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Pilot trial of the effects of low-dose acetylsalicylic acid on platelet thromboxane B_2 production

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Objective: It has been suggested that certain foods of plant origin contain milligram-quantities of acetylsalicylate which could exert an anti-thrombotic effect. Acetylsalicylic acid prevents cardiovascular diseases through inhibition of platelet endoperoxide thromboxane production and platelet aggregation. We investigated whether a daily intake of 3 mg acetylsalicylic acid causes a measurable decrease of platelet cyclo-oxygenase activity assessed by *in vitro* thromboxane B_2 production.
Design: We carried out a randomised, double-blind, placebo-controlled cross-over study.
Subjects: Ten healthy volunteers (5 men, 5 women) aged 22 ± 3 years (mean ± s.d.) participated in the study; there were no drop-outs.
Interventions: Participants took 3 mg/d of acetylsalicylic acid or a placebo for 2 weeks each. At the end of each treatment period venous blood was drawn, and platelet-rich plasma was stimulated with arachidonic acid.
Results: Treatment with acetylsalicylic acid caused a 39 ± 8% decrease in maximal thromboxane B_2 production (P = 0.000), which was independent of treatment order.
Conclusions: Quantitative data on acetylsalicylate in foods and the possible anti-thrombotic action of a diet rich in acetylsalicylate deserve closer investigation.
Sponsorship: Wageningen Agricultural University (to PLTMK Janssen), the Netherland Heart Foundation (93.084), and the Foundation for Nutrition and Health Research.
Descriptors: aspirin, acetylsalicylate, platelet aggregation, salicylate, thromboxane B_2

Introduction

Acetylsalicylic acid is, even in low amounts, effective in the prevention of cardiovascular diseases (Harter *et al.*, 1979; Lorenz *et al.*, 1984; Steering Committee of the Physicians' Health Study Research Group, 1989; Anti-platelet Trialists' Collaboration, 1988, 1994; ISIS-2 Collaborative Group, 1988, 1992; The RISC Group, 1990; The Dutch TIA Trial Study Group, 1991; The SALT Collaborative Group, 1991; Hirsch *et al.*, 1992; Juul-Möller *et al.*, 1992; Turpie *et al.*, 1993). It inhibits platelet endoperoxyde thromboxane B_2 pro-

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duction and platelet aggregation by irreversible acetylation of platelet cyclo-oxygenase which converts arachidonic acid into prostaglandin G_2/H_2, leading to further formation of thromboxane A_2 (Roth & Majerus, 1975; Roth, Stanford & Majerus, 1975*b*; Roth & Siok, 1978). Low dose of acetylsalicylic acid, however, rarely influences the production of endothelial-cell-derived prostacyclin, a vasodilator and inhibitor of aggregation (Patrignani, Filabozzi & Patroni, 1982; FitzGerald *et al.*, 1983; Toivanen, Ylikorkala & Viinikka, 1984; Kallman *et al.*, 1987; Benigni *et al.*, 1989; Clarke *et al.*, 1991).

Swain and co-workers suggested that fruits, vegetables, herbs, spices and tea are good sources of salicylates. Qualitative results also hinted at the presence of acetylsalicylate in fruits, vegetables, beverages, herbs and spices (Swain, 1984; Swain *et al.*, 1985*a*,*b*). The content of acetylsalicylate in foods was thought to be 0.1–1.0 mg per 100 g (Swain, 1984; Swain *et al.*, 1985*a*; Truswell personal communication). However, quantitative data on acetylsalicylate in foods are as yet not available.

In addition to acetylsalicylic acid, flavonoids also inhibit *in vitro* platelet aggregation, but exact mechanisms are poorly understood (Beretz & Cazenave, 1991). Intake of certain flavonoids, namely flavonols and flavones, is inversely associated with mortality from coronary heart diseases (Hertog *et al.*, 1993*a*). The average daily intake of flavonols and flavones in the Netherlands is 23 mg, mainly provided by tea, apples, onions and red wine (Hertog *et al.*, 1993*b*).

Data on the anti-thrombotic tendency of a diet rich in acetylsalicylate or flavonoids are as yet unknown. We studied whether a daily dose of 3 mg acetylsalicylic acid – the amount possibly provided by foods rich in acetylsalicylate – causes measurable changes in platelet function in man as assessed by a decrease in thromboxane B_2 production.

Methods

Subjects

Five men and five women, all students, participated in the study. Initial characteristics (mean ± s.d.) were as follows: body mass index 21 ± 1 kg/m^2, age 22 ± 3 years, platelet count 255 (±39) × 10^9 platelets/l, and systolic and diastolic blood pressure 116 ± 9 and 66 ± 7 mmHg, respectively. All participants were healthy, based on a medical questionnaire, with absence of glucose and protein in urine, and with normal blood chemistry (haematocrit, haemoglobin, mean cell volume, erythrocyte sedimentation, alanine amino transferase, gamma-glutamyl transferase, creatinine, platelet count, thrombin time, prothrombin time, activated partial thromboplastin time). None of the subjects was hypertensive. The participants were all non-smokers and had not used any regular or homeopathic medication for at least one month before the study. The subjects were asked not to use any medication during the study. Paracetamol was provided to the subjects for emergency pain relief, but none of the subjects made use of it. One person used Fucithalmic during the study; however there is no indication that this medication should have any influence on platelet activity.

The experimental protocol was approved by the Medical Ethics Committee of the Department of Human Nutrition. The protocol was fully explained to the subjects, but they were not told that the design of the study was cross-over, so as to minimise possible bias. All participants gave their written informed consent.

Design

The study had a double-blind placebo-controlled cross-over design for 2 × 14 days; there was no wash-out period between the treatments. On day 1 the subjects were randomly assigned to daily treatment with 3 mg acetylsalicylic acids or a placebo. Four subjects started out on acetylsalicylic acid and six on placebo. Subjects swallowed one capsule a day before breakfast. Identical capsules of placebo and acetylsalicylic acid were prepared by the Department of Pharmacy of the Utrecht Academical Hospital.

Subjects were requested not to eat fatty fish (salmon, trout, herring, mackerel) and to maintain their usual physical activity patterns, alcohol consumption and eating habits. Deviations from activity patterns or eating and drinking habits, and consumption of tea, fish, licorice, herbs and spices, alcoholic drinks, honey, onions and garlic were recorded in a diary, as were any signs of illness.

Subjects visited the Department on days 0, 7, 15, 21 and 29 to enhance compliance and check maintenance of activity patterns and eating and drinking habits. On these visits we checked the diary and the body weight, and asked about adverse effects, illness, medication and visits to a dentist or doctor. Returned capsules were counted and a new supply was distributed.

Measurements

Random codes were assigned to all blood samples. Venous blood was drawn into 3.8% sodium-citrate tubes 1:10 v/v (Sarstedt, Etten-Leur, Holland) on days 11, 14, 25 and 28 at 8.30 a.m. after an overnight fast. Platelet-rich plasma was prepared by centrifugation at room temperature for 10 min at 200 *g* (Hettich Rotanta, Depex, De Bilt, The Netherlands), removed and stored at room temperature in a capped tube. The residual blood was centrifuged at room temperature for 10 min at 1500 *g* to prepare platelet-poor plasma. Platelets were counted (Sequoia Turner, Abbot, Santa Clara, USA) and the platelet-rich plasma was diluted by adding autologous platelet-poor plasma to 250 × 10^9 platelets per litre.

The diluted platelet-rich plasma (450 µl) was stimulated in duplicate with arachidonic acid (1.5 mM final concentration) (Bio Data Corporation, Horsham, USA) in an aggregometer at 37°C (Bio Data Corporation, Horsham, USA). After 10 min 50 µl of the aggregate was added to 950 µl buffer (0.9% NaCl, 0.01 M EDTA, 0.3% bovine gamma-globulin, 0.005% Triton-X-100, and 0.05% sodium-azide in 50 mM phosphate buffer, pH 6.8; NEN Research Products, Du Pont, Boston, USA), the samples were immediately submerged in liquid nitrogen, and stored at –80°C until analysis. Thromboxane B_2 was measured in duplicate (Thromboxane B_2 [^{125}I]RIA kit, NEN Research Products, Du Pont, Boston, USA).

Venous blood, taken from two healthy volunteers who had not used any medication for a month preceding blood donation, served as control pools. Preparation of the citrate-plasma, aggregations and storage were carried out as described. Within-run variation was 8%.

Statistical methods

Data were analysed using the Statistical Package for Social Sciences (SPSS Inc., 1990). Mean values of thromboxane production at day 11 and 14, respectively and day 25 and 28, respectively were calculated. Those mean values were used to evaluate changes in thromboxane B_2 production after treatment with acetylsalicylic acid and placebo treatment using a paired Student's *t*-test with a probability level of 5% and a power of 90% for normally distributed data; Wilcoxon's signed rank test was used for not normally distributed data. Within-person within-period variation of thromboxane production was calculated. Treatment order effects were checked using analysis of variance (Snedecor & Cochran, 1980).

Results

Treatment adherence

Compliance, expressed by the proportion of capsules not returned, was 100% during placebo treatment, and 99% during acetylsalicylic acid treatment. The diaries did not reveal relevant changes between the treatment and placebo period in physical activity patterns, eating or drinking habits. Body mass index was stable (95% confidence interval –0.1 to 0.5 kg/m^2) and no adverse reactions were reported.

Platelet thromboxane B_2 production

There was no effect of treatment order (P = 0.207). Within-person variation of thromboxane B_2 production between blood samples taken 3 days apart was 8% after placebo and 10% after aspirin treatment. Consumption of 3 mg/d of acetylsalicylic acid for 14 days significantly decreased (P = 0.000) platelet thromboxane B_2 production by 783 ± 77 nmol/10^{11} platelets or 39 ± 8% (mean ± s.d., n = 10). Mean thromboxane B_2 production was 2016 ± 299 and 2062 ± 356 nmol/10^{11} platelets after daily treatment with placebo for 11 and 14 days, respectively, and 1216 ± 294 and 1294 ± 326 after daily treatment with 3 mg aspirin for 11 and 14 days, respectively (Figure 1).

Discussion

There is a growing interest in food components

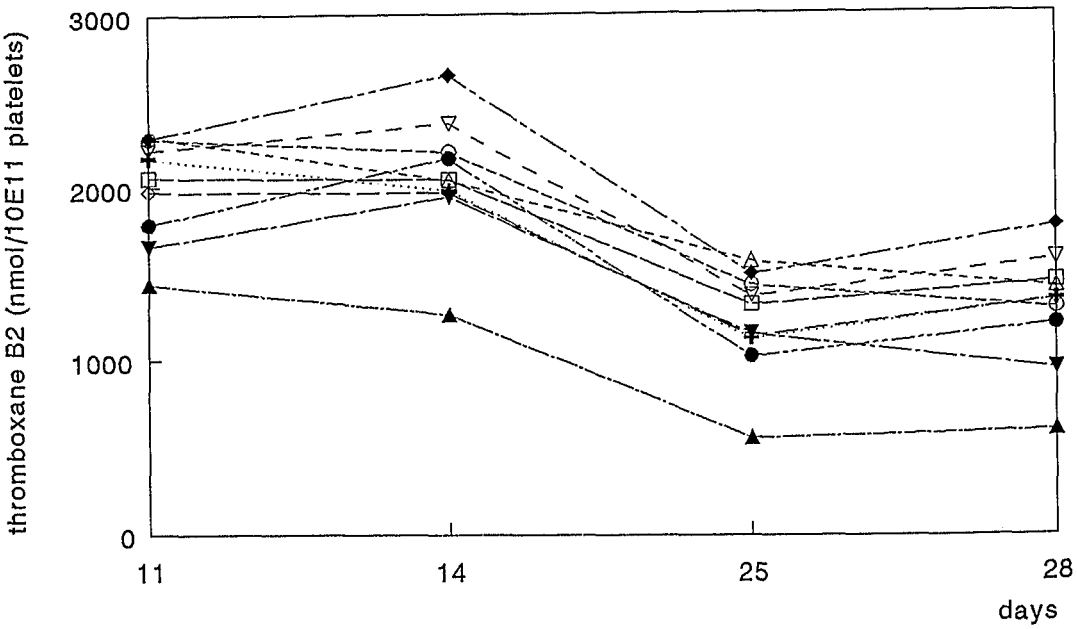

Figure 1 Maximally stimulated thromboxane B_2 production (nmol/10^{11} platelets) in 10 healthy volunteers treated with placebo (day 11 and 14) or 3 mg acetylsalicylic acid (day 25 and 28) daily, for 2 weeks each. Treatments were given in random order in a double-blind cross-over trial. Thus 4 out of the 10 subjects actually received aspirin on days 1 to 15, and 6 out of the 10 subjects received placebo on days 1 to 15.

nisms other than the classical intermediates, blood pressure and lipoproteins. Dietary effects on platelet function are of special interest because acetylsalicylic acid has been convincingly shown to reduce the incidence of cardiovascular diseases (Hennekens *et al.*, 1989; Steering Committee of the Physicians' Health Study Research Group, 1989; ISIS-3 Collaborative Group, 1992). Contents of acetylsalicylate in foods are unknown yet, and need further investigation.

As far as we are aware the only previous studies which used a daily dose of acetylsalicylic acid in amounts of 3 mg or less were uncontrolled trials (Sinzinger *et al.*, 1984, 1989; Toivanen *et al.*, 1984; Roberts *et al.*, 1986). We now found that 3 mg acetylsalicylic acid daily reduced *in vitro* platelet thromboxane B_2 production by almost 40%. By applying careful standardisation we were able to reduce the combined analytical and biological within-subject variation to 8% after placebo treatment and 10% after aspirin treatment. Using our methodology we may therefore be able to detect even a 10% reduction in platelet thromboxane B_2 production at $P = 0.05$ with a power of 90%, corresponding to the effect of <1 mg acetylsalicylic acid daily (Patrignani, 1982; Toivanen *et al.*, 1984).

According to Swain, fruits, vegetables, herbs, spices, honey, and tea are good sources of salicylate and a normal mixed daily diet provides 10–200 mg salicylate. Qualitative results also hinted at the presence of acetylsalicylates in fruits, vegetables, beverages, herbs and spices (Swain, 1984; Swain *et al.*, 1985*a*), but quantitative data are absent. Our data suggest that if the level of acetylsalicylic acid in foods is as high as can be inferred from the results of Swain (1984) and Swain *et al.* (1985*a*), a normal mixed daily diet might have measurable effects on platelet function in man. Studies on the possible anti-thrombotic action of a diet rich in acetylsalicylate or flavonoids deserve closer investigation.

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