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In-Vitro-Response of Platelet Aggregation Induced by Various Agonists in Chronic Smoking Coronary Artery Disease Patients

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Background: Chronic cigarette smoking (CCS) is known as one of the major risk factors in the development of atherosclerosis. The relationship between smoking and coronary artery disease (CAD) has been established by epidemiological studies. Smoking has also been shown to have negative effects on haemostatic balance especially on platelet function.

Aim: In this study, the effect of CCS on *in-vitro*-platelet-aggregation induced by various agonists was investigated in coronary artery disease patients.

Methods: One hundred and twenty-one patients with stable angina pectoris and controlled unstable angina pectoris were included in the study. Patients were divided into two groups: Group I consisted of 53 CCS patients and Group II consisted of 68 non-smokers. Agonist induced platelet aggregation (PA) waves were measured by turbidometric method of Born. Maximum amplitude and duration of aggregation waves for each of the agonists were calculated.

Results: Maximum amplitude and duration of ADP, collagen, and epinephrine induced *in vitro* PA response were significantly higher in Group I (p-values respectively: $p < 0.05$, $p < 0.0001$, $p < 0.0001$).

Conclusion: In vitro PA response is increased despite antiaggregant therapy in CAD patients who are chronic smokers. Further studies are needed to establish the effect of smoking cessation on PA and progression of CAD. We also believe that prospective studies are needed to show the effect of more efficient antiaggregant therapy for CAD patients who are chronic smokers. *J Clin Basic Cardiol 2003; 6: 55–7.*

Key words: smoking, coronary artery disease, in vitro platelet aggregation, agonists

Chronic cigarette smoking (CCS) is known as one of the major risk factors in the development of atherosclerosis in coronary and peripheral arteries. CCS shows adverse effects on the production of the eicosanoid system and thromboxane A_2 which play an important role in the protection of haemostatic balance. These adverse effects are not only seen in active smokers but in passive smokers as well. Although there are conflicting results, many studies agree that smoking increases platelet aggregation (PA). Several authors have suggested that increased PA plays an important role in smoking induced vascular injury. However the effect of this increased aggregability on the progression of atherosclerosis remains unclear [1].

Aim

In this study, the effect of CCS on *in vitro* PA induced by various agonists were investigated in coronary artery disease (CAD) patients with stable angina pectoris (SAP) and controlled unstable angina pectoris (UAP).

Material and Methods

One hundred and twenty-one patients angiographically diagnosed as CAD who had clinical and laboratory data of SAP and controlled UAP were included in this study. Patients were divided into two groups: Group I consisted of 53 CCS patients (mean: 20 cigarettes per day for 25 years), Group II consisted of 68 non-smokers. Patients who had uncontrolled hypertension, insulin dependent diabetes mellitus, renal or hepatic dysfunction, myocardial infarction during the last 3 months, cerebrovascular accident in the last year, uncontrolled UAP and bleeding diathesis were excluded. All patients were given aspirin 100 mg/day, oral nitrate 40–60 mg/day and/or calcium antagonists, β-blockers and angiotensin converting enzyme inhibitors. They were followed for 6 months. Demographic and clinical properties of the groups are shown in Table 1.

Measurement of Platelet Function

Before coronary angiography 9 ml of fasting blood samples were obtained from brachial veins with minimal tourniquet application within two hours after last smoking. Then the blood sample was put into special tubes containing 1 ml of 3.8 % sodium citrate. Platelet rich plasma (PRP) was obtained by centrifuging the mixture at 200 g for 15 minutes. PRP was *in vitro* treated separately with adenosine diphosphate (ADP, 10 mmol/l), collagen (COL, 0.6 mg/ml) and

Table 1. Demographic and clinical findings

	Group I n = 53	Group II n = 68	P
Age (years)	51 ± 8	58 ± 10	< 0.0001
Male gender	47 (88 %)	50 (74 %)	NS
Functional capacity (NYHA)			
Class I	27 (51 %)	(56 %)	NS
Class II	22 (42 %)	(43 %)	NS
Class III	4 (7 %)	(1 %)	NS
Clinical			
UAP	22 (42 %)	23 (34 %)	NS
SAP	31 (59 %)	45 (66 %)	NS
Prior MI	39 (73 %)	36 (53 %)	< 0.05
Hypertension	28 (53 %)	20 (29 %)	NS

UAP: unstable angina pectoris; SAP: stable angina pectoris; MI: myocardial infarction; NS: p > 0.05

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epinephrine (EPN, 20 mmol/l). Platelet aggregation waves were measured by turbidometric method of Born working on the principle of optical density changes of plasma when the platelets were aggregated [2]. These aggregation waves were recorded on special millimetrical papers. Maximum amplitude (%) and duration (seconds) of aggregation waves for each agonist were calculated. Activated clotting time (ACT), platelet counts in whole blood and blood lipid profiles of the patients before coronary angiography were also measured.

Statistical Analysis

A primary package program was used for statistical analysis. Student's t-test and Chi-square test were applied according to the parameter variables and $p < 0.05$ was accepted as statistically significant.

Results

There was no difference of sex and functional capacity between the groups. Group I patients were significantly younger ($p < 0.05$) but had higher ratios of hypertension and myocardial infarction than Group II ($p < 0.05$). Clinical presentation (UAP and SAP) of the groups was similar ($p > 0.05$) (Table 1).

The number of diseased coronary artery and segmentary wall motion disorders were similar in both groups ($p > 0.05$), but left ventricular ejection fraction was significantly less in Group I ($p < 0.001$). The number of total interventions (coronary bypass, PTCA and coronary stenting) was higher in Group I ($p = 0.011$, $p = 0.05$ and $p = 0.015$, respectively). Ratio of coronary artery stenosis, success of interventions, residual stenosis after interventions and restenosis after 6 months did not differ between groups ($p > 0.05$) (Table 2).

Activated clotting time, total and LDL cholesterol and triglyceride values were similar in both groups ($p > 0.05$), but the number of platelets in whole blood was significantly higher ($p < 0.05$) and HDL cholesterol levels were significantly lower in Group I ($p < 0.0001$) (Table 3).

Maximum amplitude and duration of ADP, COL, and EPN induced *in vitro* PA response was significantly higher in Group I (respective p-values: $p < 0.05$, $p < 0.05$ for ADP, $p < 0.0001$, $p < 0.05$ for COL and $p < 0.001$, $p < 0.0001$ for EPN) (Table 4). Both groups did not show any correlation between activation ratio and duration of PA response and plasma levels of total, HDL and LDL cholesterol and triglyceride ($r > 1$ and $p > 0.05$ for each correlation).

Table 2. Angiographic and interventional properties of the patients

	Group I	Group II	P
Extent of CAD			
1 vessel	25 (47 %)	33 (48 %)	NS
2 vessel	15 (28 %)	25 (37 %)	NS
3 vessel	13 (25 %)	10 (15 %)	NS
LVWMA	31 (59 %)	29 (43 %)	NS
LVEF (%)	49 ± 9	55 ± 8	< 0.0001
Treatment			
PTCA ± stent	39 (73 %)	37 (54 %)	0.05
CABG	10 (19 %)	13 (19 %)	NS
Total Int.	49 (92 %)	50 (72 %)	0.01
Medical	4 (8 %)	18 (27 %)	< 0.05
PTCA ± stent			
Stenosis BI (%)	91 ± 11	88 ± 10	NS
Stenosis AI (%)	15 ± 5	13 ± 8	NS
Restenosis rate after six months	26 (49 %)	19 (28 %)	> 0.05

LVWMA: rest left ventricular wall motion abnormalities; LVEF: left ventricular ejection fraction; Int: intervention; BI: before intervention; AI: after intervention; NS: $p > 0.05$

Discussion

Habitual smoking is one of the best established risk factors for cardiovascular disease. The pathogenesis of smoke induced vascular damage is not clearly defined, but it is well known that cigarette smoking has negative effects on artery endothelium, platelets, oxidative and coagulation system and lipid profile. Chronic smokers with coronary atherosclerotic plaques do not have decreased basal or nitroglycerine induced vasodilation response [3]. Similar findings in peripheral arteries have also been reported [4]. Bradykinin, EDRF secretion induced by calcium ionophore and platelet derived nitric oxide levels were significantly decreased in chronic smokers [5, 6]. Opposite findings for the effect of CCS on PA were reported. Some studies showed that CCS increased PA while some found PA was decreased due to cigarette smoking [7].

Series of prospective studies have clearly documented the coronary risk. There are studies which reaffirmed the epidemiological relationship between smoking and CAD [8, 9]. Compared with non-smokers, smokers have an increased prevalence of coronary spasm [10]. Cigarette smoking also lowers the age of initial myocardial infarction that was more common in female gender [11]. Patients with CAD were younger in our study as well. Although the clinical presentation was similar in both groups, prevalence of previous myocardial infarction was significantly higher in CCS patients. These findings could be explained by more coronary spasm and increased platelet aggregability seen in smokers. However, the effect of aging alone on PA has not been documented. We believe that reduced left ventricular ejection fraction observed in CCS is related to previous myocardial infarctions. The vasomotor dysfunction participates in the development of not only CAD but also hypertension. It has also been shown that PA increases in hypertensive patients

Table 3. Laboratory findings

	Group I	Group II	P
Basal ACT	101 ± 22	105 ± 15	NS
Platelet count in whole blood ($\times 10^3$/ml)	259 ± 80	230 ± 63	< 0.05
Cholesterol (mg/dl)			
Total	248 ± 51	238 ± 37	NS
HDL	33 ± 13	47 ± 11	< 0.0001
LDL	152 ± 28	148 ± 20	NS
Triglyceride (mg/dl)	235 ± 62	220 ± 26	NS

ACT: activated clotting time; HDL: high density lipoprotein; LDL: low density lipoprotein

Table 4. *In vitro* response of agonist induced platelet aggregation

Agonist	Group I	Group II	P
ADP			
MA (%)	39 ± 15	34 ± 12	< 0.05
Duration (sec)	177 ± 147	133 ± 78	< 0.05
Collagen			
MA (%)	46 ± 33	24 ± 21	< 0.0001
Duration (sec)	465 ± 184	405 ± 138	< 0.05
Epinephrine			
MA (%)	29 ± 13	21 ± 11	< 0.001
Duration (sec)	362 ± 133	264 ± 120	< 0.0001

MA: maximum amplitude (%)

[12, 13]. In this study, incidence of hypertension in CCS patients was found to be significantly increased.

Cigarette smoking is known to be deleterious to patients with coronary disease, but the effect of smoking on the short-term clinical outcome after intervention is not well defined. In a study consisting of more than 5000 patients followed for 16 years, Hasdai reported that patients who continued to smoke after PTCA were at greater risk for Q-wave infarction and death than non-smokers [14]. However, Violaris concluded that after successful coronary angioplasty there appears to be no evidence that smoking influences short-term (6 months) outcome [15]. We also could not find any significant effect of smoking on restenosis. Few patients quitted smoking in our study population so it was not possible to draw any conclusion regarding favourable effects of stopping smoking on restenosis.

In a study, number of red blood cells and platelets and levels of total cholesterol and triglyceride were not found different in chronic smokers and non-smokers. On the other hand another study showed the correlation between ADP induced PA response and lower HDL and EPN induced PA and higher triglycerides [16, 17]. In our study, blood lipid profiles of the groups were similar but number of platelets were significantly higher in Group I. No correlation between activation ratio and duration of PA response and plasma levels of total, HDL and LDL cholesterol and triglyceride was present.

After smoking a new cigarette, ADP, COL and PAF mediated *in vitro* PA response did not change in chronic smokers who did not have CAD. But thrombin induced PA was increased significantly in smoking CAD patients using aspirin 325 mg/day [18]. In our study, duration and amplitude of PA to ADP, COL and EPN were significantly increased in chronic smokers when compared to non-smoking CAD patients.

Conclusions

In vitro PA response is increased despite an antiaggregant therapy in CAD patients who are chronic smokers. Chronic smoking does not appear to have any effect on success of interventions and short-term restenosis rate. Further studies are needed to establish the effect of smoking cessation on PA and progression of CAD. We also believe that prospective studies are needed to show the effect of more efficient antiaggregant therapies for CAD patients who are chronic smokers.

References:

1. Kritz H, Sinzinger H. Passive smoking, platelet function and atherosclerosis. Wien Klin Wochenschr 1996; 108: 582–8.
2. Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 1962; 194: 927.
3. Ikeda H, Takajo Y, Murohara T, Ichiki K, Adachi H, Haramaki N, Katoh A, Imaizumi T. Platelet-derived nitric oxide and risk factors. Hypertension 2000; 35: 904–7.
4. Sharp DS, Benowitz NL, Bath PN, Martin JF, Beswick AD, Elwood PC. Cigarette smoking sensitizes and desensitizes impedance-measured ADP-induced platelet aggregation in whole blood. Thromb Haemost 1995; 74: 730–5.
5. Zeiher AM, Schachinger V, Minners J. Long-term cigarette smoking impairs endothelium-dependent coronary arterial vasodilator function. Circulation 1995; 92: 1094–100.
6. Corretti MC, Plotnick GD, Vogel RA. Smoking correlates with flow-mediated brachial artery vasoactivity but not cold pressor vasoactivity in men with coronary artery disease. Int J Card Imaging 1998; 14: 11–7.
7. Higman DJ, Greenhalgh RM, Powell JT. Smoking impairs endothelium-dependent relaxation of saphenous vein. Br J Surg 1993; 80: 1242–5.
8. U. S. Department of Health and Human Services. The health consequences of smoking: Cardiovascular Disease: A report of the Surgeon General. Washington, DC, Government Printing Office, DHHS Publication No 84-50204, 1983.
9. Massberg S, Brand K, Gruner S. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. J Exp Med 2002; 196: 887–96.
10. Sugiishi M, Takatsu F. Cigarette smoking is a major risk factor for coronary vasospasm. Circulation 1993; 87: 76–9.
11. Hansen EF, Anderson LT, Von Eyben F. Cigarette smoking and age at first myocardial infarction and influence of gender and extent of smoking. Am J Cardiol 1993; 71: 1439–42.
12. Rigotti NA, Pasternak RC. Cigarette smoking and coronary heart disease: risk and management. Cardiol Clin 1996; 14: 51–68.
13. Hernandez Hernandez R, Carvajal AR, Guerrero Pajuelo J, Armas de Hernandez MJ, Armas Padilla MC, Barragan O, Boada Boada JJ, Roa E. The effect of doxazosin on platelet aggregation in normotensive subjects and patients with hypertension: An in vitro study. Am Heart J 1991; 121: 389–94.
14. Hasdai D, Garratt KN, Grill DE, Lerman A, Holmes DR jr. Effect of smoking status on the long term outcome after successful percutaneous coronary revascularization. N Engl J Med 1997; 336: 755–61.
15. Violaris AG, Thury A, Regar E, Melkert R, Serruys PW. Influence of history of smoking on short term (six month) clinical and angiographic outcome after successful coronary angioplasty. Heart 2000; 84: 299–306.
16. Maly J, Pecka M, Vodickova L, Pidman V, Mala H, Blaha M, Jebavy L. Changes in the activity of thrombocytes by smoking. Kralova 1990; 33: 299–306.
17. De Padua Mansur A, Caramelli B, Vianna CB, Chamone D, Ramires JA. Smoking and lipoprotein abnormalities on platelet aggregation in coronary heart disease. Int J Cardiol 1997; 62: 151–4.
18. Hung J, Lam JY, Lacoste L, Letchacovski G. Cigarette smoking acutely increases platelet thrombus formation in patients with coronary artery disease taking aspirin. Circulation 1995; 92: 2432–6.