Evaluation of aspirin resistance in patients with coronary artery disease

Koroner arter hastalarında aspirin direnci sıklığı

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Objectives: Although aspirin is widely used for secondary prevention of cardiovascular disease, its effect is not standard in all patients. We aimed to evaluate the frequency of aspirin resistance (AR) in patients taking aspirin for stable coronary artery disease (CAD) and the effect of AR on platelet sensitivity to adenosine diphosphate (ADP).

Study design: The study consisted of 100 patients (28 females, 72 males; mean age 56 years; range 30 to 75 years) who had been on aspirin treatment at least for the past seven days for stable CAD. Thirty healthy volunteers (10 females, 20 males; mean age 54 years) without a history of aspirin ingestion within the past seven days comprised the control group. Platelet function was measured by the PFA-100 system and the 95th percentile (170 sec) of the control group was defined as the cut-off value of the closure time at the collagen/epinephrine cartridge to determine AR.

Results: Twenty-seven patients (27%) were found to have AR. Seventeen patients (17%) showed a prolonged closure time (range 171 sec to 212 sec) with a maximum increase by 25%, while 34 patients (34%) had no closure at the end of 300 sec. Patients with AR also showed an increased platelet sensitivity to ADP as shown by a significantly shorter closure time (70.0 sec) at the collagen/ADP cartridge compared to that of patients without AR (100.4 sec; p=0.007) or controls (79.0 sec; p=0.03). Patients with and without AR did not differ significantly with regard to age, sex, history of hypertension, diabetes mellitus, hyperlipidemia, smoking, family history of CAD, platelet count and mean platelet volume, and blood levels of urea and creatinine (p>0.05). The duration of aspirin usage and its formulation (enteric-coated or not) were not related with AR.

Conclusion: The effect of aspirin is not always desirable and the sensitivity of platelets to ADP is increased in patients with AR, requiring to individualize the antiplatelet treatment.

Key words: Aspirin/adverse effects; cardiovascular diseases/drug therapy/complications; drug resistance; platelet aggregation/drug effects; platelet function tests.

Amaç: Kardiyovasküler hastalıkların ikincil korunmasında yaygın olarak kullanılmasına rağmen aspirinin etkisi tüm hastalarda aynı düzeyde değildir. Çalışmamızda, aspirin kullanan kararlı koroner arter hastalarında (KAH) aspirin direncinin sıklığı ve aspirinin trombositlerin adenozin difosfata (ADP) duyarlılığına olan etkisi araştırıldı.

Çalışma planı: Kararlı koroner arter hastalığı nedeniyle en az son yedi gündür aspirin kullanan 100 hasta (28 kadın, 72 erkek; ort. yaş 56; dağılım 30-75) çalışmaya alındı. Son yedi gün içinde aspirin kullanmamış olan 30 sağlıklı gönüllüden (10 kadın; 20 erkek; ort. yaş 54) kontrol grubu oluşturuldu. Trombosit fonksiyonları PFA-100 cihazı ile değerlendirildi. Aspirin direnci için sınır, kontrol grubunda kollajen/epinefrin kartuşunda ölçülen kapanma zamanı değerlerinin 95. persentiline denk düşen değer (170 sn) olarak kabul edildi.

Bulgular: PFA-100 ile 27 hastada (%27) aspirin direnci saptandı. On yedi hastada (%17) kapanma zamanında, en çok %25 olmak üzere, uzama (dağılım 171-212 sn) görüldü; 34 hastada (%34) ise 300 sn sonunda kapanma olmadı. Aspirin direnci görülen hastalarda trombositlerin ADP'ye duyarlılığında anlamlı yükselme vardı; kollajen/ADP kartuşunda ölçülen zaman, aspirin direnci olmayanlar (100.4 sn; p=0.007) ve kontrol grubuna (79.0 sn; p=0.03) göre anlamlı derecede kısa bulundu Aspirin direnci olan ve olmayanlar arasında yaş, cinsiyet, hipertansiyon, diyabetes mellitus, sigara içimi, ailede KAH öyküsü, trombosit sayısı ve ortalama trombosit hacmi, kandaki üre ve kreatinin düzeyleri açısından anlamlı farklılık görülmedi (p>0.05). Aspirin kullanma süresi ve yapısı (enterik kaplı olup olmaması) aspirin direnci ile ilişkili bulunmadı.

Sonuç: Aspirin kullanımı her zaman istenen sonucu vermemektedir ve aspirin direnci görülen hastalarda trombositlerin ADP'ye duyarlılığında artış görülmektedir; bu durum anti-trombosit tedavinin bireyselleştirilmesini gerektirebilir.

Anahtar sözcükler: Aspirin/yan etki; kardiyovasküler hasatalık/ilaç etkisi/komplikasyon; ilaç direnci; trombosit agregasyonu/ilaç etkisi; trombosit fonksiyon testi.

Presented in part at the 19th National Cardiology Congress (October 11-14, 2003, Antalya, Turkey).

Received: April 12, 2005 *Accepted:* May 31, 2005

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Despite the development of new molecules, aspirin remains the mainstay of antiplatelet therapy, being indispensable for the secondary prevention of cardiovascular events. Aspirin exerts its major antithrombotic effect by irreversibly acetylating platelet cyclooxygenase-1, thereby inhibiting thromboxane A2 synthesis. It reduces the risk for death, myocardial infarction, and stroke by 25% in patients with cardiovascular disease.[1] However, patients taking aspirin might exhibit variable responses to *in vitro* tests for platelet aggregation and might experience breakthrough thromboembolic events. This phenomenon is called aspirin resistance (AR) and wide variations (5.5% to 75%) in the rate of AR have been reported.[2-4] Until recently, AR was, to some extent, neglected due to difficulty in its determination, and to the lack of alternative antiplatelet medications. However, the development of rapid and simple platelet function analyzers has facilitated the detection of AR and enabled to draw clinical implications associated with AR.

Data about the frequency of AR in Turkish population is lacking. This study was planned to determine the prevalence of AR in patients taking aspirin for stable coronary artery disease (CAD) and to investigate the effect of AR on platelet sensitivity to adenosine diphosphate (ADP).

PATIENTS AND METHODS

The study group consisted of 100 patients (28 females, 72 males; mean age 56±10.6 years; range 30 to 75 years) who had been taking aspirin regularly (80 mg to 300 mg daily) at least for the past seven days for the treatment of stable CAD. Thirty healthy volunteers (10 females, 20 males; mean age 54 years) without a history of aspirin ingestion within the past seven days comprised the control group.

Exclusion criteria included the presence of acute coronary syndrome, myocardial infarction, or rest angina within a month; the use of clopidogrel, ticlopidine, dipyridamole, or other nonsteroidal anti-inflammatory drugs; a family or personal history of a bleeding disorder; a platelet count of less than 150,000/micL or above 450,000/micL, a hemoglobin level of less than 8 g/dl; or a very recent major surgical procedure within a week.

Cardiovascular risk factors were sought through patient interviews, which included the following: current smoking; hypertension (being on antihypertensive drugs or with known and untreated hypertension); diabetes mellitus (the use of insulin or an oral hypoglycemic agent); a family history of premature CAD; and hypercholesterolemia (the use of cholesterol lowering drugs, or plasma levels of low-density lipoprotein cholesterol above 130 mg/dl or total cholesterol above 200 mg/dl).

Fasting blood samples were taken into 7.5% EDTA and hematologic parameters (hemoglobin, mean platelet volume, platelet, red and white blood cell counts) were measured by an autoanalyzer (Gen-S, Coulter Corp., Miami, USA). Serum values of urea, creatinine, and glucose were also measured.

Detection of aspirin resistance. Blood samples were obtained with the use of an 18-gauge needle by venipuncture and drawn into 4.5-ml vacutainer tubes anticoagulated with 3.8% sodium citrate. The tubes were gently inverted 3 to 5 times to ensure complete mixing of the anticoagulant. Platelet function analyses were performed within two hours.

The effect of aspirin on platelet function was assessed by the PFA-100 system (Dade Behring W,. Sacramento, USA), which simulates primary hemostasis in an injured blood vessel. The PFA-100 uses disposable test cartridges having a collagen-coated membrane infused with either ADP or epinephrine. The analyzer aspirates the whole blood at a high shear rate through the capillaries where it comes into contact with the membrane, to which the platelets adhere its surface and aggregate. A platelet plug forms with occlusion of the aperture and cessation of the blood flow. The closure time (CT) reflects platelet function in the sample evaluated. With the PFA-100, aspirin-induced platelet dysfunction can be detected with prolonged closure time at the collagen/epinephrine (C/E) cartridge, but normal at the collagen/ADP (C/ADP) cartridge (<114 sec).[5] The cut-off value for AR in the patient group was defined as the 95th percentile of the closure time at the collagen/epinephrine (C/E) cartridge in the control group.

Statistical analyses. Continuous variables were presented as a mean±standard deviation. Categorical variables were presented as frequencies and percentages. The study and control groups were compared using the unpaired t-test for continuous variables and chi-square test for categorical variables. The SPSS 10.0 for Windows was used for the entire statistical work-up. A p value of less than 0.05 was considered to be statistically significant.

RESULTS

The mean closure times obtained from the control group were 131.8±18.8 sec and 79±15.4 sec for C/E

and C/ADP, respectively. The 95th percentile of the closure times for C/E was 170 sec in the control group.

According to this cut-off value, 27 patients (27%) were found to have AR. Seventeen patients (17%) showed a prolonged closure time (range 171 sec to 212 sec) with a maximum increase by 25%. In 22 patients, the closure time exceeded 212 sec, while 34 patients (34%) had no closure at the end of 300 sec, which was the standard maximum time for the C/E cartridge.

In patients with AR, the mean closure time for C/ADP was significantly shorter (70.0±10.1 sec) than that of patients without AR (100.4±47.1 sec; p=0.007) or controls (79±15.4 sec; p=0.03).

Demographic features of the patients with and without AR are summarized in Table 1. There were no differences with regard to age, sex, history of hypertension, diabetes mellitus, hyperlipidemia, smoking, and family history of CAD. Blood levels of urea and creatinine did not differ, either (p>0.05).

Although it did not reach statistical significance, the dosage of aspirin was lower in patients with AR. The duration of aspirin usage and its formulation (enteric-coated or not) were not related with AR (Table 1).

Patients with AR had higher white blood cell (8696/micL vs. 7797/micL; p=0.08) and monocyte (655/micL vs. 571/micL; p=0.08) counts, and a higher mean platelet volume (8.41 fL vs. 8.01 fL p=0.09); but these increases were not statistically significant. There were no differences in red blood cell and platelet counts (Table 1).

DISCUSSION

We found the AR rate as 27% in patients using aspirin for CAD. Gum et al.[6] reported its incidence as low as 9.5% in the USA, while a study from Europe found AR in 32%.[7] These rates are quite high for a drug which plays an important role in the treatment of CAD according to all guidelines, under such a circumstance that an important proportion of patients lack anti-aggregate protection.

The relation between AR and unfavorable outcomes has been shown in previous studies. Gum et al.[8] reported that, during a mean follow-up of 679 days, aspirin resistance was associated with an increased risk for death, myocardial infarction (MI), or cerebrovascular accidents, compared to patients who had sensitivity to aspirin (24% *vs* 10%).

Table 1. Patient characteristics, aspirin usage, and hematologic parameters in the study group

	Aspirin resistance (+) (n=27)				Aspirin resistance (–) (n=73)				
	Mean	Range	n	%	Mean	Range	n	%	*p*
Patient characteristics									
Mean age (years)	57.7±11	30-75			55.4±10	33-75			0.3
Gender Women			10	37.0			18	24.7	0.3
Men			17	63.0			55	75.3	0.3
Smoking			13	48.2			29	39.7	0.4
Hypertension			12	44.4			39	53.4	0.3
Diabetes mellitus			4	14.8			15	20.6	0.5
Hypercholesterolemia			14	51.9			26	35.6	0.1
Family history			9	33.3			34	46.6	0.2
Aspirin usage									
Duration (weeks)	129±189	2-520			80±120	1-676			0.1
Dosage (mg)	197.2±101	80-300			238.6±101	80-300			0.09
Enteric-coated			22	81.5			53	72.6	0.4
Hematologic parameters									
White blood cell (10^3/micL)	8.6±1.9				7.7±2.3				0.08
Neutrophil (10^3/micL)	5.2±1.6				4.8±1.3				0.2
Lymphocyte (10^3/micL)	2.4±0.6				2.4±0.8				0.9
Monocyte (10^3/micL)	0.65±0.2				0.57±0.1				0.08
Eosinophil (10^3/micL)	0.22±0.1				0.21±0.1				0.7
Erythrocyte (10^6/micL)	5.02±0.5				4.81±0.5				0.1
Thrombocyte (10^3/micL)	257.6±56				254.7±80				0.8
Mean platelet volume (fL)	8.4±0.9				8.0±1.0				0.09

In a subgroup analysis of the HOPE study, urine levels of 11-dehydrothromboxane B2 (11-DTXB2), a metabolite of thromboxane A2, were monitored in patients taking aspirin during a follow-up of five years. It was found that high levels of urinary 11-DTXB2 was predictive of MI and cardiovascular death: the upper quartile of the patients had a two-fold risk for MI and 3.5-fold risk for cardiovascular death compared with the lowest quartile.[9]

Aspirin resistance is detected by the PFA-100 according to a determined cut-off value. The concept of semi-responsiveness, which implies a partial response to aspirin, has not been validated. We observed only a slight increase (maximum 25%) in the closure time for C/E in relation to the cut-off value in 17% of our patients. Further studies are needed to clarify the clinical importance of this partial responsiveness.

There are conflicting data about the relationship between AR and age, smoking, or female gender. Some studies point to such a relationship,[6,10,11] while others do not.[6,12] We did not observe any relationship between AR and the patients' characteristics.

Many possible mechanisms have been proposed for the development of AR, which include insufficient dosing,[2] polymorphism of glycoprotein IIIa,[13,14] production of prostaglandin H2 (PGH2) in monocytes and endothelial cells through a cyclooxygenase-2 (COX-2) pathway,[15] increased COX-2 levels as a result of increased platelet degradation,[16] and increased sensitivity of platelets to ADP.[13]

We found an increased sensitivity of platelets to ADP in patients with AR, which had been shown only in one study previously.[17] This suggests that platelet aggregation by ADP, which is not affected by aspirin, may be involved in the occurrence or development of AR. On the other hand, increased white blood cells and monocytes in patients with AR, although not statistically significant, may be associated with the development of AR by the production of prostaglandin H2 (PGH2) in these cells through a cyclooxygenase-2 (COX-2) pathway.

The patients were taking different doses of aspirin (100, 150, or 300 mg) and we did not measure serum levels of acetylsalicylic acid. This parameter was not measured by similar studies, either; moreover, large-scale clinical studies comparing the effect of low or high doses (75, 300, and 1200 mg) of aspirin found no difference in efficacy.[18,19]

We employed the PFA-100 system without the use of a photo-optic aggregometer because a low concordance was reported between the two techniques for the detection of AR.[6] While the aggregometer method uses platelet-rich plasma to evaluate AR and may yield a lower detection rate, the PFA-100 system utilizes whole blood and simulates primary homeostasis, which is a more physiologic approach to evaluate platelet function, making it a more reliable and sensitive method for the detection of AR.

In conclusion, the frequency of AR is quite high when evaluated with a new and sensitive method. Considering the universal use of aspirin for CAD, the development of AR renders it ineffective or less effective in almost one-third of all the patients, which poses an increased risk for this patient population. Moreover, the sensitivity of platelets to ADP is increased in patients with AR.

Authors' notification

The patient group of this study was used and reported as a control group in another publication. (*For reference,* High frequency of aspirin resistance in patients with acute coronary syndrome. Tohoku J Exp Med 2005;207:59-64.)

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