Is There a Link Between Clopidogrel Resistance and Common Risk Factors for Atherosclerosis in Patients with Acute Coronary Syndrome?

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The antiplatelet effect of clopidogrel prodrug is characterized by a wide inter-individual variability that has a significant clinical relevance. Among varios factors that are involved in the occurrence of clopidogrel resistance, the genetic polymorphisms play a key role. The aim of the present study was to investigate the impact of some risk factors for atherosclerosis on the antiplatelet effect of clopidogrel in patients with acute coronary syndrome and the possible correlation with metabolizer phenotype of patients based on CYP2C19 polymorfisms. We found a statistically significant correlation (p value < 0.05) between smoking or dyslipidaemia and the presence of ultrarapid metabolizer phenotype for clopidogrel in our research population.

Keywords: clopidogrel resistance, atherosclerosis, risk factors, CYP2C19 polymorphisms

According to the current American College of Cardiology/ American Heart Association and European Society of Cardiology guidelines, oral antiplatelet agents are the cornerstone of modern pharmacotherapy in cardiovascular atherothrombotic diseases [1]. Clopidogrel is one of the most recommended oral antiplatelet drugs worldwide for the treatment of arteriosclerotic events in patients with acute coronary syndrome, stroke, myocardial infarction or peripheral arterial disease or to prevent stent thrombosis [2, 3]. It is a second-generation oral thienopyridine prodrug that requires enzymatic biotransformation into the active compound [4].The active clopidogrel metabolite inhibits adenosine diphosphate (ADP)-induced platelet aggregation by binding and blocking the platelet P2Y12 receptors [5, 6]. After its digestive absorbtion, the major part of clopidogrel is hydrolyzed by esterases to an inactive carboxylic acid derivative. A small proportion of clopidogrel (15%) is activated *via* two sequential oxidation reactions catalyzed by the hepatic cytochrome P450 (CYP450) enzymes, mainly CYP2C19, CYP23A4, CYP3A5 and CYP2C9. The first bioactivation stage is characterized by the oxidation of the thiophene ring of clopidogrel to 2-oxoclopidogrel which is further oxidized in the second stage, with the opening of the thiophene ring and the formation of both a carboxyl and a thiol group (fig. 1) [2, 5, 7, 8].

The antiplatelet effect of clopidogrel is associated with a substantial inter-individual variability. It has been estimated that between 4 and 44% of patients display the so-called clopidogrel resistance which is expressed by the failure of antiplatelet effect and the occurrence of adverse clinical outcomes as ischemic cardiovascular events [9]. A poor response to clopidogrel is determined by various pharmacogenetic, clinical and environmental factors. Several potential mechanisms of clopidogrel resistance have been suggested, such as: genetic variables (polymorphisms of CYP enzymes and P2Y12 receptors), non-compliance of patient, innapropriate dosage of clopidogrel, drug-drug interactions *via* CYP3A4 enzyme, increased release of ADP or up-regulation of P2Y12-independent pathways [2, 10]. CYP2C19 is a highly polymorphic enzyme that present more than 25 alleles [7]. The CYP2C19*2 allele is the most known variant. It is associated with a loss of function and a decreased antiplatelet response [11]. On the contrary, CYP2C19*17 allele increases enzyme activity and the production of active metabolite and thereby determines a better clopidogrel-induced platelet inhibition [2]. Also, the polymorphisms of ABCB1 gene can cause variations in the expression of P-glycoprotein that influence the absorbtion and bioavailability of clopidogrel [6]. It has been reported that high doses of calcium-channel blockers and angiotensin-converting enzyme inhibitors, and also co-administration of the proton pump inhibitor omeprazole could contribute to a decreased responsiveness of clopidogrel [5]. In addition, concurrent medication with lipophilic statins (atorvastatin, simvastatin) can compromise the antiplatelet effect of clopidogrel due the fact that they share the same CYP450 metabolizing isoenzymes (CYP2C19, CYP3A4) [2]. The patient risk profile can also be actively involved in the clopidogrel resistance [1]. Atherosclerosis is the dominant cause of cardiovascular disease including myocardial infarction, heart failure, stroke and claudication [12]. It is a multifactorial, inflammatory disease that affect the walls of blood vessels and determine major changes of features and function of platelets. A diet high in saturated fat, hypercholesterolemia, obesity, dyslipidaemia, hyperglycemia, insulin resistance, hypertension, dysfunction of nitric oxide, and smoking habit are key players in atherosclerosis occurrence [13, 14]. All these factors are also actively involved in the development of major cardiovascular diseases (coronary diseases, stroke, heart failure, peripheral vascular disease) [15, 16].The aim

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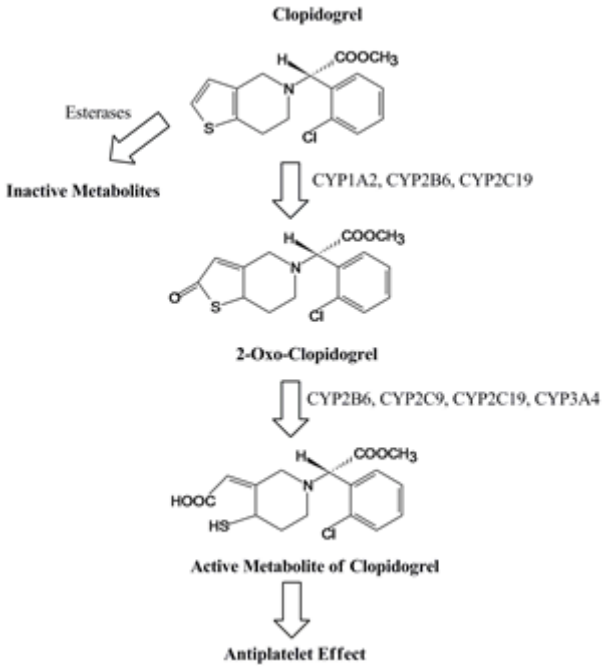

Scheme 1. Bioactivation of clopidogrel [8]

of this study was to investigate of the impact of some risk factors for atherosclerosis on the antiplatelet effect of clopidogrel in patients with acute coronary syndrome and the possible correlation with metabolizer phenotype of patients based on CYP2C19 polymorfisms.

Experimental part

Study population

Eighty patients (aged between 45-85 years) with acute coronary syndrome who were seen at the 1st Cardiology Clinic of the St. Spiridon Emergency Clinical Hospital Iasi during January-June 2012 were enrolled in the present study. 86.25% of the patients were male and 13.75% were female. The inclusion criteria were: a) clinical: anterior chest pain with angina features which meets the criteria for unstable angina or acute myocardial infarction; b) paraclinical: suggestive electrocardiographical changes-subendocardic or subepicardic ischemic lesions or recent branch block (12-lead electrocardiogram), segmental wall-motion abnormalities of the left ventricle (2D, M, Doppler echocardiogram), analysis of myocardial necrosis enzymes. Exclusion criteria were as follows: a) uncertain acute coronary syndrome diagnosis; b) occurrence of any bleeding disorders and family history of blood disease; c) liver disease; d) patients without clopidogrel treatment; e) psychiatric disorders; f) noncompliant patients. All the patients included in the study received antiplatelet treatment with clopidogrel (75 mg/day) associated sometimes with aspirin (75 mg/day).

Laboratory investigations

They included analysis of total cholesterol, triglycerides, lipid profile, hepatic enzymes (alanine transaminase, ALT; aspartate transaminase, AST), myocardial enzymes (creatine kinase MB isoenzyme, CK MB), serum creatinine, serum urea and thrombocytes count.

Data collection

Informations on the general condition, medical history (hyper-tension, diabetes or coronary heart disease), personal history (smoking and drinking) were obtained from all patients.The study was approved by the Ethics Committee of University of Medicine and Pharmacy Grigore T.Popa Iasi, and all patients enrolled were previously informed about the subject of the study and they signed an informed consent.

DNA extraction and genotyping

The Wizard Genomic DNA Purification - Promega Kit (Promega Inc., Madison, WI, USA) was used to isolate DNA from blood samples. CYP2C19 polymorphisms were investigated using TaqMan assay (Applied Biosystems, Life Technologies, Pleasanton, CA, USA) that is based on the allelic discrimination by Real-Time PCR according to the methodology presented in our previously published work [17, 18].

Statistical analysis

Statistical analysis was performed using the IBM SPSS 20.0 software (Statistical Package for the Social Sciences, Chicago, Illinois). Data were expressed as mean ± standard deviation or number of cases with percentage, for continuous and ordinal variables. Cross-tabulation and Pearson Chi-Square test were used for describing the relationship between two categorical variables. The one-way analysis of variance (ANOVA) was used to determine the significant differences between the means of continuous variables and an independent categorical variable. For all data, a two-sided p value < 0.05 was considered statistically significant.

Results and discussions

Eighty patients have been analyzed, with a mean age of 69.30 ± 9.39 years. The majority were males and most subjects were hypertensive (98.8%), diabetic (63.8%), smokers (80%), dyslipidaemic (93.8%) or obese (83.8%). Mean cholesterol was above the normal limit with the highest value being 375 mg/dL. As well, most patients had values of triglycerides over the superior limit, the highest value being 450 mg/dL. The average value of CK MB enzyme was 38.46 U/L, and 36.25% of the patients showed CK MB values about the normal limit (24 U/L). Their clinical data and electrocardiogram supported the diagnosis of unstable angina. The hepatic transaminases reached normal levels, the higher values being for AST since some patients were admitted with myocardial infarction in different stages. The renal markers were close to the superior limit, while the thrombocytes were normal in most patients. All data can be found in table 1.

Table 1
CARDIOVASCULAR RISK PROFILE IN THE STUDIED POPULATION

Cardiovascular risk factor	Results
Age (years)	69.30 ± 9.39
Male sex (%)	86.30
Arterial hypertension (%)	98.80
Type 2 diabetes mellitus (%)	63.80
Current smokers (%)	80
Dyslipidaemia (%)	93.80
Obesity (%)	83.80
CK (U/L)	196.30 ± 226.21
CK MB (U/L)	38.46 ± 175.37
Total cholesterol (mg/dL)	229.56 ± 46.28
Triglycerides (mg/dL)	163.81 ± 57.79
AST (U/L)	46.40 ± 26.44
ALT (U/L)	41.30 ± 12.37
Serum creatinine (mg/dL)	1.36 ± 0.63
Serum urea (mg/dL)	56.79 ± 21.39
Thrombocytes (/mm³)	263.00 ± 124.04

Table 2
THE PHENOTYPE METABOLIZER PREVALENCE ACCORDING TO MAJOR CARDIOVASCULAR RISK FACTORS

Variable	Category	Phenotype (%)				p value
		Extensive metabolizer	Unpredictable metabolizer	Poor metabolizer	Ultrarapid metabolizer	
Hypertension	No	0	100	0	0	0.222
	Yes	5.1	17.7	3.8	73.4	
Diabetes	No	0	13.8	6.9	79.3	0.225
	Yes	7.8	21.6	2	68.6	
Obesity	No	0	30.8	0	62.9	0.462
	Yes	6	16.4	4.5	73.1	
Dyslipidaemia	No	0	60	20	20	0.012
	Yes	5.3	16	2.7	76	
Smoking	No	0	31.3	12.5	56.3	0.050
	Yes	6.3	15.6	1.6	76.6	

Regarding the CYP2C19 genotypic variants, 72.5% of patients presented ultrarapid metabolizer phenotype (wild-type for *2 and *3 alleles and homozygote and heterozygote for *17 allele), 5.0% were extensive metabolizers (wild-type CYP2C19*2*3*17) while the poor metabolizer phenotype (carriers of *2/*2, *2/*3 and *3/*3 alleles) and the unpredictable metabolizer phenotype (heterozygote or homozygote for *17 and heterozygote for *2 and /or *3 alleles) were found in 3.8% and 18.8% of patients, respectively [19].

By comparing the phenotypes based on gender, no significant differences were noticed, most males and females having an ultrarapid metabolizer phenotype (73.9% and 63.6%, respectively; p = 0.0524). As well, no significant differences were remarked between the type of phenotype and major cardiovascular risk factors, such as arterial hypertension (p = 0.222), type 2 diabetes mellitus (p = 0.225) or obesity (p = 0.462). However, we noticed a trend increase of poor metabolizer phenotype in the case of hypertensive and obese patients compared with normotensive and non-obese patients (table 2). Also, the diabetic patients showed a decrease by about 13.5% of ultrarapid metabolizer phenotype proportion and an increase of more than 50% in the proportion of unpredictable metabolizer phenotype.

In our study, we found that more than 76% of dyslipidaemic patients presented an ultrarapid phenotype while 20% of non-dyslipidaemic patients had a poor response based on genetic testing (p = 0.012) (fig.1). As well, the same trend was seen when comparing the smoking status where most smokers had an ultrarapid phenotype while more non-smokers were categorized in unpredictable and poor group response (p = 0.050) (fig. 2).

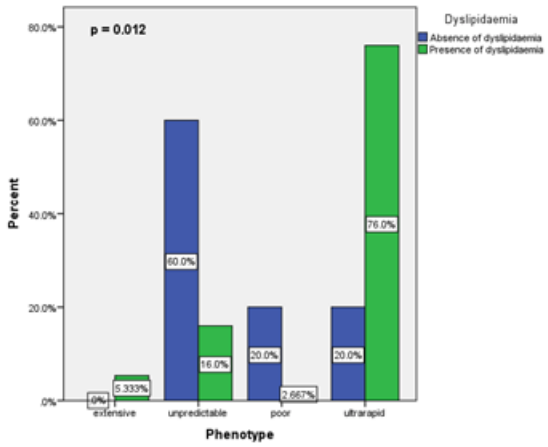

Fig.1. The phenotype prevalence according to dyslipidaemia

Older patients had an ultrarapid metabolizer phenotype, but without statistical significance (p = 0.661). No significant differences were noticed for the values of total cholesterol and those of triglycerides. AST and ALT did not differ between the four groups, as well. The renal function was impaired in the extensive and ultrarapid metabolizer categories as compared to the poor and unpredictable ones (p = 0.762). The thrombocytes values were basically the same among all phenotypes (table 3).

Arterial hypertension, diabetes and obesity have been described as independent risk factors for resistance to clopidogrel. Hypertension is known for its enhanced platelet aggregability and adhesiveness but the relationship between this condition and the incidence of clopidogrel resistance remains unclear [2, 19]. Generally, the metabolic features of obesity with major resistance to insulin and chronic inflammatory status, high platelet reactivity, increased platelet turnover, endotoxemia, and suppression of CYP450 enzyme activity predispose to the alteration of the pharmacological response to clopidogrel and may cause an increased risk of thrombotic events [6]. Also, diabetic patients tend to be low responders to clopidogrel due to a prothrombotic condition with high platelet reactivity that might trigger atherothrombotic burden [20]. The prothrombotic state in type 2 diabetes mellitus is related to the endothelial dysfunction, impaired fibrinolysis and coagulation, and also to the impaired platelet function (adhesion, aggregation and activation) [4]. However, various studies reported conflictual data. Liu et al. (2016)[2] found a significant association of both hypertension and CYP2C19*2 allele with clopidogrel resistance in Chinese patients with ischemic stroke but they did not observe any correlation between poor response to clopidogrel and diabetes in the same subjects. Another study did not identify arterial hypertension, diabetes, obesity, age, platelet count,

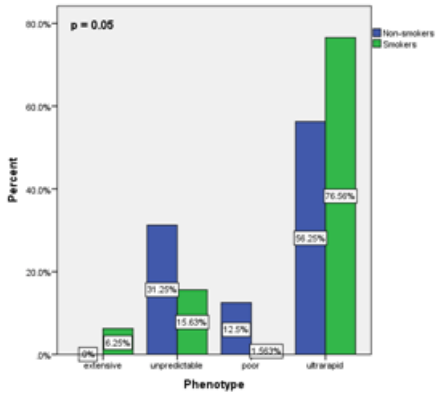

Fig. 2. The phenotype prevalence according to smoking status

Table 3
BIOCHEMICAL VALUES ACCORDING TO THE CYP2C19 POLYMORPHISMS

CV risk factor	Extensive metabolizer	Unpredictable metabolizer	Poor metabolizer	Ultrarapid metabolizer	p value
Age (years)	66.50 ± 9.74	67.73 ± 10.77	65.67 ± 11.37	70.09 ± 9.04	0.661
Total cholesterol (mg/dL)	236.50 ± 21.81	237.07 ± 46.43	221.00 ± 45.17	227.59 ± 48.13	0.879
Triglycerides (mg/dl)	175.75 ± 16.50	173.07 ± 48.76	143.67 ± 24.09	161.64 ± 62.86	0.807
AST (U/L)	46.25 ± 9.32	51.53 ± 31.52	45.00 ± 5.00	45.16 ± 26.66	0.877
ALT (U/L)	42.50 ± 6.45	42.80 ± 16.32	43.33 ± 7.63	40.72 ± 11.89	0.930
Creatinine (mg/dL)	1.30 ± 0.11	1.52 ± 1.24	1.40 ± 0.34	1.32 ± 0.39	0.762
Thrombo cytes (/mm³)	282500 ± 72743	269400 ± 101445	280000 ± 140000	259120 ± 133284	0.970

cholesterol level or concurrent drug intake as clinical predictors of clopidogrel resistance in Indian patients with acute coronary syndrome [21]. Pankert et al. (2011)[22] reported an impaired responsiveness to clopidogrel only in obese patients with metabolic syndrome, while obese patients without metabolic syndrome showed no significant differences in platelet reactivity compared to nonobese subjects. Also, Gaglia et al. (2011)[23] did not found an association between body mass index and on-treatment platelet reactivity (clopidogrel plus aspirin).The same discrepancies were noticed as regards to the influence of smoking. Commonly, the current smoking was proposed as an independent predictor of low antiplatelet effect of clopidogrel [24]. On the contrary, Liu et al. (2016)[2] did not observe any correlation between smoking habit and clopidogrel resistance in ischemic stroke patients.The identification of a statistically significant association of smoking with the ultrarapid metabolizer phenotype of clopidogrel as in our study is in agreement with data reported by Matetzky et al. (2004)[25] and Desai et al. (2009)[26]. They showed an enhanced antiplatelet effect of clopidogrel in smokers. A possible explanation is the activation of CYP1A2 enzyme by the polycyclic aromatic hydrocarbons from tobacco smoke. CYP1A2 is the enzyme responsible for the first oxidative step in the metabolic activation of clopidogrel [5, 25]. Certainly, the response to clopidogrel is not uniform and it is related to multifactorial causes. Although it has been suggested that CYP2C19 genotype largely contribute to the clopidogrel resistance this should not be interpreted unidirectionally. A systematic meta-analysis performed by Holmes et al (2011)[27] did not demonstrate a clinically significant association of CYP2C19 genotype with cardiovascular outcomes. Besides, Bauer et al. (2011)[28] showed that the data from genetic association studies does not indicate a consistent influence of CPY2C19 polymorphisms on the clinical efficacy of clopidogrel. In the line with these findings, a recent metabolomic analysis suggested that the CYP3A isoenzymes are involved in the primary metabolism of clopidogrel but not CYP2C19 [29]. Overall, the relationship between traditional cardiovascular risk factors with genetic factors involved in clopidogrel resistance is not a simple linear association. Besides genetic makeup, the effect of major risk cardiovascular factors as hypertension, diabetes, obesity, smoking, dyslipidaemia on pharmacological response to clopidogrel is modulated by race- and population-specific differences, clinical conditions, platelet phenotype, clopidogrel treatment schedule, and their particular interactions.

Conclusions

Our results did not demonstrate a statistically significant association of cardiovascular risk factors as hypertension, obesity and type-2 diabetes with CYP2C19 genotypes in our research population. On the contrary, a statistically significant correlation was found between smoking or dyslipidaemia and the presence of the ultrarapid metabolizer phenotype for clopidogrel. Further investigations should be conducted on a high number of patients. The analysis of all genetic polymorphisms and the measurement of platelet reactivity may contribute to a strength of research.

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