Coronary angioplasty induced oxidative stress and its relation with metoprolol use and plasma homocysteine levels

Koroner anjiyoplastiye bağlı oksidatif stresin metoprolol kullanımı ve plazma homosistein düzeyi ile ilişkisi

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ABSTRACT

Objective: Short episodes of myocardial ischemia during coronary angioplasty may induce oxidative stress and increase lipid peroxidation. The aim of this study was to determine the effect of metoprolol on lipid peroxidation by measurements of malondialdehyde (MDA) and total antioxidant capacity (TRAP) in patients undergoing angioplasty. The relations between homocysteine level and lipid peroxidation were also studied.

Methods: Forty-six patients (mean age 57 years, 37 males) undergoing elective angioplasty were enrolled. Metoprolol treatment was initiated in 27 patients (group 1), meanwhile 19 patients could not take metoprolol due to diverse contraindications (group 2).

Results: Following angioplasty, while venous MDA levels decreased in group 1 (0.188±0.021 vs. 0.159±0.020 nmol/ml, p=0.05), an increase was detected in group 2 (0.203±0.025 vs. 0.229±0.024 nmol/ml, p=0.045) as compared with baseline levels. In group 1, TRAP levels markedly increased after angioplasty in venous samples (1.201±0.036 vs. 1.478±0.044 mmol/L, p=0.0001). However, small increase was observed for TRAP in group 2 (1.274±0.043 vs. 1.363±0.053 mmol/L, p=0.05). There was no significant change in plasma homocysteine levels with angioplasty. There was no significant correlation between homocysteine, changes in MDA and TRAP levels either.

Conclusion: Administration of metoprolol may cause a reduction in the oxidative stress and an increase in the antioxidant activity in patients undergoing elective angioplasty. *(Anadolu Kardiyol Derg 2006; 6: 308-13)*

Key words: Malondialdehyde, lipid peroxidation, angioplasty, homocysteine, metoprolol

ÖZET

Amaç: Koroner anjiyografi esnasındaki kısa süreli miyokardiyal iskemi atakları oksidatif strese neden olabilir ve lipid peroksidasyonunu artırabilir. Bu çalışmada, anjiyoplasti yapılan hastalarda, malondialdehid (MDA) ve total antioksidan kapasite (TAK) ölçümü yardımı ile metoprololün lipid peroksidasyonu üzerine olan etkisinin araştırılması amaçlandı. Ayrıca homosistein ve lipid peroksidasyon ürünleri arasındaki ilişki incelendi.

Yöntemler: Elektif anjiyoplasti yapılan 46 hasta (yaş ortalaması: 57, 37 erkek) çalışmaya alındı. Yirmi yedi hastaya metoprolol tedavisi başlanırken (grup 1), farklı kontrendikasyonlar sebebi ile 19 hasta metoprolol alamadı (grup 2).

Bulgular: Anjiyoplasti sonrası, grup 1'de venöz MDA seviyeleri azalırken (0.188±0.021 ve 0.159±0.020 nmol/ml, p=0.05), grup 2'de MDA seviyelerinde artış gözlendi (0.203±0.025 ve 0.229±0.024 nmol/ml, p=0.045). Grup 1'de venöz örneklerde TAK düzeyleri anjiyoplasti sonrası önemli miktarda arttı (1.201±0.036 ve 1.478±0.044 mmol/L, p=0.0001). Fakat, grup 2'de hafif bir artış gözlendi (1.274±0.043 ve 1.363±0.053 mmol/L, p=0.05). Anjiyoplasti ile plazma homosistein seviyelerinde önemli bir değişiklik olmadı. Ayrıca, homosistein düzeyleri ile MDA ve TAK ölçümündeki değişiklikler arasında önemli bir korelasyon gözlenmedi.

Sonuç: Elektif anjiyoplastiye giden hastalarda metoprolol kullanımı, oksidatif streste azalma ve antioksidan aktivitede artış sağlayabilir. *(Anadolu Kardiyol Derg 2006; 6: 308-13)*

Anahtar kelimeler: Malondialdehid, lipid peroksidasyon, anjiyoplasti, homosistein, metoprolol

Introduction

Lipid peroxidation of membrane polyunsaturated fatty acids by reactive oxygen species is considered the major mechanism of ischemia-reperfusion injury (1). Free radical production may cause myocardial damage during reperfusion of ischemic myocardial tissue.

Short episodes of myocardial ischemia during percutaneous transluminal coronary angioplasty (PTCA) may induce a sustained oxidative stress, a sustained increase of lipid peroxidation

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Note: This study was presented as poster presentation at the 1st National Congress of Molecular Biology, 16-19 April, 2005, İstanbul, Turkey

and a transient decrease of antioxidant defenses (2,3) Therefore, antioxidant therapy in patients with ischemic heart disease before PTCA procedure might have useful role. Investigation of antioxidant properties of cardiovascular drugs may lead to new therapeutic approaches.

Metoprolol is a beta-adrenoceptor antagonist. It reduces oxygen consumption of the myocardium and diminishes myocardial ischemia (4). Furthermore, metoprolol may reduce lipid peroxidation as shown in vitro studies and studies on animal models (5-8). Thus, the aim of this study was to determine the effect of metoprolol treatment on lipid peroxidation associated with oxidative stress in patients undergoing elective PTCA. The determination of oxidative status was based on measurements of concentrations of malondialdehyde (MDA) and total antioxidant capacity (TRAP).

Hyperhomocysteinemia has been suggested as an independent risk factor for atherosclerosis and it was associated with pathological and stressful conditions (9). Some studies showed that elevated plasma homocysteine (Hcy) level is associated with enhanced lipid peroxidation (10,11). Therefore, we have also aimed to evaluate the relation between plasma Hcy levels and lipid peroxidation in patients undergoing elective PTCA.

Methods

Forty-six subjects (mean age 57 years, range 38 to 76 years, 37 males) were enrolled into the study. Subjects were selected from consecutive patients undergoing elective PTCA for discrete coronary stenosis of left anterior descending artery (LAD) or left circumflex coronary artery (LCx). The criteria for exclusion were: myocardial infarction within the previous 30 days, angina episodes in the last 12 hours before the procedure, severe (>90%) coronary stenosis and renal failure (as serum creatinine >2.0 mg/dl).

Metoprolol (Beloc zok tb 50 mg, metoprolol succinate, AstraZeneca Pharmaceutical Co.) treatment was started at a dosage of 50 mg/day in patients with no contraindication to beta-blockers and increased to 100 mg/day. These patients were accepted as Group 1. Metoprolol treatment was started at least 72 hours before PTCA. The mean metoprolol dosage was 83.3±4.6 mg/day.

Patients who could not take beta-blockers because of sinus bradycardia, atrioventricular conduction disturbance, peripheral vascular disease, diabetes mellitus with hypoglycemia attacks or bronchospasm were also included into the study and accepted as Group 2. At the time of PTCA, all patients were on oral aspirin, clopidogrel and statin treatment. Thirty patients were using oral nitrates, 13 patients were using oral calcium-antagonists and 28 patients were using angiotensin converting enzyme inhibitors. There was no significant difference in angiotensin converting enzyme inhibitors and statin use between groups.

Balloon angioplasty was performed according to standard techniques. After local anesthesia with lidocaine, one sheath was placed to femoral artery. Heparin was given at baseline (100 U/kg) in order to maintain the activated coagulation time ≥300 s. A venous sample was taken from an antecubital vein on the forearm. At the same time, an arterial sample was taken proximal to the lesion from the coronary guiding catheter. Ten minutes after the first balloon inflation, again venous and arterial blood samples were taken. Blood was collected into the EDTA containing tubes during sampling procedure. Arterial blood pressure, heart rate and electrocardiography were continuously monitored throughout the procedure.

The oxidative status was determined by measurement of MDA and TRAP in the venous and arterial blood samples. Homocysteine concentration was measured in these blood samples. The study protocol conforms to the ethical guidelines of Declaration of Helsinki and protocol was approved by the ethical committee of the hospital board, Mersin University. All patients signed an informed consent.

Determination of MDA:

Blood samples were collected in EDTA containing tubes. After centrifugation, the obtained plasma was stored at -20 °C. Malondialdehyde levels were determined by using reagent kit (Reagent cat no: 67 000) for HPLC analysis of MDA (Chromosystems, GmbH Germany). Analyses were performed with isocratic HPLC system with fluorescence detection (HP 1100). HPLC condition for MDA: Injection volume: 20 μl; Flow rate: 1.0 ml/min; Column and room temperature 25 °C; Wavelength: EX 515, EM, 553.

The determination of total antioxidant status:

2,2'-Azino-di 3-ethybenzthiazoline sulphonate) (ABTS) was incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation ABTS. This had a relatively stable blue-green color, which was measured at 600 nm. Antioxidants in the added sample caused suppression of this color production to a degree proportional to their concentration (12).

Determination of Homocysteine:

After centrifugation, the obtained plasma was stored at -20 °C. Plasma Hcy levels were determined by using reagent kit (Reagent cat no: 39 000) for HPLC analysis of Hcy in serum/plasma (Chromosystems, GmbH Germany). Analyses were performed with isocratic HPLC system with fluorescence detector (HP 1100). The HPLC condition for Hcy: Injection volume: 20 μl, Flow rate: 1.7 ml/min, Room temperature 25 °C, Wavelength: EX 385, EM, 515.

Statistical analysis

Three-way analysis of variance with two factor repeated measurement design (repeated measures variance analysis) was used to compare the baseline and post-angioplasty MDA, TRAP and Hcy plasma levels from the venous and arterial blood samples in two groups. In case of statistically significant differences, paired between group and within-group comparisons were performed by Bonferroni PostHoc analysis. For detection of correlations of MDA, TRAP and Hcy plasma levels with categorical binary variables (gender, hypertension, diabetes mellitus, history of myocardial infarction, severity of coronary artery disease), independent t-test and one-way ANOVA tests were used. Pearson correlation test was used to determine any relation between MDA, TRAP and Hcy with continuous variables (creatinine, blood glucose, ejection fraction). Pearson Chi-Square analysis test was used for detection of relations between categorical variables and patient groups. All data were presented as mean ± standard error of the mean. Differences were considered significant at a value of $p<0.05$. Statistical analysis was assessed by computer software SPSS for Windows version 12.

Results

Forty-six patients (mean age 57 years, range 38 to 76 years; 37 males) underwent elective PTCA. Twenty-nine patients underwent balloon angioplasty for a stenosis of LAD and seventeen patients underwent balloon angioplasty for a stenosis of LCx. No significant complications were encountered during PTCA. Angiography of the target coronary artery after post-deflation sample acquisition showed no significant residual stenosis with normal contrast runoff.

Thirty patients had a history of smoking and all of them were male. Twenty patients had high blood pressure, ten had diabetes mellitus and eighteen had history of myocardial infarction. Myocardial infarction history was frequently observed in patients with smoking habits (p=0.039). Baseline clinical characteristics were similar between two groups. The clinical and angiographic characteristics of the patients are described in Table 1.

When changes in levels of MDA were evaluated, it was detected that, the interaction of patient groups, sampling vessels and PTCA was statistically significant (p=0.034). Just prior to PTCA, in group 1 patients, mean MDA level was 0.188±0.021 nmol/ml in venous samples and 0.186±0.025 nmol/ml in arterial samples. Ten minutes after the first balloon inflation, while MDA levels decreased in venous samples (p=0.05), but remained similar in arterial plasma (p>0.05) (Table 2). Conversely, in group 2 patients, MDA levels increased in venous samples, demonstrating increased oxidative stress (p=0.045) and remained similar in arterial plasma (p>0.05) (Table 2) (Fig. 1).

When changes in levels of TRAP were evaluated, it was detected that, the interaction of patient groups, sampling vessels and PTCA was also statistically significant (p=0.045). In group 1 patients, levels of TRAP markedly increased after PTCA in both venous and arterial samples (p=0.0001). In group 2 patients, there was also an increase in both venous and arterial levels of TRAP, however a milder increase was observed compared to group 1 (p=0.05 and p=0.002) (Fig. 2). In group 1 patients, after balloon angioplasty, venous TRAP levels were significantly higher than arterial TRAP levels (1.478±0.044 vs. 1.394±0.059 mmol/L, p=0.035). In addition, venous TRAP levels were significantly higher in group 1 patients than those of group 2 patients after balloon angioplasty (1.478±0.044 vs. 1.363±0.053 mmol/L, p=0.009).

There were no significant changes in plasma Hcy levels in either group with PTCA in both venous and arterial samples (p=0.707). Table 2 reports on the levels of MDA, TRAP and Hcy in both venous and arterial samples before and after balloon angioplasty. There was no significant correlation between the basal levels of Hcy with changes in MDA (r= -0.175, p=0.245) and TRAP (r= -0.022, p=0.883,) after balloon angioplasty.

Table 1. Clinical and angiographic characteristics of patients

	Group 1 (n= 27)	Group 2 (n = 19)	p
Age, years	55±9	60±10	NS
Men, n (%)	24 (89)	13 (68)	NS
Hypertension, n (%)	10 (37)	10 (53)	NS
Diabetes, n (%)	4 (15)	6 (32)	NS
Smoking, n (%)	20 (74)	10 (53)	NS
History of MI, n (%)	11 (41)	7 (37)	NS
Severity of CAD, n (%)			
1-Vessel	8 (30)	7 (37)	NS
2-Vessel	14 (52)	9 (47)	NS
3-Vessel	5 (19)	3 (16)	NS
Target coronary artery, n (%)			
LAD	17 (63)	12 (63)	NS
LCx	10 (37)	7 (37)	NS
LVEF,%	59±3	60±4	NS
Heart rate, beat/min	70.2±1.6	73.7±1.9	NS
Systolic BP, mmHg	116.1±2.9	119.7±2.8	NS
Diastolic BP, mmHg	70.7±1.7	73.7±1.7	NS
Laboratory findings			
Total cholesterol, mg/dl	183±47	180±56	NS
LDL cholesterol, mg/dl	111±33	109±43	NS
HDL cholesterol, mg/dl	38±8	40±12	NS
Triglycerides, mg/dl	176±97	145±55	NS

BP- blood pressure, CAD- coronary artery disease, LAD- left anterior descending artery, LCx- left circumflex artery, LVEF- left ventricular ejection fraction, MI- myocardial infarction, NS-not significant, p>0.05

Table 2. Levels of MDA, TRAP and Hcy in the venous blood samples before and after PTCA (V1, V2), and in the arterial blood samples before and after PTCA (A1, A2)

	V1	V2	p	A1	A2	p
Group 1						
MDA, nmol/ml	0.188±0.021‡	0.159±0.020	0.05	0.186±0.025	0.189±0.025	NS
TRAP, mmol/L	1.201±0.036‡	1.478±0.044*†	0.0001	1.234±0.048	1.394±0.059*	0.0001
Hcy, µmol/L	20.59±1.73‡	20.58±2.36	NS	18.11±1.97	20.13±1.70	NS
Group 2						
MDA, nmol/ml	0.203±0.025	0.229±0.024	0.045	0.240±0.029	0.242±0.030	NS
TRAP, mmol/L	1.274±0.043	1.363±0.053†	0.05	1.279±0.058	1.426±0.071	0.002
Hcy, µmol/L	21.37±2.06	20.66±2.81	NS	15.98±2.35	16.19±2.02	NS

* p=0.035 between venous and arterial TRAP levels of group 1 after angioplasty
† p=0.009 between venous TRAP levels of group 1 and group 2 after angioplasty
‡ p>0.05 between Hcy levels and change in MDA venous levels and TRAP venous levels
Hcy- homocysteine, MDA- malondialdehyde, NS-not significant, p>0.05, PTCA- percutaneous transluminal coronary angioplasty, TRAP- total antioxidant capacity

There was no significant correlation between the levels of MDA, TRAP, Hcy and diabetes mellitus, hypertension, smoking, gender, target coronary artery, history of myocardial infarction and left ventricular ejection fraction (p>0.05). Baseline venous Hcy levels were positively correlated with age of the patients (p=0.012, r=0.368), the severity of coronary artery disease as evaluated with the number of narrowed arteries (p=0.046, r=0.295) and serum creatinine levels (p=0.035, r=0.312).

All patients were followed-up for one month. During the follow-up, one patient died after an acute myocardial infarction on the third day of angioplasty and one patients died suddenly on the 21. day of angioplasty. Two patients underwent a new revascularization procedure (1 coronary artery bypass surgery, 1 re-angioplasty). No correlation was observed between the cardiovascular events (death, myocardial infarction, revascularization), and both the baseline levels and changes in MDA, TRAP and Hcy levels after PTCA (p>0.05).

Discussion

Metoprolol is a frequently used beta-adrenoceptor-1 antagonist. Various favorable effects of metoprolol have been shown in patients with heart failure and ischemic heart disease like reduction in energy requirements and ischemia, anti-arrhythmogenic effect and improvement of diastolic function (4, 13). Although, the action of metoprolol against lipid peroxidation has been a subject of many studies, results reported thus far are contradictory. While some investigators have postulated that, metoprolol reduces oxidative stress to a certain extent (6,7), some authors could not observe its antioxidant properties (14,15).

Oxidative stress is defined as a disturbance in pro-oxidant/anti-oxidant balance in favour of the first one, leading to potential damage (15). Studies showed that PTCA causes a cardiac oxidative stress after short episodes of balloon inflation (2,3,16). Roberts et al. found that there was a significant increase in MDA levels in coronary sinus blood samples following balloon angioplasty of the LAD (16). However, Oostenbrug et al. could not observe any association between the PTCA and lipid peroxidation (17).

The cardiac oxidative stress after PTCA may decrease the benefit of angioplasty and increase adverse events complicating angioplasty. Antioxidant therapy might have a role in patients with ischemic heart disease undergoing PTCA.

Among the compounds that result from lipid peroxidation, MDA is widely looked upon as a marker of oxidative damage (8,16,18). Malondialdehyde is the end product of lipid peroxidation and it could be used as an index to follow the lipid peroxidation pattern in patients with coronary artery disease (18). Total antioxidant capacity represents the real status of plasma defenses as determined by the overall effects of water soluble and lipid soluble antioxidants (2,19).

In the present study, we found a decrease in the lipid peroxidation and increased antioxidant activities as determined by MDA and TRAP measurements in patients undergoing balloon angioplasty and using metoprolol. In contrast, there was an increase in lipid peroxidation in patients who could not take metoprolol. Taken together, it can be reasonably speculated that, metoprolol treatment may decrease lipid peroxidation and increase antioxidant activity after a brief ischemia-reperfusion produced by balloon angioplasty.

Consistent with results of the present study, some studies also demonstrated that metoprolol reduces lipid peroxidation during myocardial ischemia (8,20). Kalaycioglu et al. studied antioxidant activity of metoprolol on guinea-pig hearts by giving metoprolol in the pre-ischemia period and measured MDA and glutathione. They found that MDA levels significantly decreased and glutathione levels increased in the metoprolol group compared with those of the control group. They concluded that metoprolol reduces ischemic injury via prevention of lipid peroxidation (8). Theres et al. proposed that metoprolol and angiotensin-converting enzyme inhibition after myocardial infarction may decrease myocardial oxidative stress (20). However, in the present study, we have demonstrated for the first time that, metoprolol decreases the balloon angioplasty associated lipid peroxidation.

Raised Hcy concentrations are associated with laboratory evidence of atherosclerosis and increased risk of atherothrombotic vascular disease (9). Being in agreement with this knowledge, in our study plasma total Hcy concentrations were above normal limits in patients (upper limit of reference for fasting total plasma Hcy levels for 15-65 years adults: 12-15 µmol/L , >65 years adults: 16-20 µmol/L) (21). In addition, venous levels of Hcy were correlated with the severity of coronary artery disease as evaluated with the number of narrowed arteries. Therefore, increased levels of Hcy could be useful in clinical practice to determine the severity of coronary artery disease.

Hyperhomocysteinemia and lipid peroxidation may play a role in the genesis of atherosclerosis and heart disease. Some aut-

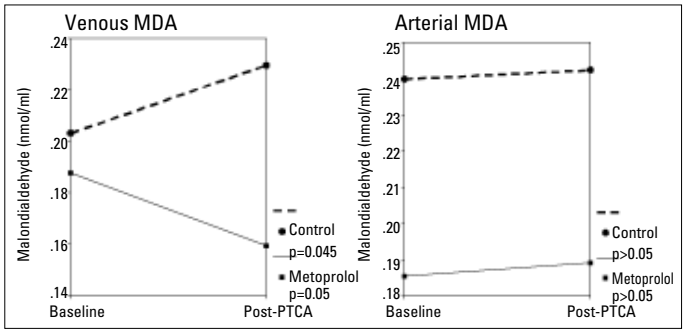

Figure 1. The graphs refer to the venous (a) and arterial (b) levels of malondialdehyde (MDA) in patients before and after PTCA. Straight lines represent group 1 (metoprolol) data, dashed lines represent group 2 (control) data

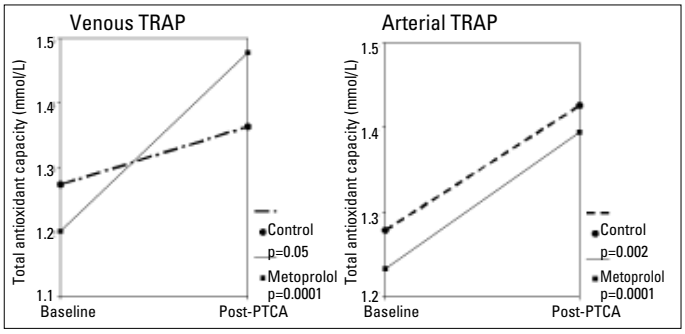

Figure 2. The graphs refer to the venous (a) and arterial (b) levels of total antioxidant capacity (TRAP) in patients before and after PTCA. Straight lines represent group 1 (metoprolol) data, dashed lines represent group 2 (control) data

hors have suggested that Hcy might cause atherosclerosis by damaging the endothelium either directly or by altering oxidative status (22). Some studies also showed that elevated plasma Hcy level is associated with enhanced lipid peroxidation (10,11,23). Therefore, we have also studied the correlation between Hcy levels and lipid peroxidation in patients undergoing PTCA. There was no significant change in plasma Hcy levels in venous and arterial blood samples with angioplasty in either group. In addition, we could not observe any correlation between Hcy levels and lipid peroxidation products. Consistent with our results, Cavalca et al. could not found any correlation between Hcy and MDA either (18). These results might indicate that Hcy is not entirely responsible for the oxidative damage in these patients.

Although elevated plasma Hcy levels have been considered as an independent risk factor of coronary artery disease, the relation between Hcy levels with clinical prognosis after PTCA was unclear. Some authors proposed that elevated plasma Hyc is an independent risk factor for mortality, revascularization and adverse late outcome after successful coronary angioplasty (24,25). However, in other studies, there was no significant association between Hcy levels, restenosis and long-term prognosis (26,27). In the present study, we could not observe any correlation between plasma Hcy levels and cardiovascular events (death and revascularization) after angioplasty. However, because of the small sample size and potential limitations, we could not decide on the risk assessment of Hcy.

The present study was limited by the absence of a control group of subjects without heart disease. Also, we could not compare results of treatments with other beta-blockers such as carvedilol. Carvedilol is a nonselective beta-adrenoceptor antagonist and there are several studies about its antioxidant effects (28-31). In addition, we could not use coronary sinus blood sampling for evaluation of cardiac ischemia. Therefore, additional studies using other beta-blockers and coronary sinus blood sampling are needed.

Conclusions

Oxidative stress is elevated in patients undergoing balloon angioplasty and administration of metoprolol may cause a reduction in the oxidative stress in these patients. Thus, the antioxidant properties of metoprolol may contribute to the cardioprotective effects of the compound. The oxidative stress status did not change with changes in homocysteine levels.

Acknowledgements

We gratefully acknowledge Ms. Handan Camdeviren for her assistance in statistical analysis reported in the study. We are also grateful to AstraZeneca Pharmaceutics for providing us with metoprolol and some biochemical assay kits.

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