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Original Research Article

Research on the prevalence of COX-2 gene promoter -765G>C polymorphism, inflammation, and serum levels of PGE_2 in coronary artery disease

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Abstract

Cyclooxygenase-2 (COX-2) is an enzyme involved in the production of prostaglandins during inflammation. The aim of this study was to investigate the prevalence of COX-2 gene promoter -765G>C polymorphism in a Greek adult population in relation to coronary artery disease. In total, 102 subjects, 74 men (72.55%) and 28 women (27.45%) (median (range) 55 (36-86) years of age) were recruited. Subjects were divided into two main groups: 69 patients (67.65%) hospitalized for myocardial infarction (MI), 54 (78.26%) of which with elevated ST (STEMI) and 15 (21.74%) without (NSTEMI) (study group). The second group consisted of 33 patients (32.35%) with unstable angina but no history of MI (controls). Serum levels of highly sensitive C-reactive protein (hsCRP) and prostaglandin E_2 (PGE_2) and COX-2 -765G>C gene promoter polymorphism were determined. Genotype frequencies were 60% for CC, 22% for GC and 18% for GG in the total of subjects participating. Although there were statistically significant higher hsCRP levels in study group than controls (p<0.001), there were no significant differences in serum levels of hsCRP (p=0.807 and 0.893) or PGE_2 (p=0.455 and p=0.303) between different genotypes in either group, respectively. The onset of acute coronary events does not seem to be related to the -765G→C polymorphism of the COX-2 gene promoter.

Keywords: -765G>C, Acute coronary syndrome, COX-2, Frequencies, Inflammation

INTRODUCTION

Arachidonic acid is an essential, polyunsaturated omega-6 fatty acid situated in the phospholipids of cell membranes. Cyclooxygenases (COXs) comprise a group of enzymes involved in the conversion of arachidonic acid to prostaglandins, prostacyclin and thromboxane. Three isoforms of the enzyme have been identified, COX-1, COX-2 and COX-3, but only the first two are functional and responsible for metabolizing arachidonic acid to PGG_2 and PGH_2, respectively, and to a wide range of eicosanoids (Fritsche et al., 2001).

COX-2 is expressed by cells involved in inflammation, such as endothelial cells and macrophages. There are some studies showing that genetic inhibition of COX-2 decreases the risk of atherosclerosis (Hui et al., 2010; Li W et al. 2009) whereas others have shown an increase in the risk of thrombosis (Yu et al., 2012). A genetic polymorphism (-765G>C or rs20417) in the promoter of COX-2 gene (*PTGS2*) has been linked to lower COX-2 activity in the atherosclerotic plaque, to increased stability of the plaque and to decreased risk of stroke and myocardial infarction (Cipollone et al., 2004; Ross et al., 2014) but does not seem to predict the risk of recurrence

of ischemic events in coronary patients (Montali et al., 2010). Carriers of minor allele (C) seem to have a decreased COX-2 expression and present with a lower cardiovascular risk (Cipollone et al., 2004; Papafili et al., 2002).

The aim of the present study was to investigate the prevalence of COX-2 gene promoter -765G>C polymorphism in a population of adults suffering from MI in relation to adults suffering from coronary artery disease but did not have a myocardial infarct yet (controls) and whether different alleles are related to different levels of inflammation and prostaglandin production in patients with MI compared to controls. The hypothesis, being investigated for the first time, to our knowledge, is whether certain genotypes are related to increased inflammation increasing this way the risk for cardiovascular diseases.

MATERIALS AND METHODS

In the present study, 102 subjects, 74 men (72.55%) and 28 women (27.45%) [median (range) 55 (36-86) years of age] were recruited. Subjects were divided into two groups: 69 patients (67.65%) were hospitalized in the Department of Cardiology for myocardial infarction (MI), 54 (78.26%) with elevated ST (STEMI) and 15 (21.74%) without (NSTEMI) (study group) and 33 patients (32.35%) with unstable angina but no history of MI (controls). Positive family history for cardiovascular diseases was recorded in 17 patients (24.64%) of the study group, and in only 1 (9.09%) in the control group. All subjects volunteered to participate in the study and gave their written informed consent.

Blood was drawn after 8-hour fast and serum and EDTA whole blood samples were stored in -80^{0}C. Serum levels of highly sensitive C-reactive protein (hsCRP) were determined in a biochemical analyzer (COBAS INTEGRA 400, Roche Diagnostics GmbH, D-68298 Mannheim) and prostaglandin E_2 (PGE_2) serum levels were assayed by ELISA (R&D Systems Europe, Ltd).

DNA was extracted using a preparation kit (High Pure PCR Template Preparation kit, ROCHE Diagnostics GmbH, D-68298 Mannheim) and COX-2 gene promoter -765G>C polymorphism was investigated using Real Time PCR (Rotor Gene Q, Qiagen Inc). For the genetic analysis, the following primers were used: COX-2 -756F: CAC GCA TCA GGG AGA GAA ATG and COX-2 -756R: GCC GTG TCT GGT CTG TAC GTC. HRM analysis and SNP genotyping were then performed.

Statistical analysis was performed with IBM Statistical Package for Social Sciences (SPSS) Statistics 20 (SPSS, IBM, Armonk, NY, USA) with level of statistical significance set at $p < 0.05$.The statistical tests used were Chi-square test for comparisons between categorical variables and Mann-Whitney and Kruskal-Wallis tests for comparisons of non-parametrical variables.

RESULTS

COX-2 gene promoter -765G>C polymorphism frequencies were 60% for CC, 23% for GC and 19% for GG in all the patients participating in the study. The frequency of the significant, so-called “protective” allele C, in total, was 42%. Levels of hsCRP and PGE_2 in the three subgroups of COX-2 genotypes, in the two groups of the study are shown in Table 1, where using Mann Whitney test statistically significant difference between the subgroups is observed.

In Table 2 levels of hsCRP and PGE_2 are presented in the two subgroups (STEMI and NSTEMI) of the study group according to the different COX-2 genotypes. Between the two subgroups no statistically significant difference was observed, using non-parametrical Mann-Whitney test.

DISCUSSION

The -765G>C is one of the most well-investigated COX-2 polymorphisms, but nothing has been discussed, to our knowledge on the intensity of inflammation in people carrying different alleles. There are studies which revealed that -765G>C is significantly associated with a decreased risk of coronary artery disease, although there are others reporting contradictory results (Wang et al., 2014; Li et al., 2009). In atherosclerotic lesions, inflammation plays a central role. The certain polymorphism disrupts the binding of Sp1 in PTGS2 proximal promoter, reducing COX-2 transcription and expression and, finally, the production of COX-2, exerting significant anti-inflammatory effects (Orbe et al., 2006).

Comparison of subjects of study and control group revealed that patients of the study group had significantly higher serum levels of the inflammatory marker hsCRP, than controls, in total and in the COX-2 genotype subgroups (Table 1), irrespectively of the presence of the C allele, that is in contrast to findings of previous studies which showed that carriers of minor allele (C) seem to have a decreased COX-2 expression and present with a lower cardiovascular risk (Cipollone et al., 2004; Papafili et al., 2002). On the other hand, there was no significant difference in the levels of PGE_2 comparing subjects with the same genotype of COX-2 promoter gene polymorphism -765G>C between study and control group, or between subjects of the same group with different genotype (Table 1). This finding contrasts with the results of the study of Sanak et al. who stated that the presence of C allele is associated with higher PGE_2 biosynthesis (Sanak et al., 2010).

Moreover, frequencies of COX-2 alleles did not differ significantly between the two groups, which is in accordance with previous studies that presented no relation of -765C allele to CAD (Papafili et al., 2002; Hegener et al., 2006; Kohsaka et al., 2008; Huuskonen et

Table 1. Frequencies of genotypes and comparison of hsCRP and PGE_2 between study group and controls and in the three genotype subgroups (GG, GC and CC) of -765G>C COX-2 gene promoter polymorphism (rs20417). Values are presented as median (range). hsCRP: highly-sensitive C-reactive protein, PGE_2: prostaglandin E_2

	Study group N=69		**Controls N=33**		**p**
hsCRP (mg/L)	4.07 (0.63-40.40)		0.76 (019-4.07)		**< 0.001**
rs20417	**n(%)**	**hsCRP (mg/L)**	**n(%)**	**hsCRP (mg/L)**	
GG	40 (57.97)	3.43 (0.63-23.5)†	20 (60.60)	0.52 (0.19-1.95)₸	**< 0.001**
GC	15 (21.74)	4.58 (0.63-18.6) †	8 (24.24)	1.59 (0.27-12.4)₸	**0.026**
CC	14 (20.29)	5.59 (1.26-18.0) †	5 (15.15)	1.18 (0.26-1.6)₸	**0.013**
PGE_2 (pg/mL)	1120 (190-5160)		1357 (180-2120)		0.598
rs20417	**n(%)**	**PGE_2 (pg/mL)**	**n(%)**	**PGE_2 (pg/mL)**	
GG	40 (57.97)	1035 (190-5160) ‡	20 (60.60)	1097 (180-2120) *	0.877
GC	15 (21.74)	1170 (240-2180) ‡	8 (24.24)	1362 (920-1740) *	0.204
CC	14 (20.29)	1350 (730-2110) ‡	5 (15.15)	1625 (300-1868) *	0.730

†: p=0.807, ‡: p=0.455, ₸: p=0.893, *: p=0.303

Table 2. Frequencies of genotypes and hsCRP and PGE_2 comparison between STEMI and NSTEMI patients in the three groups of different -765G>C COX-2 gene promoter genotype (CC, GC and GG). STEMI: Myocardial infarction with elevation of ST, NSTEMI: Myocardial infarction with no elevation of ST, hsCRP: highly-sensitive C-reactive protein, PGE_2: prostaglandin E_2

	STEMI N=54		**NSTEMI N=33**		**p**
hsCRP(mg/L)	5.09 (1.17-40.40)		4.18 (0.63-18.6)		0.818
rs20417	**n (%)**	**hsCRP (mg/L)**	**n (%)**	**hsCRP (mg/L)**	
GG	33 (61.11)	3.44 (1.17-23.5)	7 (21.21)	6.44 (2.78-10.10)	1.000
GC	11 (20.37)	5.09 (2.22-16.0)	4 (12.12)	2.72 (0.63-18.6)	0.578
CC	10 (18.52)	4.91 (1.26-18.0)	4 (12.12)	1.18 (0.26-1.6)	0.861
PGE_2 (pg/mL)	1120 (150-5160)		1240 (240-1750)		0.666
rs20417	**n (%)**	**PGE_2 (pg/mL)**	**n (%)**	**PGE_2 (pg/mL)**	
GG	33 (61.11)	1020 (190-5160)	7 (21.21)	1025 (770-1280)	0.434
GC	11 (20.37)	990 (150-2180)	4 (12.12)	1200 (240-1360)	0.586
CC	10 (18.52)	1250 (720-2110)	4 (12.12)	1525 (360-1868)	0.735

al., 2008), even though the -765C allele has been previously associated with decreased carotid-intima media thickness (Papafili et al., 2002; Corella et al., 2009), and has been reported to protect against myocardial infarction (Cipollone et al., 2004). Therefore, differences in inflammation levels between the two groups cannot be attributed to differences in frequencies in the COX-2 alleles but only to well-known reasons, such as augmented inflammatory status that takes place during myocardial infarction and plaque rupture, events that release proinflammatory and inflammatory elements in the circulation.

Acute coronary syndrome can be divided in ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI) and unstable angina. NSTEMI and unstable angina have very similar clinical presentation, with NSTEMI having positive cardiac biomarkers (Basit et al., 2020). The pathophysiology of STEMI is usually coronary plaque rupture resulting in occlusion of coronary arteries. On the other hand, there are several causes of NSTEMI, such as a stable plaque, vasoconstriction, embolism of coronary arteries, myocarditis, the presence of cardiotoxic substances, hypertension, hypotension, and pulmonary embolism (Rupprecht et al., 2019).

ACS and NSTEMI cannot be excluded with a normal

ECG. STEMI must be considered when there is ST elevation or anterior ST depression and should be treated as such. Transient ST elevation, ST depression, or new T wave inversions suggest NSTEMI (Basit et al., 2020). Several tools have been suggested for the early diagnosis of ACS, such as TIMI (Thrombolysis In Myocardial Infarction) risk score (Rao et al., 2020), GRACE (Global Registry of Acute Coronary Events) risk score (D' Ascenza et al., 2012), HEART (History, ECG, Age, Risk Factors, and Troponin) score (Graham, 2006) etc.

In the present study, no significant differences between the values of the parameters between patients with STEMI or NSTEMI were found, indicating that the expression of COX-2 does not affect the type of acute coronary event. This is the first time, as far as we know, that such an observation has been made. Therefore, the determination of COX-2 promoter gene polymorphism -765G>C could not help as a risk predictor for acute coronary syndrome.

The limitations of this study are the small number of patients which prevents us from excluding safe results and the fact that controls are patients with unstable angina who have not yet presented with MI but might do so in the future.

CONCLUSION

The hypothesis, being investigated for the first time, to our knowledge, is whether certain genotypes are related to increased inflammation increasing this way the risk for cardiovascular diseases. The findings of the present study indicate that the onset of acute coronary events does not seem to be related to the -765G→C polymorphism of the COX-2 gene promoter. However, these results provide us with indications which need to be further investigated.

Conflict of Interest

The authors declare no conflict of interest

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