Aspirin resistance frequency in healthy males

Sağlıklı erkeklerde aspirin direnci sıklığı

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Abstract

Introduction: Aspirin has been shown to have variable antiplatelet activity in individuals. Previous studies have estimated that 5% to 45% of the population do not achieve an adequate antiplatelet effect from aspirin and still suffer ischemic events despite therapy.

Aim: In this study, we aimed to evaluate aspirin resistance in healthy males.

Method: We prospectively enrolled 175 healthy males who were ≥19 years old and taking 100 mg of aspirin daily ≥7 consecutive days before study entry. Demographic information and laboratory data were collected. Aspirin resistance was detected by optical platelet aggregometry, a widely accepted method using adenosine diphosphate (ADP) and arachidonic acid (AA).

Result: 28% of the participants were aspirin-resistant, 62% were aspirin-semi-responders and 10% were aspirin-sensitive. The aspirin-resistant individuals were older than the semi-responders ($p<0.01$), and had higher erythrocyte sedimentation rate than the semi-responders ($p<0.01$) and sensitive individuals ($p<0.05$).

Conclusion: Our study demonstrates aspirin resistance to be particularly important given its high frequency in our study population. The biological effects of aspirin, which is widely used as an antithrombotic drug, are not similar in all individuals. *(Turk J Hematol 2008; 25: 83-6)*

Key words: Aspirin resistance, platelet aggregation, healthy males.

Özet

Giriş: Aspirinin herbir bireyde farklı antitrombosit aktivitesi olduğu gösterilmiştir. Önceki çalışmalar aspirinin antitrombosit etkisinin populasyonun %5-45'inde yeterli başarı sağlamadığını göstermiştir ve tedaviye rağmen hala iskemik olaylar görülmektedir.

Amaç: Bu çalışmada sağlıklı erkeklerde aspirin direncinin sıklığını araştırmayı amaçladık.

Yöntem: Prospektif olarak yaşları ≥19 olan 175 sağlıklı erkek çalışmaya alındı ve 100 mg/gün aspirin ≥7 gün çalışma öncesi verildi. Demografik bilgiler ve laboratuvar verileri toplandı. Aspirin direnci yaygın olarak kabul gören bir yöntem olan adenozin difosfat (ADP) ve araşidonik asitin (AA) kullanıldığı optik trombosit aggregometri ile saptandı.

Bulgular: Aspirin direnci %28, aspirin yarıduyarlılığı %62 ve aspirin duyarlılığı %10 olarak saptandı. Aspirin direnci olanlar yarıduyarlı olan bireylerden daha yaşlı idi ($p<0.01$), eritrosit sedimentasyon hızları yarıduyarlı olanlardan ($p<0.01$) ve duyarlı bireylerden ($p<0.01$) daha yüksek idi.

Sonuç: Bizim çalışmamız, araştırma grubumuzda saptadığımız yüksek aspirin direnci sıklığını gözönüne aldığımızda bu klinik durumun önemini göstermiştir. Yaygın olarak kullanılan bir antitrombotik ilaç olan aspirinin biyolojik etkisi tüm bireylerde aynı değildir. *(Turk J Hematol 2008; 25: 83-6)*

Anahtar kelimeler: Aspirin direnci, trombosit agregasyonu, sağlıklı erkekler

Introduction

Aspirin (acetylsalicylic acid) is one of the main therapeutics in the prevention of thromboembolic vascular events. Its efficiency is proven in the prevention of atherothrombotic vascular disease in clinical trials and meta-analyses [1,2]. However, the antiplatelet effect of aspirin is not absolute in all patients, and some patients experience thromboembolic events despite

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aspirin. These patients are clinically referred to as aspirin resistant or aspirin nonresponders. Aspirin resistance may be defined clinically and/or with laboratory methods. Previous studies have estimated that 5% to 45% of the population do not achieve an adequate antiplatelet effect from aspirin and still suffer ischemic events despite therapy (clinical aspirin resistance) [3]. Estimates of the prevalence of laboratory aspirin resistance range from 5% to 61% [4-6]. Aspirin resistance has especially been studied in cardiovascular, cerebrovascular and peripheral vascular diseases, and different results have been gained with different methods [7-10]. However, current clinical guidelines do not support routine screening for aspirin resistance, in part because determination of the most appropriate screening test has not been established and because the mechanisms of aspirin resistance are likely to be multifactorial. As a result, its frequency is currently unknown.

In the current study, we aimed to evaluate aspirin resistance in healthy males, since investigating healthy subjects for aspirin resistance is known to reduce the degree of influence of acquired factors and to help in identifying genetic factors involved in aspirin resistance [11].

Material and Methods

Participants. In this study, we prospectively enrolled 175 healthy males between January 2004 and May 2005. Study subjects were ≥19-years old and had taken 100 mg of aspirin daily ≥7-consecutive days before study entry. All of the participants were investigated according to the presence of cardiac risk factors such as tobacco use, obesity (body mass index ≥30), hyperlipidemia, and family history of coronary artery disease. Blood samples were collected for evaluating complete blood count, erythrocyte sedimentation rate (ESR), fasting glucose and lipids. Exclusion criteria included: ingestion of ticlopidine, dipyridamole, or other nonsteroidal anti-inflammatory drugs; administration of heparin or low-molecular-weight heparin within 24 h before enrollment; major surgical procedure within one week before enrollment; malignant paraproteinemias; family or personal history of bleeding disorders; platelet count $<150x10^3$ / μL or $>450x10^3$ / μL; hemoglobin <8 g/dl; and history of myeloproliferative disorders. The study was approved by the local ethics committee. Informed consent was taken from all participants. Aspirin usage in all participants was confirmed by measuring salicylate levels and also during face to face interview.

Blood samples. Samples for blood counts were drawn into Becton Dickinson anticoagulated tubes and complete counts were made by Beckman Coulter Gen-S SM, USA automated blood counting device. ESR was performed on Vacuette R* Automated ESR Systems, Germany. Fasting plasma glucose and a lipid profile including total cholesterol, high density lipoprotein cholesterol (HDL-C) and triglyceride were measured by spectrophotometric method on Roche Hitachi 911 Chemistry Analyzer, USA. The salicylate serum Tox Assay (Microgenics, Fremont, CA, USA) was used for determination of salicylate level by enzyme immune assay.

Platelet function studies. Venous blood was collected under light tourniquet through 19 gauge needles into Becton Dickinson vacutainers containing 3.2% trisodium citrate in a 9:1 blood anticoagulation ratio. One to 24 h before platelet analysis, the last dose of aspirin was administered. Platelet aggregation studies were performed in whole blood lumi-aggregometer (Chronolog Corporation, Model 560-Ca) using optical method within 1 h. The whole blood specimen was centrifuged for 10 min at 200 g to obtain platelet rich plasma (PRP). Platelet poor plasma (PPP) was obtained on the remaining specimen by recentrifugation at 200 g for 15 min. A platelet count was performed on the PRP and was adjusted to $300x10^3$ / μL with PPP. 450 μL of this PRP were transferred into cuvettes (Chronolog No: P/N 312). After agonist addition, platelet aggregation was measured over 6 min and expressed as a percentage of the maximal amplitude in PRP. The agonist used and their final concentrations were: adenosine diphosphate (ADP) (Chrono Par 384) 5 μM and arachidonic acid (AA) (Chrono Par 390) 0.5 mg/ml. Laboratory normals were established by screening 27 normal male and female subjects.

Definition of aspirin resistance. Aspirin resistance was defined as a mean aggregation of ≥64% with 5 μM ADP and a mean aggregation of ≥20% with 0.5 mg/ml AA. Aspirin semi-responders were defined as meeting one, but not both, of the above criteria. The definition of aspirin resistance was set as ADP-induced platelet aggregation within our laboratory normal range (>64%). AA-induced platelet aggregation is usually completely inhibited by aspirin, therefore aggregation >20% was set as the cutoff defining aspirin resistance.

Statistics

SPSS version 12.0 for Windows was used for statistical analysis. Continuous variables are presented as mean±standard deviation. Categorical variables are presented as frequencies and percentages. Categorical variables were compared using chi-square tests. Parametric tests (one way analysis of variance [ANOVA]) were performed if the data was distributed normally according to Shapiro-Wilk test, while non-parametric tests (Kruskal-Wallis test, Mann-Whitney U test) were used for the abnormally distributed data. Differences with P value below 0.05 were considered significant.

Results

Among subjects, 49 (28%) were aspirin-resistant, 109 (62%) were aspirin semi-responders and 17 (10%) were aspirin-sensitive. Aspirin-resistant individuals were older than the semi-responders ($p<0.01$), and aspirin resistance was found to be higher in the 4th and 5th decades ($p<0.001$). The aspirin-resistant individuals had higher ESR than the semi-responders ($p<0.01$) and sensitive individuals ($p<0.05$). Aspirin-resistant subjects were determined to have higher platelet counts than semi-responders ($p<0.05$). Aspirin-resistant, aspirin semi-responder and aspirin-sensitive subjects had similar rates of other cardiac risk factors (tobacco use, obesity and family history of coronary disease) and laboratory data (hemoglobin, leukocyte counts, fasting glucose, HDL, total cholesterol and triglyceride).

Baseline characteristics of subjects are summarized in Table 1.

Discussion

The present study showed that 28% of healthy males in Eskişehir region are aspirin-resistant. Our ratio is consistent with the previously described findings in the literature [4-6].

Aspirin resistance is indeed clinically important in select populations. In patients with cardiovascular, cerebrovascular and peripheral vascular disease, aspirin resistance has been reported with many different laboratory methods. Gum et al. [7] in a cohort of 325 patients with stable coronary artery disease found that 5.5% and 9.5% of the subjects were aspirin-resistant by optical aggregometry and PFA-100, respectively. Acute stroke patients with elevated platelet reactivity despite aspirin therapy were more likely to experience vascular death, myocardial infarction, or cerebrovascular accident [8]. Patients non-responding to aspirin had an increased incidence of recurrent cerebral ischemic attacks compared with aspirin responders [9]. In patients with claudication, there is an association between failed inhibition of platelet reactivity by aspirin and the risk of reocclusion after peripheral vascular angioplasty [10].

There have not been many studies about aspirin resistance in healthy populations. Gonzalez-Conejero et al. [11] performed a study on 24 healthy subjects. Eight subjects (33.3%) were aspirin nonresponders and 16 subjects (66.7%) were normal responders in their investigation with PFA-100. PFA-100 was determined as unlikely to be useful in screening for aspirin resistance, and a full resistance to aspirin was indicated to be unlikely in healthy subjects. Kawasaki et al. [12] studied bleeding time and collagen-induced platelet aggregation in 8 healthy volunteers and found 37.5% to be aspirin nonresponders. In a study by Buchanan et al. [13], among 10 healthy volunteers whose bleeding time was not prolonged (acetylsalicylic acid nonresponders=4 volunteers), platelet 12-hydroxyeicosatetraenoic acid (12-HETE) synthesis and platelet adhesion were unchanged or increased despite platelet thromboxane A2 (TxA2) and platelet aggregation being inhibited. In another study, aspirin resistance was found to be rare in a healthy population while pseudo-resistance was more frequent [14]. Additionally, aspirin response of healthy adults was found to be widely variable in another study [15]. Our study is a prospective trial investigating the response of aspirin among healthy participants and we showed that 28% of healthy males were aspirin-resistant with optical platelet aggregation.

The mechanisms associated with aspirin resistance are not well known, but some clinical, biological or genetic factors may be responsible. In this study, we found that in healthy males, aspirin resistance was significantly associated with age, platelet count and ESR. Gum et al. [7] in their study showed a trend toward increased age in patients with aspirin resistance. In our study, we found differences in platelet counts between the aspirin-resistant and aspirin semi-responder groups, but there was no difference between the aspirin-resistant and -sensitive groups. In a study by Narvaez et al. [16], patients with aspirin resistance had higher levels of platelet counts ($p<0.05$).

Table 1. Baseline characteristics of subjects

	Aspirin- Resistant (n=49)	Aspirin Semi-responder (n=109)	Aspirin- Sensitive (n=17)	p value
Clinical factors				
Age, mean±SD (yrs)[a]	38±9	32±10	32±11	0.001
Tobacco use (%)	27 (55.1%)	50 (45.9%)	6 (35.3%)	0.322
Obesity (%)	7 (14.3%)	10 (9%)	3 (17.6%)	0.464
Hyperlipidemia (%)	14 (28.6%)	23 (21%)	3 (17.6%)	0.507
Family history of coronary heart disease (%)	8 (16.3%)	17 (15.6%)	5 (29.4%)	0.366
Laboratory values				
Hemoglobin (g/dl)	15.3±0.9	15.6±0.8	15.5±0.7	0.131
Platelet counts ($x10^3$/L)[b]	261±56	240±50	261±70	0.045
Leukocyte counts ($x10^3$/L)	7.9±1.8	7.3±1.8	7.4±1.2	0.224
Sedimentation (mm/h)[c]	6.4±7.4	4.0±4.1	3.4±3.0	0.012
Glucose (mg/dl)	80±12	79±13	83±12	0.546
Total cholesterol (mg/dl)	188±36	177±42	170±44	0.168
HDL cholesterol (mg/dl)	46±10	47±10	49±8	0.674
Triglyceride (mg/dl)	168±106	167±94	166±72	0.238

a. $p<0.01$ aspirin-resistant group versus semi-responder group
b. $p<0.05$ aspirin-resistant group versus semi-responder group
c. $p<0.01$ aspirin-resistant group versus semi-responder group
$p<0.01$ aspirin-resistant group versus semi-responder group

In a study by Gonzalez-Conejero et al. [11], there were no significant differences in platelet counts among normal and non-responders. Similarly, Gum et al. [7] reported no significant differences between the aspirin-resistant and non-aspirin-resistant groups. The risk of coronary heart disease increases markedly with age [17]. Growing evidence also indicates that platelets act as prominent players in the inflammatory component of the coronary heart disease process and that biomarkers such as ESR and C-reactive protein may have clinical utility for identification of patients at high risk of atherothrombosis [18]. Since we have demonstrated an association between aspirin resistance and age, platelet count, and ESR, which all give information on the risk of coronary heart disease events, we suggest that laboratory aspirin resistance predicts cardiovascular risk and that functional aggregometry identifies at-risk individuals.

The present study demonstrated that aspirin resistance is a real and important problem. The biological effects of aspirin, which is used widely as an antithrombotic drug, are not similar in all individuals. According to our results, if laboratory aspirin resistance can be diagnosed in individuals before an atherothrombotic event occurs, this will potentially allow patients to receive more effective antiplatelet treatment options when they are affected in the future.

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