Effects of Aspirin on Serum Total Antioxidant Activity in A Short Term Period

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ABSTRACT

Objective: Aspirin is generally used in the prevention of thrombotic occlusive events such as coronary heart disease and stroke. Underlying mechanism of action is that aspirin inhibits platelets by irreversibly inactivating cyclooxygenase-1, thereby blocking the generation of thromboxane A_2 which is a potent vasoconstrictor and platelet agonist. Aspirin may also help to decrease the progression of atherosclerosis by its antioxidant effect in addition to its inhibiting effect on the coagulation system. The aim of this study was to examine the antioxidant effect of low-dose aspirin supplementation in a short term period.
Methods: Ten healthy volunteers were enrolled in the study. Low-dose aspirin (300 mg. daily for 10 days) was given orally to subjects. Serum specimens were taken after 12-14 hr fasting as baseline and at 4^{th} hr and 10^{th} day of the oral aspirin supplementation. Serum total antioxidant activity (AOA), ferritin, iron, total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides were measured.
Results: Serum AOA at 4^{th} hr were found significantly higher in comparison to baseline levels (p=0.006), but no significant difference was determined on the 10^{th} day. There were no significant differences among baseline, 4^{th} hr and 10^{th} day values for the remaining parameters.
Conclusion: Our results suggest that low-dose aspirin supplementation rapidly increases total antioxidant activity. Aspirin may play a role in improving the general antioxidative potency of the body.

Keywords: Aspirin, ferritin, total antioxidant activity

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INTRODUCTION

Acetylsalicylic acid, known as aspirin, was introduced in the late 1890s. Despite its well-known analgesic and antipyretic effects, the antiplatelet activity of this agent was recognized almost 70 years later. Aspirin is known to reduce the incidence of thrombotic occlusive events such as myocardial infarction and stroke. Low dose aspirin is frequently prescribed for primary prevention to reduce the risk of cadiovascular disease. The benefits of low dose aspirin therapy are well established and a recent meta-analysis of more than 50,000 women and 40,000 men taking part in six randomized trials indicated that low dose aspirin usage is associated with a significant reduction in cardiovascular events in both women and men.[1] This effect is considered to be due to the platelet inhibitory action of aspirin, which results from irreversible inhibition of platelet cyclooxygenase activity and thromboxane A_2 formation. Thromboxane A_2 is a potent agonist and mediator of vascular smooth muscle contraction and platelet aggregation. However, recently aspirin has been shown to have free radical scavenging and antioxidant properties.[2-4] It is reported that aspirin protects LDL from oxidative modification,[5] endothelial cells of the vascular wall from damage caused by oxygen radicals,[6-9] and also prevents proteins from oxidation by acetylation of the amino groups of lysine residues or scavenging hydroxyl radicals.[10]

Tuomainen et al,[11] have shown that subjects with depleted levels of iron also have a lowered risk of atherosclerosis and myocardial infarction. Furthermore, Oberle et al,[7] demonstrated that aspirin at therapeutically relevant concentrations is capable of activating synthesis of ferritin in bovine pulmonary artery endothelial cells. They

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concluded that since ferritin is an iron-binding protein, in addition to providing a reserve source, it may also play an important role during oxidative stress by preventing iron-mediated formation of oxygen radicals. Therefore, aspirin could be beneficial by increasing ferritin synthesis. The measurement of AOA is a global indicator of oxidative stress, thus providing an integrated parameter rather than the simple sum of measurable antioxidants. The activity of known and unknown antioxidants and their synergistic interaction is therefore assessed, thus giving an insight into the delicate balance in vivo between oxidants and antioxidants.[12]

The beneficial effects of aspirin in reducing acute coronary and cerebrovascular events such as unstable angina, myocardial infarction, sudden cardiac death, and stroke have been attributed largely to its antiplatelet action and effects on thromboxane. Whether aspirin has a more profound action, particularly as an antioxidant, is not clear. In this study, we investigated the effects of low-dose aspirin supplementation on serum AOA levels and some other biochemical parameters in acute phase and long term interval in healthy individuals.

MATERIALS AND METHODS

Ten healthy adults (male/female: 6/4:, aged 31 ± 5 (25-40 y) were initially screened to rule out any systemic disease. Subjects who had renal, hepatic, coronary artery, gastrointestinal, hemostatic disorders and diabetes were excluded from the study. All were instructed to abstain from any medication including vitamin supplements at least for four weeks leading up to the study.

Specimens were obtained after 12-14 hr fasting state in the morning (08:00 am) by antecubital venipuncture for baseline values. Then, oral aspirin at 300 mg dose was given daily. Venous blood was obtained at the 4th hr and 10th day after aspirin supplementation and separated by centrifuging for 15 minutes at 1,500 g. Six biochemical analytes including triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, ferritin and iron were analysed with fresh sera by autoanalyzer. For AOA measurement sera were stored at -20^{o}C until analysis.

The study protocol was approved by the Ethics Committee of Ataturk Research and Training Hospital (No.481). Informed written consent was obtained from the 10 healthy subjects.

AOA was determined spectrophotometrically. A solution of 0.1 mM 1,1–diphenyl-2-picrylhydrazil was rapidly mixed with the sample (1/10, v/v). The decline in absorbance was recorded at 550 nm against an ethanol blank over a period of 15 min in a microplate reader (Thermo Labsystems, Multiskan EX instrument, which was also used for all subsequent spectrophotometric assays). The decrease in absorbance corresponding to 100% radical scavenging was determined with a solution of pyrogallol in dimethyl sulfoxide (ca. 0.5%), which caused complete scavenging within seconds.[12] Ferritin was measured by immunochemiluminescence assay (Roche Diagnostics, Modular analytics E170, Germany). Serum triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol and iron levels were measured on a clinical chemistry autoanalyser (Olympus AU2700 systems, Japan).

Statistical analysis

Analyses were performed using the SPSS (Version 11.0) for Windows XP program. All data were expressed as mean ± SD. Paired t-test was used to assess the differences between measurements before and after supplementation. Pearson correlation analysis was performed to assess the associations of parameters.

RESULTS

Total antioxidant activity levels were determined as 31.7 ± 8.2% at baseline, 41.4 ± 8.3% at 4th hr and 35.3 ± 9.0% at 10th day after aspirin supplementation. AOA levels at 4th hr were found significantly higher compared with baseline (p=0.006). Although AOA levels were higher on the 10th day of the study versus baseline, the difference did not reach to a significant level. AOA values at 4th hr were not significantly different from the 10th day values (Fig 1).

There were no statistically significant differences among baseline, 4th hr and 10th day measurements for serum triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, ferritin and iron (p>0.05) (Table 1). There was also no significant correlation between serum AOA and serum triglyceride, total cholesterol, LDL cholesterol, HDL cholesterol, ferritin, and iron levels.

DISCUSSION

Many people worldwide take aspirin for the prevention and treatment of cardiovascular disease on a daily basis. The beneficial effects of aspirin in reducing acute coronary and cerebrovascular events have been attributed largely to its antiplatelet action and effects on thromboxane A_2. In the current study, low-dose aspirin increased the AOA in a short time period which may be considered as another beneficial effect.

TABLE 1. Serum ferritin, iron and lipid levels at baseline, 4th hr and 10th day after the aspirin supplementation. Results are given as the mean ± standard deviation; Chol: cholesterol.

	Ferritin (mg/dl) (mg/dl)	Iron (mg/dl)	Total Chol. (mg/dl)	HDL Chol. (mg/dl)	LDL Chol. (mg/dl)	Triglyceride
Baseline	67 ± 54	91 ± 46	190 ± 35	48 ± 15	122 ± 33	102 ± 68
4th hr	66 ± 55	101 ± 48	182 ± 32	47 ± 14	115 ± 28	98 ± 63
10th day	64 ± 55	87 ± 32	185 ± 31	47 ± 12	120 ± 32	91 ± 47

There is no significant difference between groups as baseline, 4th hr, 10th day (p>0.05).

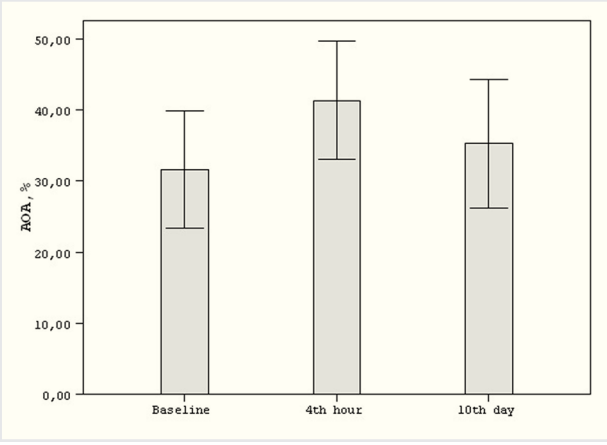

Fig 1. AOA(%) at baseline, 4th hr and 10th day after the aspirin supplementation.

There is a significant difference between baseline and 4th hr AOA ($p = 0.006$).

There are some studies evaluating the antioxidant effect of aspirin. According to Podhaisky et al, aspirin plays a role in the prevention of atherosclerosis by protecting endothelial cells of the vascular wall from damage caused by oxygen radicals.[6] On the other hand, Bulckaen et al, investigated the protective antioxidant effect of low dose aspirin treatment in mice and found a decrease in 8 hydroxy-2 deoxyguanosine levels (8-OHdG), which is an oxidative stress marker, in mice aortic homogenates.[13]

In the present study, we demonstrated that low-dose aspirin treatment significantly increases total antioxidant capacity in healthy individuals at the 4th hr. It was an important finding to show the direct short term effect of aspirin on serum AOA. Although there was an increase in AOA in the 10th day, the difference was not significant. In another study, low dose enteric coated aspirin was given to 25 healthy subjects for two weeks. It was reported that serum AOC (antioxidant capacity) levels were increased significantly during administration.[10] They also found no association between AOC and blood lipids including triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol. Although, in our study we found statistical difference in AOA levels at the 4th hr which could be a better indicator of the low dose aspirin effect in a short time period without any interference, we could not find statistically significant difference at the 10th day. It should be kept in mind that there could be some factors such as lifestyle and food habits during the study which could effect the results over a longer period. In a short time period such as 4 hours, this kind of interference should be excluded. We did not find any association between AOA and serum blood lipids similar to Ristimae et al's study.[10] On the other hand, Mehmetoglu et al, found in their recent study that low doses of aspirin treatment (150 mg/day) reduced the total oxidant status and oxidized LDL levels in two months. In the same study, although there was a slight increase, they could not find any significant difference in total antioxidant status between groups. They concluded that aspirin treatment may contribute to the prevention of atherosclerosis, which is a dose and time dependent beneficial effect.[14] Mehmetoglu et al, also reported that low dose aspirin treatment for two months did not change blood lipid levels in healthy volunteers.[15] Our results agree with these findings.

Podhaisky et al, concluded that aspirin protected endothelial cells from oxidative stress and claimed that this action was via binding or chelation of free cytosolic iron.[6] Oberle et al,[9] showed in cultured cells that aspirin at therapeutically concentrations is capable of activating the synthesis of ferritin which is a protein with cytoprotective and antioxidant properties by rapidly sequestering free cytosolic iron. In our study, we found no significant difference between serum ferritin and iron levels of groups at all time periods.

In conclusion, our results suggest that low-dose aspirin supplementation in a short time period significantly increases total antioxidant activity and improves the general antioxidative potency of blood. Further investigation of this possible protective effect of aspirin as an antioxidant in a large population of healthy subjects with long term studies is recommended.

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