high-performance liquid chromatograph from SongGang Tianrui (Baoan District, Shenzhen).

Detection of *MDR1* and *ECE-1* gene polymorphisms: *Blood sample collection:* Peripheral vein blood was collected from the patients the next morning after fasting and then placed in EDTA tubes. Anticoagulant processing was performed. The samples were preserved in -80°C. DNA extraction: The samples were unfrozen and subpackaged. DNA extraction was conducted using the QIAamp DNA Blood Mini Kit in accordance with the manufacturer's instructions. Measurement of DNA sample content: The DNA concentration, purity, and A260/A280 value of the samples were measured using 1% agarose gel electrophoresis and UV spectrophotometry. *MDR1* and *ECE-1* gene SNP sites were then selected. Two SNP sites that are closely correlated with *MDR1* and *ECE-1*, namely, *1C3435T* and *C-338A*, were selected on the basis of relevant literature and gene databases. SNP genotyping detection: Genes in the SNP sites were detected by Beijing Genomics Institute using the MassARRAY molecular weight array technology platform to obtain *1C3435T* and *C-338A* genotyping results.

Measurements of plasma concentration and blood pressure: The plasma drug concentration was detected every other hour of medication for 8 h. On the basis of the results, the plasma drug concentration-time curve was drawn. The plasma concentration was also recorded. The SBP and DBP were determined before and after performing the conventional Korotkoff-Souna Method. The corresponding drop of blood pressure was also calculated (ΔDBP and ΔSBP).

Observation index

The following observation indices were observed: genotype and allele distributions of *MDR1* SNP site *1C3435T* and *ECE-1* SNP site *C-338A* in hypertensive patients; plasma drug concentration values of the *1C3435T* and *C-338A* genes within 8 h after taking irbesartan tablets; blood pressure difference between the *1C3435T* and *C-338A* genes before and after treatment; antihypertensive efficacies of patients with the *1C3435T* and *C-338A* genotypes: drop of DBP ≥ 10 mmHg with decrease to normal level or drop of DBP ≥ 20 mmHg without decrease to normal level, which was excellent, drop of DBP<10 mmHg with decrease to normal level or DBP decrease by 10-19 mmHg or drop of SBP ≥ 30 mmHg without DBP decease to normal level, which was effective. Results were considered invalid if the above criteria were not met. Total effective rate=(excellent+effective)/total number × 100.

Statistical methods

Data were analysed using SPSS15.0. Two-sample measurement and count data were compared using t-test and χ^2 test, respectively. Multiple-sample measurement data were compared using single-factor ANOVA and the rank sum test. Statistical significance was considered at P<0.05.

Results

Polymorphic genotypes and allele distributions of 1C3435T and C-338A in patients

The distribution frequencies of *1C3435T* CT, CC, and TT were 37.63%, 34.95%, and 27.42%, respectively, whereas those of *C-338A* CC, CA, and AA were 32.80%, 30.65%, and 36.56%, respectively (Table 1).

Table 1. *Polymorphic genotypes and allele distributions in patients with 1C3435T and C-338A genotypes (n (%)).*

SNPs			n	Frequency (%)
1C3435T	Genotype	CC	70	37.63
		CT	65	34.95
		TT	51	27.42
	Allele	C	205	55.11
		T	167	44.89
C-338A	Genotype	CC	61	32.80
		CA	57	30.65
		AA	68	36.56

Allele	C	179	48.12
	A	193	51.88

Plasma concentration peaks in patients with the 1C3435T and C-338A genotypes

The plasma concentration peaks of *1C3435T* TT and CT were significantly higher than that of CC (t=211.374, 108.445, P=0.000, 0.000), and the plasma concentration peak of TT was significantly higher than that of CT (t=104.541, P=0.000). Moreover, the plasma concentration peaks of *C-338A* CC and CA were significantly higher than that of AA (t=198.618, 67.666, P=0.000, 0), and the plasma concentration peak of CC was significantly higher than that of CA (t=132.585, P=0.000) (Table 2).

Table 2. *Plasma concentration peaks in patients with 1C3435T and C-338A genotypes ($\bar{x}$ ± s). Compared with CC, [a]P<0.05; Compared with CT, [b]P<0.05; Compared with CA, [c]P<0.05.*

Genotype		n	Plasma concentration peak (μg/L)
1C3435T	CC	70	1749.24 ± 21.08
	CT	65	2176.69 ± 24.68[a]
	TT	51	2654.24 ± 28.37[ab]
C-338A	CC	61	2986.67 ± 34.38
	CA	57	2273.17 ± 27.46[a]
	AA	68	1975.76 ± 23.24[ac]

Plasma concentration peaks in patients with the 1C3435T and C-338A genotypes

The plasma concentration peaks of *1C3435T* TT and CT were significantly higher than that of CC (t=211.374, 108.445, P=0.000, 0.002), and the plasma concentration peak of TT was significantly higher than that of CT (t=104.541, P=0.000). Moreover, the plasma concentration peaks of *C-338A* CC and CA were significantly higher than that of AA (t=198.618, 67.666, P=0.000, 0.000), and the plasma concentration peak of CC was significantly higher than that of CA (t=132.585, P=0.000) (Table 3).

Table 3. *The drops of blood pressure in patients with 1C3435T and C-338A genotypes ($\bar{x}$ ± s). Compared with CC, [a]P<0.05; Compared with CT, [b]P<0.05; Compared with CA, [c]P<0.05.*

Genotype		n	ΔDBP (mmHg)	ΔSBP (mmHg)
1C3435T	CC	70	15 ± 4	21 ± 5
	CT	65	17 ± 5[a]	24 ± 6[a]
	TT	51	19 ± 6[ab]	28 ± 7[ab]
C-338A	CC	61	18 ± 8	29 ± 8
	CA	57	15 ± 7[a]	26 ± 6[a]
	AA	68	13 ± 4[ac]	21 ± 7[ac]

Antihypertensive efficacies of patients with the 1C3435T and C-338A genotypes

After treatment, the total effective rates of the *1C3435T* CT, CC, and TT genotypes were 90.20%, 75.38%, and 61.43%, respectively. The total effective rates of TT and CT were significantly higher than that of CC (t=12.552, 4.122, P=0.000, 0.044), and the rate of TT was significantly higher than that of CT (t=4.229, P=0.040). In addition, the total effective rates of the *C-338A* CC, CA, and AA genotypes were 90.16%, 75.44%, and 60.29%, respectively. The total effective rates of CC and CA were significantly higher than that of AA (t=15.070, 4.069, P=0.000, 0.044), and the rate of CC was significantly higher than that of CA (t=4.539, P=0.033) (Table 4).

Table 4. *Antihypertensive efficacies of patients with 1C3435T and C-338A genotypes (n (%)). Compared with CC, [a]P<0.05; Compared with CT, [b]P<0.05; Compared with CA, [c]P<0.05.*

Genotype		n	Excellent	Effective	Ineffective	Total effective rate (%)
1C3435T	CC	70	22 (31.43)	21 (30.00)	27 (38.57)	43 (61.43)
	CT	65	24 (36.92)	25 (38.46)	16 (24.62)	49 (75.38)[a]
	TT	51	34 (66.67)	12 (23.53)	5 (9.80)	46 (90.20)[ab]
C-338A	CC	61	36 (59.02)	19 (31.15)	6 (9.84)	55 (90.16)
	CA	57	28 (49.12)	15 (26.32)	14 (24.56)	43 (75.44)[a]
	AA	68	25 (36.76)	16 (23.53)	27 (39.71)	41 (60.29)[ac]

Discussion

Primary hypertension is a systemic and complicated disease often accompanied by glycometabolism and lipid metabolism disorders, as well as organic or functional changes in the heart, kidney, brain, and retina, which seriously impairs patient health [5]. The pathogenesis of primary hypertension is complicated. Age, diet, occupation, obesity, mind, environment, and genetic factors are associated with primary hypertension. Irbesartan, an Angiotensin II receptor antagonist (ARB), is a stable and long-term antihypertensive drug widely applied in clinics. This antihypertensive drug functions by inhibiting the renin-angiotensin system. However, the efficacies of this drug are different between individuals possibly because of individual differences and SNPs.

The human MDR genes include *MDR1* and MDR3, of which *MDR1* plays a dominant role. *MDR1*-encoded P-glycoprotein (P-gp) can influence the absorption and metabolism of drugs. It can pump out intracellular drugs, thereby causing drug resistance [6]. Meanwhile, *MDR-1* genes include multiple SNPs. *MDR-1* gene polymorphisms cause different expression levels of P-gp between individuals, leading to individual differences in multidrug resistance. Moreover, *1C3435T* is an important site of *MDR1* gene polymorphisms. Studies reported

that P-gp is involved in the transmembrane transport of Irbesartan, and its inhibition could increase the absorption of irbesartan [7]. The antihypertensive effect of irbesartan might be related to the *1C3435T* genotype. In the present study, the plasma concentration peak of the *1C3435T* TT genotype was significantly higher than that of CT and CC ($P<0.05$), suggesting that the TT genotype could increase the plasma concentration of Irbesartan. After treatment, the drop of blood pressure of the TT genotype was significantly higher than that of CT and CC ($P<0.05$). Meanwhile, the total effective rate of TT was significantly higher than those of CT and CC ($P<0.05$), suggesting that the drop of blood pressure of the TT genotype was significantly increased and the antihypertensive effect was significantly increased.

ECE is a metalloprotease that includes *ECE-1* and ECE-2 genotypes. *ECE-1* plays a major role in the activation of endothelin, and its increased expression can stimulate endothelin biosynthesis. Endothelin is a potent vasoconstrictor that regulates vascular tone and increases mitosis to induce vascular remodeling. Moreover, it can increase oxidative stress to induce vascular inflammation, which promotes the occurrence and development of primary hypertension [8]. Decreased *ECE-1* expression can effectively inhibit Angiotensin II (AngII)-induced elevated blood pressure, but the inhibitory effect is affected by *ECE-1* gene polymorphisms, in which *C-338A* is an important site of *ECE-1* gene expression [9]. In addition, when the allele A carrier of *ECE-1* mRNA expression is increased, the inhibition on AngII is significantly reduced, which seriously affects the antihypertensive effect of ARBs [10]. In the present study, the plasma concentration peak of the *C-338A* CC genotype was significantly higher than that of CA and AA ($P<0.05$), suggesting that the CC genotype could increase the plasma concentration of irbesartan. After treatment, the drop of blood pressure of the CC genotype was significantly higher than that of CA and AA ($P<0.05$), and the total effective rate of CC was significantly higher than that of CA and AA ($P<0.05$), suggesting that the drop of blood pressure of the CC genotype was significantly increased and the antihypertensive effect was obviously improved.

Conclusion

The relationship between *MDR1* gene *1C3435T* and *ECE-1* gene *C-338A* polymorphisms are closely related to the efficacy of irbesartan. Moreover, the *1C3435T* TT genotype and the *C-338A* CC genotype can significantly increase the plasma concentration peak, and the drop of blood pressure is significant. Thus, the antihypertensive effects of the TT and CC genotypes were superior to those of the CT and CC, CA and AA genotypes.

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