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ORIGINAL ARTICLE

Genotyping and phenotyping CYP3A4\CYP3A5: no association with antiplatelet effect of clopidogrel

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Abstract

The objective of this study was to determine the impact of polymorphism of CYP3A subfamily isoenzymes (allelic variants of CYP3A4*22 and CYP3A5*3) on the efficacy clopidogrel in patients with an acute coronary syndrome (ACS), who have undergone percutaneous coronary intervention (PCI). Platelet activity was determined on a VerifyNow P2Y12 test system in 81 patients with ACS aged 37–91 who had PCI. The activity of CYP3A4/5 was expressed as the ratio of the concentrations of cortisol and 6β-hydroxycortisol was performed by using high performance liquid chromatography. Genotyping was performed by using real-time polymerase real-time chain reaction. The frequencies for the CYP3A5 gene, rs 776746, were identified as follows: 77 (95.1%)—CC, 4 (4.9%)—CT; the allele frequencies by loci for the CYP3A4, rs rs35599367, were as follows: 78 (96.3%)—GG, 3 (3.7%)—AG. There was no statistically significant genotype-dependent difference between the presence of a minor T and G alleles and the presence of clopidogrel resistance (OR 3.53; 95% CI 0.46–26.94; $p = 0.233$ and $p = 0.443$, respectively). The average level of the metabolic relationship (6β-hydroxycortisol/cortisol) between the clopidogrel-resistant group and the normal platelet reactivity group was not statistically significantly different: 3.3 ± 2.8 versus 3.2 ± 3.2; $p = 0.947$. So, the activity of CYP3A4/5 was not related to platelet aggregation rates in this model. Genotyping and phenotyping CYP3A4\CYP3A5 does not predict the antiplatelet effect of clopidogrel. More extensive research is required to establish their clinical relevance.

Keywords Acute coronary syndrome (ACS) · Clopidogrel · Pharmacogenetics · CYP3A4*22 · CYP3A5*3 · Resistance · High residual platelet reactivity (HRPR)

Introduction

The basis of the treatment of acute coronary syndrome (ACS), regardless of the chosen treatment strategy, is the use of dual antiplatelet therapy (DAT) as a combination of acetylsalicylic acid and the P2Y12 receptor blocker of the platelets [1–5]. Clopidogrel is less effective than ticagrelor and prasugrel in the prevention of recurrent ischemic events, but remains the most widely used drug of this class [6–9].

Therefore, according to Choe et al., 73% of patients after percutaneous coronary intervention (PCI) receive clopidogrel. The lower efficacy of this drug is largely determined by the phenomenon of resistance, which is expressed as high residual platelet reactivity (HRPR). The frequency of detecting HRPR depends on the chosen method of aggregometry, for example, when using VerifyNow, HRPR is detected in 33.5% of patients who have undergone ACS. The risk of stent thrombosis in this population increases by 2.5 times in the presence of HRPR, which determines the clinical significance of the phenomenon [7, 10, 11].

Clopidogrel is a prodrug with a two-step activation process that occurs under the action of isoenzymes of the cytochrome P450 family. The most evidence-based genes that demonstrate its impact on clopidogrel efficacy are

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CYP2C19 (encoding CYP2C19 enzyme, which makes the greatest contribution to clopidogrel activation), *CES1* (encoding carboxylesterase-1, that inactivates the drug) and *ABCB1* (encoding *P*-glycoprotein that regulates the transport of the drug in the intestine) [12, 13]. *CYP3A4* and *CYP3A5* are other promising candidate genes, since CYP3A isoenzymes are responsible for the formation of up to 39.8% of the active metabolite of clopidogrel during the second stage of activation [14]. The peculiarity of *CYP3A4* is significant individual differences in the activity of the isoenzyme (up to 100 times), however, most variants of the *CYP3A4* gene are not associated with changes in activity [15–19]. Thus, pharmacogenetic testing of the minor alleles of the *CYP3A4* and *CYP3A5* genes must be supplemented with a phenotypic determination of the activity of the isoenzyme.

One of the allelic variants of the *CYP3A4* gene, which is associated with a significant decrease in the activity of the isoenzyme, is CYP3A4*22 [18, 20, 21]. This polymorphism is found almost exclusively in the Caucasians, in which the most common of the *CYP3A4* minor alleles is (5%). The presence of CYP3A4*22 leads to the partial retention of intron 6 and the premature stopping of transcription with the formation of a functionally inactive enzyme. At the same time, expression of *CYP3A4* is reduced only in liver cells, while expression in enterocytes remains normal [22].

The results of assessing the activity of the isoenzyme CYP3A4 depend on the selected test. In previous studies, various methods were proposed (erythromycin respiratory test, midazolam test, determination of the 6β-hydroxycortisol/cortisol ratio in the urine), reflecting the activity of the CYP3A4 isoenzyme, but the obtained values do not correlate with each other [17, 23–25]. From a practical standpoint, determining the ratio of concentrations of 6β-hydroxycortisol and cortisol in the urine may be most convenient, since this method is non-invasive and can simultaneously reflect the activity of CYP3A4 and CYP3A5 isoenzymes, substrates of which in up to 70% are common for both isoenzymes.

Objective to study the association of carriage of *CYP3A4*22* and *CYP3A5*3* allelic variants and the activity of CYP3A4 and CYP3A5 isoenzymes with changes in the antiplatelet effect of clopidogrel.

Materials and methods

The research was conducted in accordance with ethical principles of the World Medical Association Declaration of Helsinki and was approved by the Local Ethical Committee at Federal State Budgetary Educational Institution of Further Professional Education "Russian Medical Academy of Continuous Professional Education" and the Ministry of Healthcare of the Russian Federation, Moscow, on 13.09.2016 (Protocol No. 9). The objective of this study and its possible complications were explained to the patients in a manner that they could understand. All patients gave written informed consent to be a part of the study.

Patients

The study included 81 patients (mean age 63.9 ± 10.9 years) hospitalized in the cardio-resuscitation department of City Clinical Hospital №1 named after. N.V. Pirogov with a diagnosis of ACS, who had PCI. As part of DAT they received acetylsalicylic acid (300 mg loading dose, 100 mg maintenance dose) and clopidogrel (average loading dose 340.7 ± 103.4 mg, maintenance dose—76.9 ± 11.7 mg).

Platelet activity

Venous blood samples were collected in tubes of Greiner Bio-One Vacuette (Greiner Bio-One, Austria) with a volume of 2 ml with a 3.2% solution of sodium citrate 3–5 days after PCI. In patients treated with GPIIb/IIIa inhibitors, aggregation was determined not earlier than 5–6 days after the end of drug use. Measurement of residual platelet reactivity was performed on a VerifyNow P2Y12 test system (Accumetrics, USA) for 1 h after taking a sample. HRPR criterion—i.e. laboratory resistance to clopidogrel—the value of platelet reaction units (PRU) ≥ 208.

Activity of CYP3A4/5 isoenzymes

To assess the activity of CYP3A4/5, patients gave samples of morning urine with a volume of 5 ml, collected in tubes without preservative for 3–5 days after PCI. Samples were stored frozen at − 20° C. The activity of CYP3A4/5 was expressed as the ratio of the concentrations of cortisol and 6β-hydroxycortisol, the formation of which occurs under the influence of these isoenzymes. Cortisol and its metabolite were determined by chromatography–mass spectrometry on an Agilent G1978B Multimode Source high performance liquid chromatograph for 6410 Triple Quad LC/MS (Agilent Technologies, Inc., USA).

Genotyping

DNA extraction was carried out from samples of venous blood stabilized with EDTA using the DNA-EXTRAN-1 reagent kit (CJSC Syntol, RF). The carriage of polymorphic markers of the CYP3A4 and CYP3A5 genes was detected by real-time polymerase chain reaction on CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, USA) and Applied Biosystems StepOne ™ (Life Technologies, USA). Primers for PCR were selected using the Primer

Select 4.05©program (DNASTAR, Inc., USA) and synthesized by Syntol CJSC (RF).

Statistical analysis

Statistical analysis of the data was carried out in the program IBM SPSS Statistics 23.0. Fisher's exact test was used to compare categorical indicators and to test Hardy–Weinberg equilibrium. Comparison of normally distributed data in groups was carried out using one-way ANOVA (one-way ANOVA). The effect of cytochrome P450 isoenzyme activity on the residual reactivity of platelets was assessed using a linear regression model. The combined influence of factors on the development of HRPR was estimated using a logistic regression model. Differences were considered statistically significant at $p < 0.05$.

Results

The frequency distribution of the *CYP3A4*22* and *CYP3A5*3* polymorphism alleles corresponded to the Hardy–Weinberg equilibrium: $\chi^2 = 0.052$, $p = 0.82$ and $\chi^2 = 0.029$, $p = 0.865$, respectively.

CYP3A4 and CYP3A5 frequencies and distribution in the current study population are listed in Table 1.

These results are consistent with those of Lu et al., Chen, Yoo et al. [26–28].

HRPR was detected in 19 (28.3%) patients. The presence of a minor T allele in patients, carriers of the *CYP3A4*22* gene polymorphism and the G allele—carriers of the *CYP3A5*3* gene polymorphism was not associated with the presence of clopidogrel resistance (OR 3.53; 95% CI 0.46–26.94; $p = 0.233$ and $p = 0.443$, respectively).

In groups of patients with and without minor alleles for the polymorphic markers *CYP3A4*22* and *CYP3A5*3*, there were no statistically significant differences in the PRU score: 168.2 ± 51.4 in the group with CC genotype versus 205.3 ± 24.4 in the group with CT genotype ($p = 0.157$); 169.6 ± 51.8 among GG genotype carriers versus 180.3 ± 18.0 among AG genotype carriers ($p = 0.723$).

There was no correlation between CYP3A4/5 activity measured by the 6β-hydroxycortisol/cortisol ratio and the level of residual ADP-induced platelet aggregation ($\beta = -0.023$; $r^2 = 0.001$; $p = 0.837$; Fig. 1).

The average level of the metabolic relationship (6β-hydroxycortisol/cortisol) between the clopidogrel-resistant group and the platelet normal reactivity group was not statistically significantly different: 3.3 ± 2.8 versus 3.2 ± 3.2; $p = 0.947$.

In the logistic regression model, the only significant predictors of HRPR were: taking dihydropyridine calcium channel blockers, presence of atrial fibrillation (OR 7.00; 95% CI 1.05–46.55; $p = 0.044$ and OR 4.67; 95% CI 1.02–21.33; $p = 0.047$, respectively).

Discussions

CYP3A4/5 are among the most important enzymes in clopidogrel activation. Previously, Lau et al. has shown that lower CYP3A4 activity, determined using an erythromycin breath test, is associated with a lower antiplatelet effect of the drug. At the same time, the carriage of *CYP3A4*22* is reliably associated with a decrease in the activity of the CYP3A4 isoenzyme. However, in our experiment, it was not possible to demonstrate a significant association of this allelic variant with a change in the antiplatelet effect of clopidogrel, which is consistent with the results of Kreutz et al. Also in

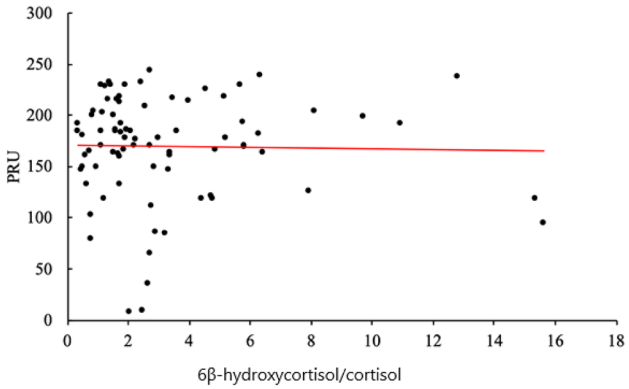

Fig. 1 Scatterplot that demonstrates relationship between the activity of CYP3A and difference in scores on PRU in patients receiving clopidogrel

Table 1 Allele frequencies for the *CYP3A4*22* and *CYP3A5*3* in the ACS patients (n = 81)

Gene	SNP	Genotype	n (%)	Minor allele	MAF (%)	Hardy–Weinberg equilibrium, *p* value
CYP3A4	rs35599367	CC CT	77 (95.1%) 4 (4.9%)	T	2.5	0.82
CYP3A5	rs776746	GG AG	78 (96.3%) 3 (3.7%)	G	0.98	0.865

MAF minor allele frequency, *SNP* single nucleotide polymorphisms

our work it was not possible to establish the presence of the effect of CYP3A4/5 activity on the residual reactivity of platelets, which does not correlate with data from previous studies.

In our study, there was no evidence of the influence of the carrier polymorphism of *CYP3A4*22* and *CYP3A5*3* on the low activity of the CYP3A4/5 isoenzyme: 3.3 ± 3.2 in the group of homozygotes for the wild-type allele; 1.6 ± 0.6 in the group of heterozygotes for *CYP3A4*22* ($p = 0.273$), 3.3 ± 3.2 in the group of homozygotes for the wild-type allele; 2.2 ± 2.3 in the group of heterozygotes for *CYP3A5*3* ($p = 0.567$).

The most likely cause of the results obtained are the features of the method used to characterize cytochrome activity: nonspecificity of cortisol as a CYP3A4 substrate, a probable tissue-specific decrease in CYP3A4 activity during carrier *CYP3A4*22* (mainly in hepatocytes) and the possibility of 6β-hydroxycortisol formation outside the liver (while erythromycin) breath test can more accurately reflect enzyme activity in the liver).

Besides, the results of our study can also explain the previous data obtained William E. Evans and Howard L. McLeod: there are many medications metabolized by both CYP3A4 and CYP3A5, that is why the lack of active CYP3A5 may not become manifest we support.

Additionally, there is evidence that CYP3A4/5 may lead to the formation of inactive clopidogrel metabolites. Thus, CYP3A4/5 activity in the intestine can play a certain value, where these isoenzymes are the main.

Conclusions

Genotyping and phenotyping CYP3A4\CYP3A5 does not predict the antiplatelet effect of clopidogrel. More extensive research is required to establish their clinical relevance.

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Compliance with ethical standards

Conflict of interest All of the authors declare that they have no conflict of interest.

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