PLATELET FUNCTIONS DURING SECONDARY PREVENTION WITH ASPIRIN OR TICLOPIDINE: A PRELIMINARY STUDY

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SUMMARY

Different subjects require different aspirin dosages to achieve complete inhibition of platelet functions and the antiplatelet effect of a fixed dose aspirin is not constant over time in every patient. Some patients develop a progressively increasing dosage requirement, which is called "aspirin resistance". The debate still goes on for a similar effect of ticlopidine. The purpose of this study was to compare the effects of aspirin and ticlopidine on platelet functions over time.

Ten patients (6 males, 4 females, ages between 42 and 79) with a history of minor stroke or transient ischemic attack were included in the study. Five patients were on aspirin and five were on ticlopidine treatment. Maximum intensity and maximum rate of platelet aggregation (MIPA and MRPA) and ATP release (all with collagen and ADP) were evaluated before the initiation of aspirin or ticlopidine and during treatment on the 40^{th} and 90^{th} days. The platelet aggregation and ATP release values were found significantly reduced on the 40^{th} day in both groups ($p<0.01$). The increase in MIPA and MRPA between the 40^{th} and 90^{th} days was found significant in aspirin group (66.4%) than in the ticlopidine group (5.7%) ($p<0.01$).

The results show that the resistance detected previously in patients on aspirin is not the case for patients on ticlopidine in the study period. Our data suggest that the antiaggregant efficacy of aspirin is not stable over time and may show decrease in a short period. We believe, therefore, periodical monitoring of platelet functions may be valuable in patients on aspirin prophylaxis and ticlopidine may be a good choise in patients, whose platelet aggregation tests cannot be evaluated properly.

Key words: Stroke, platelet aggregation, resistance, aspirin, ticlopidine

ASPİRİN VEYA TİKLOPİDİN İLE SEKONDER PROFİLAKSİ SIRASINDA TROMBOSİT FONKSİYONLARI; BİR ÖN ÇALIŞMA

Her bireyin trombosit fonksiyonlarının inhibisyonu için gerekli olan aspirin dozu farklıdır ve aynı dozda aspirin her hastada sürekli aynı antitrombotik etkiyi göstermez. Bazı hastalarda giderek artan aspirin dozuna gereksinim olur ve bu durum aspirin direnci olarak isimlendirilmektedir. Benzer bir gereksinimin tiklopidin için var olup olmadığı tartışmalı bir konudur. Bu çalışmanın amacı, aspirin ve tiklopidinin zaman içinde trombosit fonksiyonları üzerine olan etkisini araştırmaktır.

Çalışmaya, minör strok veya geçici iskemik atak öyküsü olan 10 hasta (6'sı erkek, 4'ü kadın, yaşları 42 ve 79 arasında olan) alınmıştır. Beş hastaya aspirin, beş hastaya ise tiklopidin başlanmıştır. Aspirin veya tiklopidin tedavisi başlanmadan önce ve başlandıktan sonra 40 ve 90. günlerde maksimum trombosit agregasyon hızları (MRPA) ve şiddeti (MIPA) ile ATP salınımı (herbiri kollajen ve ADP ile olmak üzere) değerlendirilmiştir. Trombosit agregasyonları ve ATP salınımı 40. günde tedavi öncesine göre her iki tedavi grubunda da anlamlı olarak azalmıştır ($p<0.01$). Kırkıncı ve 90. günler arasında MIPA ve MRPA değerlerindeki artış aspirin grubunda (%66.4), tiklopidin grubundan (%5.7) anlamlı olarak fazla bulunmuştur ($p<0.01$).

Bu sonuçlar aspirin alan hastalarda gösterilmiş olan ilaç direncinin tiklopidin alan hastalarda çalışma süresince gelişmediğini göstermiştir. Bu çalışma, aspirinin antiagregan etkisinin stabil olmadığını ve kısa süre içinde azaldığını göstermektedir. Biz, bu nedenle, aspirin ile profilaktik tedavi alan hastalarda trombosit fonksiyonlarının aralıklı olarak kontrol edilmesi gerektiğine ve bu kontrollerin yapılamadığı hastalarda tiklopidinin iyi bir seçenek olduğuna inanıyoruz.

Anahtar Sözcükler: İnme, trombosit agregasyonu, direnç, aspirin, tiklopidin

INTRODUCTION

Acetylsalicylic acid (ASA) is the most commonly prescribed medication for the secondary prevention of ischemic stroke. However, regular ASA intake does not prevent stroke recurrence in every patient with arterial disease. Previous studies have shown that different subjects require different ASA dosages to achieve sufficient inhibition of platelet functions and the antiplatelet effect of a fixed dose ASA is not constant overtime in every patient. Some patients develop a progressive increasing dosage requirement which is called "aspirin resistance" (1,2). Moreover, it has been known that some individuals are unresponsive to ASA treatment and do not benefit from the administration of

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ASA for the prevention of vascular accidents (3). Previous reports have shown that these individuals are more prone to develop myocardial infarction, stroke and vascular death (4). In patients who can not tolerate ASA or experience stroke on ASA treatment, the most preferred antiaggregant agents are ticlopidine and clopidogrel. Ticlopidine unresponsiveness or resistance has not been reported before and we could identify only one study comparing the effects of ASA and ticlopidine on platelet aggregation (5)

The purpose of this study was to compare the effects of ASA and ticlopidine on platelet functions over time.

MATERIALS AND METHODS

The effects of ASA (300 mg/d) and ticlopidine (500 mg/d) on platelet aggregation in patients with minor stroke or transient ischemic attacks (TIA) were evaluated on the 40^{th} and 90^{th} days of treatment and compared with the baseline values. Ten patients (6 males and 4 females, between the ages of 42 to 79 years) with minor stroke or TIA were included in the study. Five patients (three males, two females, mean age: 62.2) were on ASA (300 mg/d) and five patients (three males, two females, mean age: 59) were on ticlopidine (500 mg/d) treatment. The exclusion criteria were myocardial infarction or surgery in the last three months, presence of atrial fibrilation, cardiac valve diseases, haematologic disorders, platelet counts under 100.000/m3, history of spontaneous bleeding, and known hypersensitivity and gastrointestinal intolerance to antiaggregants. The patients on drugs which can effect platelet aggregation prior to the study were not eligible.

Maximum intensity and maximum rate of platelet aggregation (MIPA and MRPA), ATP release (all with collagen and ADP), and platelet counts were evaluated in both ASA and ticlopidine groups before and on the 40^{th} and 90^{th} days of treatment. Prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen values were measured before the treatment in all patients.

Because the results may depend on the collection technique of blood samples, all procedures were performed by the same pyhsician who was unaware of patients' treatment. Antecubital veins were used for the collection of blood samples. Blood samples were collected into a syringe containing 1/10 volume of 3.8% sodium citrate solution. Platelet aggregation tests were done with the impedance technique and platelet ATP release tests were done with bioluminescence technique in whole blood by using a whole blood aggregometer in a two channel system (Chronolog Corp., Havertown Model 560 WB). Aggregometry was performed at 37°C in 1 ml polystrene cuvettes with magnetic stirring of the platelets in aliquots of whole blood. Platelets were seperately activated both with collagen (Chrono-Par Collagen Reagent 385) making a final concentration of 2 µg/ml and with ADP (Chrono-Par ADP Reagent 384) making a final concentration of 10 µM. Maximum changes in impedance were named "maximum intensity of platelet aggregation" and maximum slope of the aggregation curve as "maximum rate of platelet aggregation". ATP release was evaluated by the comparison of the traces achieved by previously known ATP solutions (Chrono-Lume 387) with the traces of collagen and ADP-induced platelet samples. ATP release tests were made by adding luciferine-luciferase reagent (Chrono-Lume 395) in every sample so that ATP in the environment and luciferine could react and luminescence was evaluated. The patients were not allowed to use any agent which could influence platelet aggregation during the study period.

MIPA, MRPA and ATP release values obtained before treatment and on the 40^{th} and 90^{th} days of treatment were compared within each other. The values of each sampling day were compared between the two treatment groups. Additionaly, the percent changes in values between the baseline and 40^{th} days, and between the 40^{th} and 90^{th} days in ASA and ticlopidine groups were also compared. Wilcoxon rank order test and Mann Whitney U test were used for statistical analysis.

RESULTS

The platelet counts in all patients before the treatment and on the 40^{th} and 90^{th} days of treatment were within the normal range. No changes in platelet counts were observed in either group during the treatment period. The fibrinogen, PT and aPTT values were also normal in all patients before the treatment. There was no apparent difference between the vascular risk factors (such as hypertension, diabetes and smoking) and stroke subtypes of two groups.

The platelet aggregation (MIPA and MRPA)

and ATP release values (both with collagen and ADP) were found significantly reduced on the 40th day in both ASA and ticlopidine groups (All p values were <0.01 except for the inhibiton of ATP release with collagen in ticlopidine group which was found <0.05). The decrease in MIPA and MRPA between the baseline and 40th days were found higher in ASA group (55.3%) than in ticlopidine group (41.4%), but did not show a statictical significance.

Neither ticlopidine nor ASA group showed a significant difference between the platelet aggregation and ATP release values between the baseline and 90th days and between the 40th and 90th days. We observed a rebound increase of MIPA and MRPA values on the 90th day in both groups. The increase in MIPA, MRPA and ATP release between the 40th and 90th days were significantly higher in ASA group (52.9%) than in ticlopidine group (9.5%) (p<0.01) (tables 1,2 and figures 1,2).

Table 1: Platelet aggregation and ATP release values in patients on aspirin

	Baseline	40th day	90th day
MIPA with collagen (ohm)	41.0±3.37	13.3±1.98	24.4±1.38
MIPA with ADP (ohm)	23.0±4.06	9.9±3.30	17.0±3.58
MRPA with collagen (ohm/m)	18.0±4.69	8.8±3.80	13.8±5.03
MRPA with ADP (ohm/m)	10.0±2.89	5.4±1.91	7.5±2.62
ATP release with collagen (nmol)	1.5±0.08	0.78±0.21	1.0±0.20
ATP release with ADP (nmol)	0.96±0.21	0.52±0.13	0.72±0.22

MIPA: Maximum intensity of platelet aggregation, MRPA: Maximum rate of platelet aggregation

Table 2: Platelet aggregation and ATP release values in patients on ticlopidine

	Baseline	40th day	90th day
MIPA with collagen (ohm)	43.6±1.85	28.7±2.58	29.6±2.21
MIPA with ADP (ohm)	22.6±1.91	12.4±2.21	13.6±2.35
MRPA with collagen (ohm/m)	22.7±2.41	13.4±3.20	14.3±2.72
MRPA with ADP (ohm/m)	15.7±2.38	8.5±0.79	9.6±0.89
ATP release with collagen (nmol)	1.7±0.18	0.98±0.08	1.04±0.08
ATP release with ADP (nmol)	0.98±0.04	0.54±0.08	0.64±0.08

MIPA: Maximum intensity of platelet aggregation, MRPA: Maximum rate of platelet aggregation

Figure 1: MIPA values with collagen in patients on ASA and ticlopidine treatment

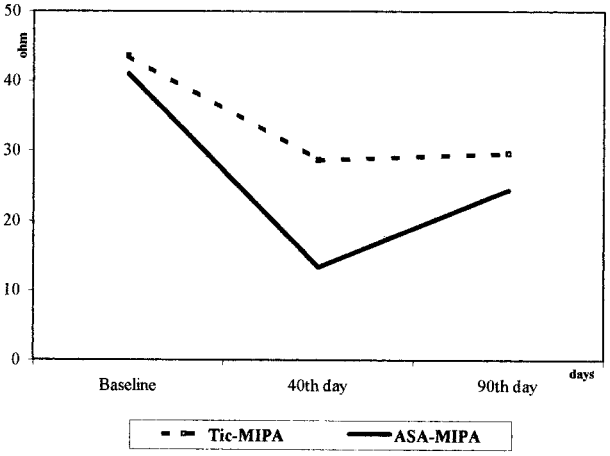
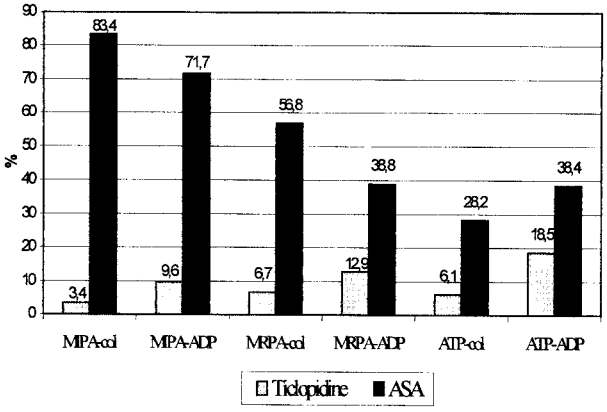


Figure 2: Percent changes of values between the 40th and 90th days in ASA and ticlopidine groups



DISCUSSION

We found that both ASA and ticlopidine have inhibitory effects on the platelet aggregation on the 40th day. The inhibition with ASA was more profound than with ticlopidine, although the difference between the groups was not statistically significant. Our results are compatible with previous reports indicating that both agents inhibit platelet aggregation response to collagen and ADP (6). The inhibiton of ATP release with collagen in patients on ASA was more pronounced than in patients on ticlopidine. Similar results for ticlopidine were obtained previously (7,8).

We observed an obvious decrease in the inhibition of platelet aggregation on 90th day in patients on ASA treatment. A similar rebound effect has not been observed in patients on

ticlopidine treatment, which is probably the result of the maintained activitiy of ticlopidine on platelet aggregation. In accordance with this finding, it has been reported that ADP-induced platelet aggregation was still significantly inhibited following 6 months of treatment with ticlopidine (9,10).

We identified only one study, which compares the effects of ASA and ticlopidine on platelet aggregation over time (5). However, the results of this study are quite different from ours. Akyüz et al. have reported that the platelet aggragation ratios showed a lower antiaggregant efficacy of ASA on the 10^{th} and 90^{th} days of treatment compared with ticlopidine. They have also reported that the inhibitory effect on platelet aggregation increased gradually in both ASA and ticlopidine groups during the ninety-day period of the study. In contrary to our results, they did not observe a rebound increase of platelet aggregability in patients on ASA on 90^{th} day. We think that the contradictory results stem from methodological differences between the two studies. Akyüz et al. used platelet aggregation ratio (PAR) for the assesment of platelet function, but we evaluated three diffrerent parameters (MIPA, MRPA and ATP release, all with collagen and ADP) which are more sensitive compared with PAR. Besides, PAR measurements are performed in platelet-rich plasma which is obtained from centrifuged whole blood. Thus, the platelets are separated from their natural environment and may be injured due to centrifuge. We performed platelet aggregation tests in whole blood, a procedure which is expected to give more reliable results. Another methodological difference is the sampling times of the blood specimens. Akyüz et al. obtained specimens on the 10^{th} and 90^{th} days, while we obtained them on the 40^{th} and 90^{th} days.

Stroke, myocardial infarction and vascular death were reported much more frequent in ASA non-responders than in ASA responders (4). Thus, it is important to determine the patients who are unresponsive to ASA or develop ASA resistance.

According to the results of this preliminary study, it seems reasonable to evaluate platelet functions in patients on ASA treatment periodically, and prefer thienopyridine derivatives in patients under high risk for stroke. However, due to small number of subjects no sound conclusion can be drawn presently and a larger study comparing the effects of ASA, thienopyridines and their combination on platelet functions should be performed.

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