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Abstract: Background: Dihydropyridine calcium-channel blockers (dCCBs) were widely used in antihypertensive treatment. The aim of this study was to examine the effect of polymorphisms of CACNA1C, eNOS and RGS2 on the antihypertensive efficiency of dihydropyridine calcium channel blocks (dCCBs) in Chinese patients with essential hypertension (EH). Methods: A total of 107 untreated Chinese mild to moderate EH patients were enrolled in this study, and had been prescribed azelnidipine or nitrendipine as monotherapy. All patients who had gave informed consent for genetic research were divided into two groups: treated with azelnidipine or nitrendipine for at least 6 weeks. Five polymorphisms of three blood pressure (BP) and hypertension susceptible genes were studied in our research, and these polymorphisms were identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and direct sequencing. Every patients’ BP and heart rate were measured at 0 week, 2 weeks, 4 weeks and 6 weeks. The biochemical parameters of blood were detected before and 6 weeks after the administration. Adverse effects were evaluated at the last visitation. Results: Both the systolic and diastolic BP levels were significantly decreased after six weeks of dCCBs treatment, from 149.3 ± 9.2 mmHg to 132.2 ± 11.7 mmHg and form 97.9 ± 3.0 mmHg to 85.5 ± 7.5 mmHg, as well as the levels of TP, TBIL, CHO and LDL, the P-values were P=0.017, P=0.045, P=0.039, P=0.041 respectively. As 11 of 75 patients appeared adverse reactions, the rate of adverse effects showed no difference in various genotypes. There were significant interactions between eNOS G894T polymorphism and △DBP, △MBP on azelnidipine therapy patients, but not in nitrendipine, the GG genotype carriers were more sensitive in blood decrease than GT/TT genotype carriers (P<0.05). Conclusion: CCBs had potential hepatoprotective and antiatherosclerosis effects for Chinese EH patients. And the eNOS G894T polymorphism is associated with the hypotensive effect of azelnidipine.

Keywords: Hypertension, Calciumchannel Blockers, Zelnidipine, Nitrendipine, eNOS, Polymorphism

1. Introduction

Hypertension, which is considered to be present when at least two times subsequent measurement of systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg, is a critical risk factor for cardiovascular diseases and blood pressure (BP) control in hypertensive patients is the most effective intervention for reducing cardiovascular diseases risk[1]. Although one in six Chinese adults have been bitterly suffered from hypertension, the awareness, treatment and control rate of hypertension is relatively low in China[2].

Calcium-channel blocker drugs (CCBs), especially the dihydropyridine (dCCBs) subclass, are widely used in the treatment of essential hypertension (EH)[3, 4]. As we known, the antihypertensive response rate of patients to dCCBs monotherapy is just a little above 50% in stage I or II hypertension patients; however, there are not reliable predictors to predict the hypotensive efficiency of dCCBs[5]. Previous researches have evidenced that gender is not an important factor to antihypertensive effect of CCBs when compared with age and initial BP; moreover, genetic factors can significantly influence the antihypertensive sensitivity of CCBs[6-9].
The α1C subunit of the L-type calcium channel (CACNA1C) is involved in encoding the drug target of CCBs, the polymorphisms of which are widely recognized to be the most critical genetic factor of the response of dCCBs\(^{[10-12]}\). The key DHP-sensing residues of CACNA1C are defined in transmembrane segments IS6, IIIS5, IIIS6, IVS6 and the pore helix IIIP\(^{[5, 13]}\). Previous studies also have mentioned that polymorphism of eNOS G894T associated with the complex pathogenesis of EH\(^{[18, 19]}\) and NO production deficiency\(^{[20]}\) and played an important role in resistance to the conventional antihypertensive therapy\(^{[21]}\).

Endothelial nitric oxide synthase (eNOS) is a limiting-velocity factor in producing nitric oxide (NO). Both clinical and animal studies have suggested that abnormal NO synthesis is involved in the pathogenesis of hypertension\(^{[15, 16]}\). Reducing RGS2 expression contributes to resistance to antihypertensive agents through poor negative feedback on the effects of aldosterone and of other vasoactive agents\(^{[22]}\). Recently, a report showed that A-638G polymorphism of RGS2 could affect the therapeutic performance of Azenlindipine\(^{[24]}\). And there are evidences that two common mutations of RGS2, RGS2 1891-1892del TC (rs3053226)\(^{[24]}\), and C1114G (rs4606) is respectively associated with hypertension and RGS2 expression\(^{[25]}\).

The purposes of our study is to investigated the influence of genetic polymorphisms of CACNA1C (rs1051356, rs2239101), eNOS (rs1799983), and RGS2 (rs3053226, rs4606) on the antihypertensive efficiency of dCCBs in Chinese Han EH patients.

### 2. Methods

#### 2.1. Subjects

The protocol of this research was approved by the Ethics Committee of Xiangya hospital. We enrolled 107 EH patients, according to the inclusion and exclusion criteria, but only 75 patients return visit on time. Peripheral blood samples for genetic analysis were collected after signature of informed consent. All of the subjects were untreated before and had been prescribed dCCBs as monotherapy. The clinical data could be obtained from records of 4 consecutive outpatient visits before and after 2 weeks, 4 weeks and 6 weeks of treatment. Blood pressure and heart rate were measured by trained nurses in quiet conditions after the patients had rested at least 10 minutes. Automatic blood pressure monitor with intellisense was used to in our study. Both the systolic blood pressure (SBP) and diabetic blood pressure (DBP) were the resulstes of the means of 3 times measurements in 10 minutes. The patients who took azenlindipine were 8 mmg/day (50/75) or 10 mmg nitrendipine every day (25/75).

The biochemical parameters of blood were evaluated before and 6 weeks after the administration, which including four liver function related indexes (total protein, albumin, total bilirubin and glutamic-pyruvic transaminase), three renal function related indexes (blood urea, serum creatinine and serum uric acid), four lipid level related indexes (triglyceride, total cholesterol, high-density lipoprotein, low-density lipoprotein) and fasting blood-glucose. And the adverse effects were evaluated by clinical doctors at the last return visitation. The adverse effects investigated in this study included respiratory adverse reactions (cough, respiratory syndrome), common cardiovascular system adverse reactions (palpitation, edema and orthostatic hypotension), common gastrointestinal system adverse reactions, common adverse reactions of the skin and common adverse reactions of the nervous system (dizziness).

#### 2.2. Inclusion and Exclusion Criteria

Inclusion criteria: men or women; diagnosed with EH, confirmed with two or more times BPs, SBP >140mm Hg or DBP >90mm Hg; never received antihypertensive therapy before; with entire 6 weeks of clinical follow up date and blood sample.

Exclusion criteria were secondary hypertension, heart failure, myocardial infarction, stroke, renal or liver dysfunction, malignant tumor or taking any medication that would further affect BP during CCBs monotherapy.

#### 2.3. Genotyping

The polymerase chain reaction (PCR) was performed using oligonucleotide primers, which were designed based on published gene sequence and synthesized by Invitrogen Trading (Shanghai) Co., Ltd. The PCR reactions were carried out with a total volume of 25µL, the reaction mixture contains as follows: 2.5µL 10×dNTPs (2.5µM each), 2.5µL 10×PCR buffer, 0.5µL both forward and reverse primers, 0.3µL Taq DNA polymerase (5U/µL), 1.0µL of genomic DNA sample and 17.7µL of PCR grade water. All the PCR reaction processes were carried out with the silver tank PCR instrucment (eppendorf AG, Germany). All of the digestion enzymes for the PCR products were designed according to the criteria.

The genotypes of CACNA1C (rs1051356, rs2239101), eNOS (rs1799983) and RGS2 (rs3053226, rs4606) were directly sequenced by shanghai majorbio company. The genotype results of PCR-RFLP were verified by sanger sequencing.
3.3. Adverse Effects after dCCB Drugs Treatment

The adverse effects were evaluated after patients were treated with dCCBs hypotension for at least six weeks. Six of 75 patients experienced dizziness symptoms (2 of nitrendipine group and 4 of azelnidipine group), 3 patients in the azelnidipine group had respiratory adverse reactions. The incidences of adverse effects showed no significant difference between azelnidipine (14%) and nitrendipine (8%) groups (P > 0.05). We further compared the rates of adverse effects in different genotypes, and did not find a statistically significant relationship between the occurrence of adverse effects and gene polymorphisms (all P-values > 0.05).

3.4. Pharmacogenetics Study of Antihypertensive Efficiency of dCCB Drugs and Gene Polymorphisms

After analyzing the relationship between gene polymorphisms and the change of BP at each time point (2, 4, 6 weeks), we found a significant association with the change of BP (ΔBP: BP at 6 week – BP at 0 week). The eNOS G894T polymorphism showed a significant association with the change of BP, as shown in Table 3. The decrease in SBP and DBP was greater in the eNOS 894GG genotype carriers than in the GT/TT genotype carriers (ΔSBP: GG -16.2 ± 8.9 mmHg, GT/TT -5.0 ± 7.6 mmHg, P = 0.001; ΔDBP: GG -13.7 ± 5.0 mmHg, GT/TT -9.5 ± 1.3 mmHg, P = 0.001; ΔMBP: GG -13.7 ± 5.0 mmHg, GT/TT -6.5 ± 9.1 mmHg, P = 0.002). No significant change was observed in levels of heart rate after treatment (P > 0.05).

3.1. Antihypertensive Effects of dCCBs in EH Patients

As shown in Table 1, both the systolic and diastolic blood pressure levels were significantly decreased after six weeks of dCCBs treatment, from 149.3 ± 9.2 mmHg to 132.2 ± 11.7 mmHg and from 97.9 ± 3.0 mmHg to 85.5 ± 7.5 mmHg respectively. No significant change was observed in the levels of heart rate after 6 weeks of treatment. After comparing the biochemical parameters of blood before and after antihypertensive treatment, we found the TP level (74.2 ± 4.9 mg/dL) and 70.9 ± 4.6 mg/dL before and after treatment; P = 0.045), CHO level (5.4 ± 1.1 mg/dL and 5.0 ± 1.0 mg/dL before and after treatment; P = 0.039) and LDL level (3.1 ± 0.8 mg/dL and 2.8 ± 0.8 mg/dL before and after treatment; P = 0.041) decreased by a big margin. Both three renal function indexes and FBG had no significant change (P > 0.05).

3.2. Genotypic analysis

The genotypes of 5 mutations of the three genes were successfully determined in the study population. The allelic frequencies and genotype distributions of the patients were presented in Table 2. In our study population, two genotype frequencies of RGS2 gene (rs3053226 and rs4606) in this study population were not accorded with Hardy-Weinberg equilibrium, P-values were 0.728, 0.9191 and 0.855 respectively. So, we did not take these two SNPs of RGS2 into consideration in the further investigation.

Statistical analyses were performed using SPSS ver. 19.0, and baseline characteristics between different groups were analyzed with ANOVA. Hardy-Weinberg equilibrium was evaluated by Pearson’s χ² test, and allele frequencies were determined by gene counting. Comparison of BP variance between genotypes was performed with multivariate analysis of variance (MANOVA), adjusting for confounding factors, which include aging, sex and BMI. A two-tailed P-value < 0.05 was considered statistically significant.

3. Result

### 3.1. Antihypertensive Effects of dCCBs in EH Patients

| Variables          | Pre-treatment | Post-treatment | P   |
|--------------------|---------------|----------------|-----|
| SBP (mmHg)         | 149.3±9.2     | 132.2±11.7*    | 0.000|
| DBP (mmHg)         | 97.9±3.0      | 85.5±7.5*      | 0.000|
| Heart Rate         | 72.7±7.2      | 71.5±6.9       | 0.276|
| Liver function indexes |            |                |     |
| Total protein (TP) (mg/dL) | 74.2±4.9      | 70.9±4.6       | 0.017|
| Albumin (ALB) (mg/dL) | 43.1±2.9      | 42.0±2.9       | 0.842|
| Total bilirubin (TBIL) (mg/dL) | 15.6±5.3      | 13.9±4.7       | 0.045|
| Glutamic-pyruvic transaminase (ALT) (U/L) | 26.3±12.4     | 25.2±15.4      | 0.501|
| Renal function     |               |                |     |
| Blood urea (BU) (mg/dL) | 5.1±1.3       | 5.0±1.1        | 0.639|
| Serum creatinine (Ser) (mg/dL) | 74.5±17.3    | 74.6±15.8      | 0.983|
| Serum Uric Acid (UA) (mg/dL) | 314.7±67.4     | 318.9±63.3    | 0.796|
| Blood glucose level|               |                |     |
| Fasting blood-glucose (FBG) (mg/dL) | 5.4±1.3       | 5.4±1.2        | 0.786|

Table 1. BP, heart rate and biochemical parameters of blood change after antihypertensive treatment.
4 Discussion

Our study indicates that both azelnidipine and nitrendipine monotherapy can successfully reduce both SBP and DBP, as well as the TP, TBIL, CHO and LDL levels after 6 weeks of dCCB treatment. And our data shows that eNOS G894T polymorphism associated with therapeutic heterogeneity of dCCB drugs.

Interesting, the results of our study suggest that CCBs treatment can significantly decrease TP, TBIL, CHO and LDL levels \( (P=0.017, P=0.045, P=0.039, P=0.041, \text{respectively}) \), which suggested that CCBs medication may have potential hepatoprotective activity and anti-atherosclerosis effects. Several researches had evidenced previously that azelnidipine exerts slight anti-atherogenic effect by reducing local oxidative stress \[28, 29\], and the results of our study suggested that CCBs showed potential antiatherosclerosis effects by lowering CHO and LDL levels. Previous study evidenced that azelnidipine can significantly decrease both SU levels and the urinary uric acid to creatinine ration in hypertension patients \[30\]. And there also data showed that nitrendipine was effective in stabilising most parameters of renal function in incipient diabetic nephropathy \[31\] and had small but significant nephroprotective effect in renal-transplant patients \[32, 33\]. And the results of our study were in line with previous researches that BU, Scr and UA levels were decreased, and the decrease change of BU and Scr levels in nitrendipine treatment group were significantly reduced than azelnidipine groups \( (P=0.02 \text{ and } P=0.04 \text{ respectively}) \). Based on these results we can estimate that dCCBs do have potential renoprotection effect in hypertension patients, and the renoprotection effect of

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**Table 2. Allelic frequency and genotype frequency distribution of eNOS, RGS2, CACNA1C in EH patients.**

| Gene    | SNP     | Genotype | Frequency Number (%) | Allele Frequency | P (H-W) |
|---------|---------|----------|----------------------|------------------|---------|
| eNOS    | Rs1799983 | GG       | 52(74.3%)            | G (86.4%)        | 0.77    |
|         |         | GT       | 17(24.3%)            | T (13.6%)        |         |
|         |         | TT       | 1(1.4%)              |                  |         |
| CACNA1C | Rs2239101 | TT       | 55(78.6%)            | T (88.6%)        | 0.92    |
|         |         | CT       | 14(20.0%)            |                  |         |
|         |         | CC       | 1(1.4%)              |                  |         |
| CACNA1C | rs1051356 | AA       | 67(95.7%)            | A (97.9%)        | 0.85    |
|         |         | AT       | 3(4.3%)              | T (2.1%)         |         |
|         |         | TT       | 0(0%)                |                  |         |
| RGS2    | rs3053226 | DD       | 8(11.9%)             | D (44.0%)        | 0.013   |
|         |         | DI       | 43(64.2%)            | I (56.0%)        |         |
|         |         | HI       | 16(23.9%)            |                  |         |
| RGS2    | Rs4606  | CC       | 9(12.9%)             | C (46.4%)        | 0.0034  |
|         |         | CG       | 47(67.1%)            | G (53.6%)        |         |
|         |         | GG       | 14(20%)              |                  |         |

**Table 3. Stratified analyses of the different hypotensive effects between eNOS G894T genotype and dCCBs interaction.**

| Variables | GG-azelnidpine | GT/TT-azelnidpine | P (GG versus GT/TT) |
|-----------|----------------|------------------|----------------------|
| Vist (N)  | 2W (38)        | 4W (38)          | 6W (36)             |
| ΔSBP (mmHg) | -12.4±9.5      | -12.7±8.6        | -16.2±8.9           |
| ΔDBP (mmHg) | -14.2±9.3      | -10.1±11.7       | -9.5±13.0           |
| ΔMBP (mmHg) | -13.7±5.0      | -10.9±5.3        | -7.8±7.0            |
| P         | 0.603          | 0.464            | 0.476               |
| Variables | GG-nitrendipine | GT/TT-nitrendipine | P (GG versus GT/TT) |
| Vist (N)  | 2W (16)        | 4W (16)          | 6W (16)            |
| ΔSBP (mmHg) | -17.1±12.8     | -18.3±15.8       | -23.3±13.0          |
| ΔDBP (mmHg) | -19.1±13.8     | -17.2±8.8        | -17.3±16.1          |
| ΔMBP (mmHg) | -9.9±9.1       | -11.6±6.1        | -12.7±9.1           |
| P         | 0.721          | 0.851            | 0.266               |

Note: the P-values were adjusted with age, BMI and gender.

**Figure 1. Relationship between eNOS G874T polymorphism and the antihypertensive efficiency of dCCBs treatment after 6 weeks medication.**
nitrendipine was stronger than azelnidipine.

The pharmacogenetic results of this research found that, of the polymorphisms we studied, only eNOS G894T polymorphism associated with antihypertensive effect, and this relationship only presented in azelnidipine treatment group. The hypotensive effects of azelnidipine was more sensitive in GG genotypes than GT or TT genotype carriers. Endothelial nitric oxide synthase (eNOS) is a limiting-velocity factor for endothelial cell to produce nitric oxide (NO), and it is expressed in many organs, such as cardiovascular system, brain, lung, liver and so on, where NO produced by this enzyme plays an important physiological role. NO is a critical molecule in regulating the vascular system, and is involved in inhibiting the platelet aggregation and adhesion and reducing the cell proliferation of vascular smooth muscles. Clinical and animal studies had suggested that NO synthesis abnormal is a contributing factor for the pathogenesis of hypertension. The eNOS G894T polymorphism, a coding region variant, results in a Glu298Asp substitution and decreases the NO levels. Azelnidipine is a long-acting CCB, it doesn’t elicit reflex tachycardia during antihypertensive therapy by inhibits sympathetic nerve activity directly or indirectly. Evidence suggested that azelnidipine treatment could up-regulate the expression and activation of eNOS, while reduced ROS simultanelously increase NO availability. Owing to the small number of subjects prescribed with nitrendipine in this study, we can not avoid the false negative results of this research. The relationship between eNOS G874T polymorphism and nitrendipine hypotensive efficiency need further verification.

In summary, our study evidenced that dCCBs can successfully decrease BP and showed slight hepatoprotective activity and antiatherosclerosis effects. And our study evidenced for the first time that polymorphism of eNOS G894T was correlated with the antihypertensive efficiency of azelnidipine, the GG genotype carriers benenfit more than GT/TT genotype carriers when under azelnidipine medication.

Acknowledgements

This work was supported by the National Scientific Foundation of China (No. 81273595, 30901834, 81001476), the Scientific Foundation of Hunan (No. 11K073, 10JJ4020), the “863” Project (No. 2012AA02A518) and NCET-10-0843. The study was supported by Pharmacogenetics Research Institute, Institute of Clinical Pharmacology, Hunan Key laboratory of Pharmacogenetics, Central South University, Changsha, Hunan. The authors also would like to thank the patients who participated in this study, without whom our research would not been possible to finish.

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