Assessment of efficacy of single dose acetylsalicylic acid over a 24-hour period

Günde tek doz verilen asetilsalisilik asidin 24 saat içindeki etkinliğini değerlendirme

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ABSTRACT

Objective: Acetylsalicylic acid (ASA) has a half-life of less than 30 minutes in the human body. This study aimed to test whether the effects of a single dose of ASA wane over a 24-hour period due to the daily release of new reactive blood platelets into the bloodstream.

Methods: The study included 30 patients (10 female and 20 male, mean age: 62.8±9.0). Each took a single dose of 300 mg enteric coated ASA orally. Platelet aggregation was determined using VerifyNow® Aspirin kits immediately prior to intake, and at 12 and 24 hours following intake. Laboratory parameters such as serum CRP and CBC were also examined before ASA intake. Patients were included irrespective of routine ASA and/or clopidogrel use.

Results: Aspirin reaction unit (ARU) values were lower than 550 at 24 hours after drug intake in 26 (86.7%) patients. Values lower than 550 indicate therapeutic range of ASA on platelet function. Two (6.7%) patients were found to be responsive to ASA at 12 hours after intake, but unresponsive at 24 hours. Aspirin resistance was found in another 2 (6.7%) patients.

Conclusion: Although ASA was found to be effective on platelet inhibition over a 24-hour period in most of the patients, there was a considerable number who were resistant to ASA, and who had developed unresponsiveness to ASA by the end of 24 hours. There is evidence in the literature regarding the clinical importance of ASA resistance, but the importance of loss ASA's effectiveness during a day warrants further studies.

ÖZET

Amaç: Vücuttaki yarılanma ömrü 30 dakikadan kısa olan asetilsalisilik asidin (ASA) etkinliğinin, her gün dolaşıma salıverilen yeni ve daha reaktif trombositler nedeniyle, gün boyunca devam edip etmediğini araştırma amacıyla çalışmamızı planladık.

Yöntemler: Çalışmaya toplam 30 hasta (10 kadın, 20 erkek, ortalama yaş: 62.8±9.0) alındı. Tüm hastalara ağız yoluyla tek doz 300 mg enterik kaplı ASA verildi. ASA verilmeden hemen önce, 12 ve 24 saat sonra trombosit agregasyon düzeyleri VerifyNow® Aspirin kitleri ile değerlendirildi. ASA verilmeden önce alınan kan örneklerinden kreatinin, lipit profili, tam kan sayımı ve CRP gibi laboratuvar parametreleri de ölçüldü. Hastalar rutinde ASA kullanımı ve/veya klopidogrel kullanımına bakılmaksızın çalışmaya alındı.

Bulgular: Hastaların 26'sında (%86.7) 24. saatte Aspirin reaksiyon birimi (ARU) değerleri 550'nin altında saptandı. ARU değerinin 550'nin altında olması ASA'nın etkin olduğu anlamına gelmektedir. İki (%6.7) hastada alım sonrası 12.saatte ASA'ya yanıt tespit edilmesine rağmen, 24. saatte yanıtın olmadığı gözlendi. İki (%6.7) hastada ise ASA direnci saptandı.

Sonuç: Hastaların çoğunda ASA'nın antiagregan etkisi 24 saatlik periyotta devam etmesine rağmen azımsanmayacak oranda hastada ise ASA etki etmemekte veya ASA'nın gün içinde etkinliğinde azalma olabilmektedir. ASA direncinin klinik sonuçları nispeten daha iyi bilinmesine rağmen, gün içinde etkinliğinin azalmasının klinik öneminin araştırılması gerekmektedir.

Acetylsalicylic acid (ASA) was discovered in late 1800s. Apart from its analgesic, antipyretic and anti-inflammatory effects, it is also a cardioprotective drug because of its antiplatelet effect. Numerous studies have made use of *ex vivo* platelet aggregation tests to detect ASA response, and have found it to have interindividual variability.[1–3] ASA 'resistance' is defined as lower platelet inhibition than expected

Received: May 25, 2013 *Accepted:* January 22, 2015

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in ASA-treated patients, a condition shown to be associated with adverse cardiac events, especially after percutaneous coronary interventions.[4,5] One possible mechanism of ASA resistance is platelet turnover, which correlates well with the amount of new platelets being formed in bone marrow and released into the bloodstream. The half-life of ASA in the human body is less than 30 minutes.[6,7] Studies including healthy people have shown that for effective platelet inhibition, ASA must inhibit more than 90% of platelet thromboxane synthesis.[7,8] The amount of new platelets formed daily in bone marrow is about 10–15% of the total platelet pool.[9] This percentage of new platelets increases with syndromes associated with arterial thrombosis such as acute coronary syndromes, in coronary artery disease patients, and in the presence of risk factors such as diabetes and smoking.[10,11] Thus, it is possible to state that when given daily as a single dose, ASA may not be able to inhibit platelet function effectively by the end of the 24-hour period.

This study examined changes in effectiveness in a single dose of 300 mg ASA during a day measured by the VerifyNow® device in a 30-patient population.

METHODS

Study design and study population

A total of 30 patients admitted to our cardiology clinic between 13th June and 1st July 2011 were included in the study. All patients signed the informed consent form before enrollment. Exclusion criteria were; age under 18, ASA allergy, ASA intolerance, any contraindication for ASA use, myocardial infarction within the previous month, elevated cardiac markers such as troponin I and/or CK-MB (creatine phosphokinase myocardial band), nonsteroidal anti-inflammatory drug intake within 48 hours, platelet count <100.000/µL, hematocrit levels >%56 or <%29, known congenital or acquired platelet dysfunction disease, and refusal to sign informed consent. Patients using GP IIb/IIIa antagonists and those who were on cilostazol treatment were also excluded due to possible interference with test results. Patients were included irrespective of their clopidogrel use. Demographic, clinical and laboratory data were recorded. Fasting blood glucose, serum creatinine and CRP levels, whole blood count and lipid profile were also tested from blood samples collected before drug intake.

Abbreviations:

ARU	*Aspirin reaction unit*
ASA	*Acetylsalicylic acid*
BMI	*Body mass index*
CK-MB	*Creatine phosphokinase myocardial band*

Patients on ASA treatment before enrollment were given 300 mg enteric coated ASA orally on an empty stomach or with food between 08:00–14:00, with a lapse of at least 24 hours since the previous dose. Patients not on ASA treatment were also given 300 mg enteric coated ASA orally on an empty stomach or with food between 08:00–14:00. Three separate blood samples were collected from all patients immediately prior to drug intake, and at 12 hours and 24 hours from initial drug intake. Platelet aggregation levels were assessed.

Laboratory

Blood samples were collected from the antecubital vein using 2 ml Vacutainer tubes with a 3.2% sodium citrate solution (Vacuette, Grainer Bio-One, Monroe, NC) in accordance with the VerifyNow® instruction manual. Samples were tested using the VerifyNow® device Aspirin kits a minimum of 30 minutes and a maximum of 4 hours from sample collection. Threshold level of ARU was accepted as 550, meaning values under 550 indicated effective platelet aggregation inhibition, while values over 550 indicated ineffective platelet aggregation inhibition.

Statistical analysis

Statistical analysis was performed using the NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) program. Apart from descriptive statistical methods (mean and standard deviation), we used the Friedman test for comparison of repeated measures of multiple groups, the Kruskal Wallis for comparison of groups, Dunn's multiple comparison test for comparison of subgroups, the Mann-Whitney-U test for comparison of independent groups and chi-squared test for comparison of quantitative data. A value of $p<0.05$ was considered statistically significant.

RESULTS

Patient mean age was 62.8±9.0. Twenty (67%) patients were male and 10 (33%) female. Patients' demographic and laboratory data are shown in Tables 1 and 2.

Table 1. Demographic characteristics of patients

	n	%	Mean±SD
Age			62.8±9.04
Gender			
Male	20	67	
Female	10	33	
Body mass index			30.1±6.13
Hypertension	24	80	
Type 2 Diabetes	16	53	
Hyperlipidemia	23	77	
Smoking			
Active	4	13	
Ex	16	53	
Family history	13	43	
Coronary artery by-pass grafting history	6	20	
Percutaneous coronary intervention history	10	33	
ACEI / Angiotensin receptor blocker	21	70	
Calcium channel blocker	9	20	
Statin	24	80	
Proton pump inhibitor	10	33	
Diuretic	10	33	
Beta blocker	18	60	
Stable coronary artery disease	10	33	
Percutaneous coronary intervention	10	33	
Primary prophylaxis	10	33	

ACEI: Angiotensin converting enzyme inhibitor.

Time intervals between consecutive ARU measurements are shown in Table 3. All ARU values are presented in Figure 1. In 26 (86.7%) patients, a single dose of 300 mg ASA was found to be effective (ARU <550) even after 24 hours (Table 4). Frequency of routine ASA and clopidogrel use among patients is shown in Table 5.

As expected, first ARU values were significantly higher than those derived from second and third measurements in patients who were not on routine ASA treatment, while first ARU values were significantly lower in patients on routine ASA use (Figure 2).

Routine clopidogrel use and clopidogrel loading were not found to affect ARU values significantly (Figure 3). In 10 (33%) patients, there was increase in ARU values between second and third measurements, which meant reduced ASA efficacy towards the end of the 24 hours. In a comparison of demographic and laboratory data between lower ASA efficacy patients and higher ASA efficacy patients, only body weight was

Table 2. Laboratory parameters of patients

	Mean±SD
Fasting blood glucose (mg/dl)	103.3±28.49
Creatinine (mg/dl)	1.05±0.70
Total cholesterol (mg/dl)	166.4±42.84
Low density lipoprotein (mg/dl)	101.3±33.53
High density lipoprotein (mg/dl)	37.7±8.11
Triglyceride (mg/dl)	145.3±90.58
White blood cell (x10^9/l)	8.1±2.50
Hemoglobin (gr/dl)	13.7±1.96
Platelet count (x10^9/l)	247.7±89.42
Mean platelet volume (fl)	8.5±1.03
C-reactive protein (mg/l)	7.7±10.24

Values are given as mean±standard deviation.

Table 3. Time intervals between ARU measurements

	Time (hr, min)
1.–2. measurements	10 hr 50 min ±1 hr 20 min
1.–3. measurements	22 hr 52 min ±3 hr 56 min

significantly different. Body weight was found significantly lower in patients with lower efficacy (Figure 4). When patients were divided into 3 subgroups as primary prophylaxis (for indication of ASA), stable coronary heart disease and percutaneous coronary intervention, ARU values were found to be similar among the subgroups (Table 6). ASA resistance was found in 2 (6.7%) patients (all 3 measurements revealed ARU values >550) (Table 4). Moreover, loss of efficacy of ASA (ARU >550) was found in 2 (6.7%) patients, with loss defined as response to ASA at first and/or second measurements (ARU <550), but loss of response at the third measurement (Table 4).

DISCUSSION

This pilot study tested whether efficacy of a single dose of ASA wanes over a 24-hour period. In 26 (86.7%) patients, a single dose of ASA was found to be effective throughout the 24 hours. Two patients in the study developed unresponsiveness to ASA at the end of the day, and in one of these patients stent restenosis was detected. It was thought that stent restenosis in this patient may have been caused by the patient's unresponsiveness to ASA. In a study undertaken in 2005, ASA resistance was significantly more common in patients with intracoronary stent restenosis compared to patients without restenosis (31.3% and 10.7%, p<0.001).[12] Regarding the 2 patients who developed unresponsiveness to ASA in the present study, we could not define any specific characteristics to differentiate these patients from the others. The clinical aspect of development of ASA unresponsiveness is not known and

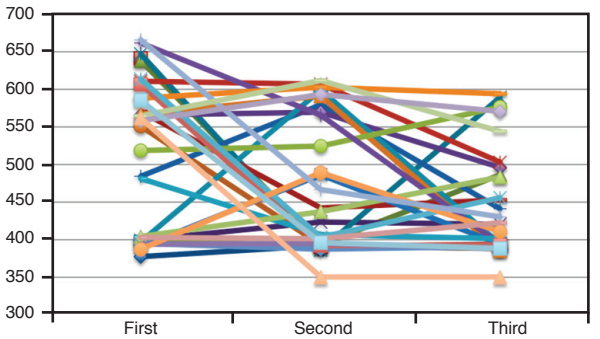

Figure 1. ARU values of patients (ARU, Aspirin Reaction Units).

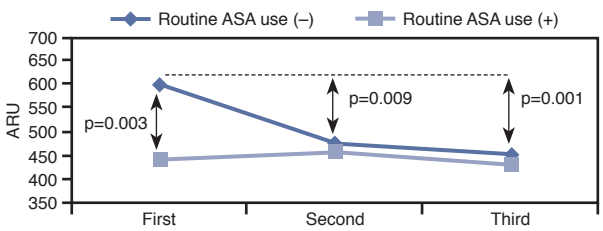

Figure 2. Effect of routine ASA use on ARU values (ARU, Aspirin Reaction Units; ASA, acetylsalicylic acid).

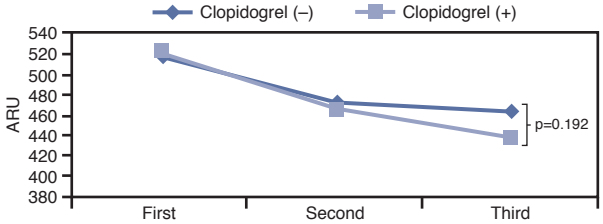

Figure 3. Effect of routine clopidogrel use on ARU values (ARU, Aspirin Reaction Units).

further studies are warranted. In such patients, ASA treatment given in divided doses may be an alternative treatment strategy. There was ASA resistance in 2 (6.7%) patients, a percentage that is consistent with the literature. Frequency of ASA resistance was found to vary between 0.4% and 83.3%, depending on the

Table 4. Acetylsalicylic acid response among patients

	Acetylsalicylic acid effective		Acetylsalicylic acid resistance		Loss of efficacy of acetylsalicylic acid	
	n	%	n	%	n	%
No	26	86.7	2	6.7	2	6.7

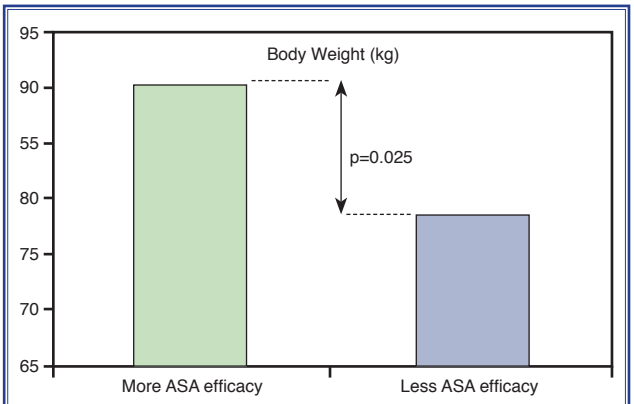

Figure 4. Comparison of body weight between patients who have more and less ASA efficacy (ASA, acetylsalicylic acid).

Table 5. Clopidogrel and acetylsalicylic acid use among patients

	n	%
Clopidogrel		
Loading	15	50
Routine clopidogrel		
Use	10	33
Routine acetylsalicylic acid		
Use	15	50

method used to measure platelet function, definition of resistance, and characteristics of patient populations involved in studies.[13] ASA resistance is known to be associated with adverse cardiac events, especially after percutaneous coronary interventions.[4,5]

In another study including 48 diabetic patients, unresponsiveness to ASA was more common in patients whose body mass index (BMI) was 30 or above.[14] In our study, in patients with reduced ASA efficacy (increase in ARU values between second and third measurements), body weight and BMI were found to be significantly and insignificantly lower respectively. These findings are inconsistent with the literature and may be associated with the small sample size or simply chance.

In a study on the impact of platelet turnover on ASA's antiplatelet effect in stable CAD patients, there was lower ASA efficacy in patients with increased platelet turnover.[11] Apart from this finding, platelet turnover was found to be more common in diabetic patients. However, the present study did not find any significant difference between diabetic and nondiabetic patients regarding ASA response. In another study of healthy volunteers, a significant relationship was found between platelet turnover and both decreased ASA antiplatelet effect and ASA resistance.[15]

In another study on efficacy of ASA during a day that included 15 healthy male volunteers, the participants were administered 37.5 mg ASA for 10 days, followed by 320 mg ASA for 7 days. On the 7th day, 2 hours after the last dose, an additional 640 mg ASA was given. At 24 hours after the final ASA dose, platelet aggregation levels were found to be near basal in both low-dose (37.5 mg) and intermediate/high-dose (320 mg, cumulative dose 960 mg) groups.[8] In that study, impedance aggregometry was used to evaluate platelet aggregation. Possible causes of different results found in our study may be differences in study populations and the methods used for platelet aggregation.

The gold standard method for assessment of platelet aggregation is light transmission aggregometry (LTA). However, because of some difficulties regarding this method (e.g. sample preparation, experienced personnel, delayed result), new and more practical devices are being developed. In our study we used the VerifyNow® device because of its ease to use, practicality and reliability.

Table 6. Comparison of three ARU measurements between patient subgroups

	Primary prophylaxis (n=10)	Stable CAD (n=10)	PCI (n=10)	*p*
	Mean±SD	Mean±SD	Mean±SD	
1. measurement	536±103	527±106	500±100	0.368
2. measurement	469±95	458±90	479±91	0.763
3. measurement	446±71	460±63	425±79	0.186

CAD: Coronary artery disease; PCI: Percutaneous coronary intervention.

Concomitant use of clopidogrel did not result in significant changes in ARU values, a finding that is consistent with the literature. In 2006, a study was done with 96 healthy volunteers which showed that concomitant clopidogrel use did not affect VerifyNow® Aspirin test results.[16]

The main limitation of our study was its small sample size. It would be preferable if patients on routine ASA use were selected from among patients on similar doses of ASA in order to prevent interaction with ARU values. Acute coronary syndrome patients, known to have high platelet turnover, could have been included in our study, but because of frequent use of GP IIb/IIIa antagonists by these patients, they were excluded. Ten patients underwent PCI, and intravenous heparin was used during these procedures. Heparin has been reported to increase platelet aggregation via arachidonic acid pathway in a study.[17] Thus, our ARU values in those patients may not reflect true values.

In order to reach more precise results, the use of platelet turnover markers would be preferable. Because of difficulties in collecting blood samples for the second measurement, some were collected with a 4-hour delay, and this might have influenced ARU values derived from second measurements.

Conclusion

In our study, a single dose of 300 mg enteric-coated ASA given orally was found to be effective even after 24 hours after drug intake in most of the patients. In 2 (6.7%) patients, ASA resistance was detected. A further 2 (6.7%) patients developed unresponsiveness to ASA at the end of the 24 hours. The clinical importance of ASA unresponsiveness is not known, and treatment in divided doses may be an alternative treatment strategy in such patients. Despite the small sample size, ours is a pioneer study on ASA effectiveness, and larger, randomized double-blind studies are warranted to test its results.

Conflict-of-interest issues regarding the authorship or article: None declared

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Key words: Aspirin; clopidogrel; platelets; platelet aggregation.

Anahtar sözcükler: Aspirin; klopidogrel; trombosit; trombosit agregasyonu.