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EFFECT OF VARIOUS DOSES OF ACETYLSALICYLIC ACID IN COMBINATION WITH DIPYRIDAMOLE ON THE BALANCE BETWEEN PROSTACYCLIN AND THROMBOXANE IN HUMAN SERUM

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1 Thirty-six healthy human subjects were randomly divided into six groups which were treated with a single dose of 75 mg (1.3 mg/kg) of dipyridamole alone, or 75 mg of dipyridamole in combination with 30 mg (0.5 mg/kg), 50 mg (0.8 mg/kg), 160 mg (2.6 mg/kg) and 330 mg (5.7 mg/kg) of acetylsalicylic acid (ASA), or with placebo.

2 The concentrations of prostacyclin (PGI_2) and thromboxane A_2 (TxA_2) metabolites, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and TxB_2 respectively, were measured in serum with specific radioimmunoassays before and 1 and 3 h after the ingestion of the test dose.

3 The basal concentrations of 6-keto-$PGF_{1\alpha}$ and TxB_2 correlated significantly ($r = 0.588$, $P < 0.001$).

4 Dipyridamole alone did not change PGI_2 or TxA_2 production.

5 Dipyridamole-ASA combinations with ASA doses between 0.5 and 0.8 mg/kg inhibited TxB_2 production by 48 to 74%, and with the ASA doses between 2.6 and 5.7 mg/kg by about 90%. None of these combinations changed PGI_2 production.

6 The ratio of 6-keto-$PGF_{1\alpha}$ to TxB_2 increased 3.5 to 6 times with ASA doses of 0.5 to 0.8 mg/kg and 21 to 29 times with doses between 2.6 to 5.7 mg/kg.

7 These results suggest that the anti-thrombotic effect of dipyridamole *in vivo* is not mediated through direct changes in PGI_2 and/or TxA_2 production.

Introduction

In platelets, cyclo-oxygenase transforms arachidonic acid to endoperoxides, from which a proaggregatory agent, thromboxane A_2 (TxA_2), is formed (Hamberg, Svensson & Samuelsson, 1975). However, the cyclo-oxygenase in the vessel walls is a key enzyme in the formation of a potent antiaggregatory agent, prostacyclin (PGI_2) (Moncada, Gryglewski, Bunting & Vane, 1976). The balance between these two substances with opposing biological activities overtly regulates platelet aggregation *in vivo* (see Moncada & Vane, 1979, for a review). It is also known that platelet aggregation can be inhibited by acetylsalicylic acid (ASA) (Weiss & Aledort, 1967; Smith & Willis, 1971; Weiss, 1976; Burch & Majerus, 1979) and that dipyridamole exerts an anti-aggregating effect *in vivo* (Emmons, Harrison, Honour & Mitchell, 1965; Arfors, Hint & Dhall, 1968; Didisheim, 1968; Sullivan, Harken & Gorlin, 1968; Harker & Slichter, 1970). Furthermore, it has been shown that ASA and dipyridamole have a synergistic anti-thrombotic effect *in vivo* (Harker & Slichter, 1970; Giromini, Bouvier, Dami, Denizot & Jeannet, 1972; Weiss, 1976) which effect, however, depends critically on the dose of ASA used (Moncada & Korbut, 1978). Since all these findings could be mediated by changes in PGI_2 and TxA_2, we compared the effects of different doses of ASA in combination with dipyridamole on the balance between PGI_2 and TxA_2 *in vivo*.

Methods

Thirty-six healthy women between 21 and 49 years of age who had not taken ASA or other anti-inflammatory drugs for at least 14 days, were allotted randomly to the study groups A to F (Table 1). After an overnight fast, the volunteers took a capsule which contained 75 mg (1.3 mg/kg) of dipyridamole (group A), or 75 mg of dipyridamole in combination with 30 mg (0.5 mg/kg), 50 mg (0.8 mg/kg), 160 mg (2.6 mg/kg) and 330 mg (5.7 mg/kg) of ASA (groups B to E), or placebo (group F), and the volunteers remained fast-

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ing for 2 h thereafter. Venous blood samples were collected into dry glass tubes from an antecubital vein without using a stasis, before the capsule intake, and 1, and 3 h thereafter. Blood was allowed to clot at +24°C for 60 min precisely after which the serum was separated by centrifugation at +4°C and frozen.

PGI_2 and TxA_2 production were studied indirectly by measuring the stable metabolites of these prostanoids, namely 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane B_2 (TxB_2), respectively (Hamberg *et al.*, 1975; Johnson, Morton, Kinner, Gorman, McGuire, Sun, Whiltaker, Bunting, Salmon, Moncada & Vane, 1976). This was done with specific radioimmunoassays, the characteristics of which are given elsewhere (Viinikka & Ylikorkala, 1980; Ylikorkala & Viinikka, 1980). Briefly, specific antisera against 6-keto-$PGF_{1\alpha}$ and TxB_2 conjugated with bovine serum albumin were produced in rabbits. For the radioimmunoassay, 2 ml serum samples were acidified to pH 3 with 0.1 N HCl and extracted four times with 4 ml ethyl acetate. The organic phases were combined and evaporated to dryness. Two aliquots from the sample residue dissolved in ethanol were taken in duplicate for the saturation analysis using specific antibodies and tritiated tracers (New England Nuclear Corporation, Boston, Massachusetts, U.S.A.). The assay sensitivity (mean blank +2 s.d.) was 12.6 pg/ml for 6-keto-$PGF_{1\alpha}$ and 9.6 pg/ml for TxB_2. The recoveries of 6-keto-$PGF_{1\alpha}$ and TxB_2 added to plasma or serum samples were between 90 and 103% ($n = 10$). The intra-assay coefficient of variation in both assays varied from 4.1 to 9.7% at different concentrations ($n = 10$). The interassay variation was 15.5% for 6-keto-PGF_1 and 18.6% for TxB_2 ($n = 10$). Samples from the same subject were always assayed in the same batch.

In order to standardize the individual changes in 6-keto-$PGF_{1\alpha}$ and TxB_2 levels, the concentrations after the capsule intake were expressed as percentages of the pretreatment level. Statistical comparisons were made between the concentrations in the treatment groups and those in the placebo group at the same time in the sequence with a two-tailed Student's t test.

Results

Basal situation

The concentrations of 6-keto-$PGF_{1\alpha}$ ranged from 159 pg/ml to 463 pg/ml. The mean levels did not differ between the various study groups (Table 1). The TxB_2 concentrations ranged from 1350 pg/ml to 24548 pg/ml with no significant difference between the treatment and placebo groups. A significant correlation (linear regression coefficient = 0.588, $P < 0.001$, $n = 36$) between basal 6-keto-$PGF_{1\alpha}$ and TxB_2 concentrations was seen.

6-Keto-$PGF_{1\alpha}$ concentrations following treatment

No changes in the concentrations of 6-keto-$PGF_{1\alpha}$ were seen (Figure 1a).

Thromboxane B_2 concentrations following treatment

ASA doses between 2.6 mg/kg and 5.7 mg/kg together with 75 mg dipyridamole inhibited over 90% of the TxB_2 formation during clotting (Figure 1b, groups D

Table 1 Clinical characteristics and concentrations of 6-keto-prostaglandin $F_{1\alpha}$ 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 (TxB_2) before treatment with dipyridamole alone or in combination with acetylsalicylic acid (ASA)

Treatment	Age (years)	Mean dosage of Dipyridamole	Mean dosage of ASA	Basal levels in serum 6-keto-PGF_1 (pg/ml)	Basal levels in serum TxB_2 (pg/ml)
Dipyridamole 75 mg (Group A)	30.2 ± 2.2	1.3 ± 0.05 mg/kg	0 mg/kg	270.8 ± 54.5	10890 ± 3660
Dipyridamole 75 mg + ASA 30 mg (Group B)	32.1 ± 3.6	1.3 ± 0.04 mg/kg	0.5 ± 0.02 mg/kg	369.2 ± 37.1	20937 ± 7105
Dipyridamole 75 mg + ASA 50 mg (Group C)	36.3 ± 13.8	1.3 ± 0.05 mg/kg	0.7 ± 0.03 mg/kg	305.3 ± 41.3	6855 ± 2751
Dipyridamole 75 mg + ASA 160 mg (Group D)	35.6 ± 1.7	1.3 ± 0.04 mg/kg	2.6 ± 0.1 mg/kg	247.3 ± 19.1	9025 ± 2807
Dipyridamole 75 mg + ASA 330 mg (Group E)	31.8 ± 1.8	1.3 ± 0.05 mg/kg	5.7 ± 0.2 mg/kg	298.7 ± 45.5	5644 ± 1828
Placebo (Group F)	33.4 ± 1.9	0 mg/kg	0 mg/kg	272.8 ± 24.4	8690 ± 3449

Each group consisted of six volunteers. Values are mean ± s.e. mean.

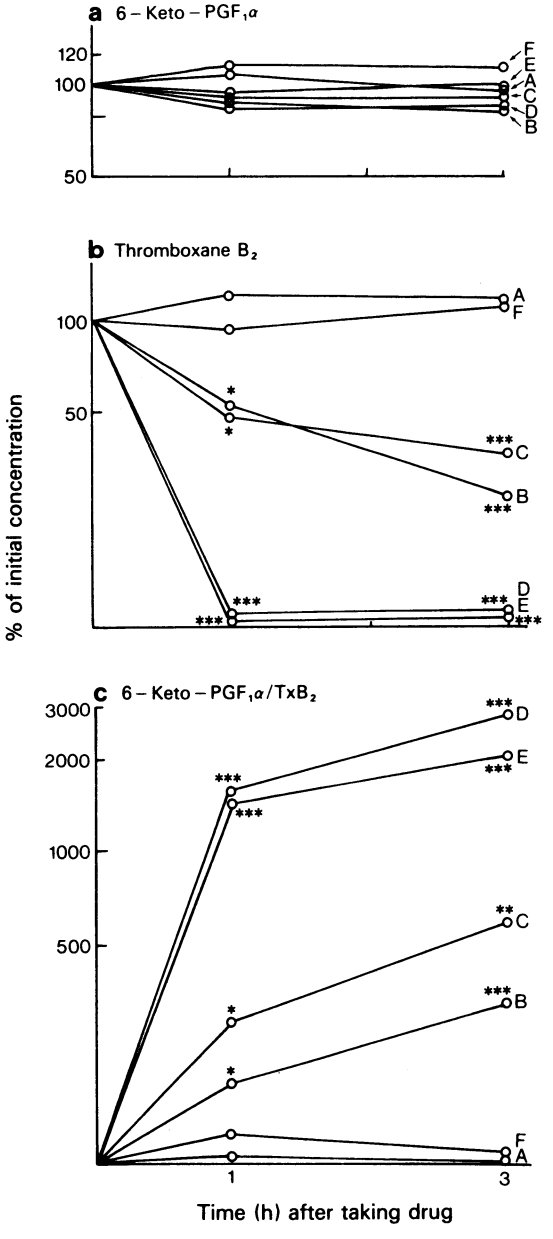

Figure 1 The mean relative levels of 6-keto-PGF$_{1\alpha}$ (a), and thromboxane B_2 (TxB_2) (b) as well as their mean ratio (c) following treatments with various dipyridamole–acetylsalicylic acid (ASA) combinations. Each group consisted of six volunteers: (A) = 75 mg of dipyridamole; (B) = 30 mg of ASA plus dipyridamole; (C) = 50 mg of ASA plus dipyridamole; (D) = 160 mg of ASA plus dipyridamole; (E) = 330 mg of ASA plus dipyridamole; (F) = placebo. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ indicate the significances of the differences in comparison with the placebo (F) group.

and E). The mean relative TxB_2 levels in these groups were 8.2 to 9.1% at 1 h and 5.6 to 11.2% at 3 h. No differences in TxB_2 levels emerged between these dosages. Half a mg/kg (group B) or 0.8 mg/kg of ASA (group C) with 75 mg of dipyridamole caused 48 to 51% and 64 to 74% inhibition in TxB_2 production at 1 and 3 h, respectively. These inhibitions were significantly less ($P < 0.01$) than those observed in groups D and E. Dipyridamole alone caused no changes in TxB_2 levels (group A).

The ratio of 6-ketoPGF$_{1\alpha}$ to thromboxane B_2 following treatment

Treatments D and E elevated the ratio of 6-keto-PGF$_{1\alpha}$ to TxB_2 by about 15 to 16 fold at 1 h and by about 21 to 29 fold at 3 h (Figure 1c). Treatments B and C also raised this ratio although less ($P < 0.01$) than did treatments D and E. Dipyridamole alone did not change the 6-keto-PGF$_{1\alpha}$/TxB_2 ratio.

Discussion

We monitored the platelet cyclo-oxygenase activity by measuring the TxB_2 formation during spontaneous clotting of the blood samples, when there is a huge release of TxB_2 (Viinikka & Ylikorkala, 1980). We also measured 6-keto-PGF$_{1\alpha}$ from the same serum samples, although the 6-keto-PGF$_{1\alpha}$ levels in serum were slightly higher than those in plasma (Ylikorkala & Viinikka, 1980), perhaps due to the generation of PGI_2 from white blood cells during clotting (Blackwell, Flower, Russell-Smith, Salmon, Thorogood & Vane, 1978). The concentrations of 6-keto-PGF$_{1\alpha}$ and TxB_2 in serum correlated significantly under basal conditions, which gives further support for the assumption that there is normally a balance between PGI_2 and TxA_2 activities in healthy subjects (Moncada & Vane, 1979).

It has recently been shown that dipyridamole increases PGI_2 biosynthesis *in vitro* (Blass, Block, Förster & Pönicke, 1980). This could explain the *in vivo* anti-thrombotic effect of dipyridamole. Our data, however, show that a single dose of dipyridamole (1.3 mg/kg) does not change the production of PGI_2, as measured by PGI_2 metabolite levels in serum. This does not exclude the possibility that dipyridamole acts as an anti-thrombotic agent by potentiating the effect of endogenous PGI_2 (Moncada & Korbut, 1978).

Impairment of platelet aggregation induced by ASA intake (see reviews, Weiss, 1976; Burch & Majerus, 1979) is mediated through the irreversible inhibition of platelet cyclo-oxygenase by acetylation (Vane,

1971; Roth, Stanford & Majerus, 1975; Roth & Majerus, 1975). According to Burch, Stanford & Majerus (1978) who measured directly the acetylation of this enzyme by means of [^{3}H]-acetyl-ASA, a single 325 mg ASA dose inactivated 89% of the platelet cyclooxygenase and when ASA doses were from 20 to 160 mg, the inhibition ranged from 34 to 82%. In our study, the platelet cyclo-oxygenase inhibition was about 90% after a single ASA dose of 160 to 330 mg together with dipyridamole, as judged from the changes in the TxB_2 production during spontaneous clotting of the blood sample, and between 48 to 78% when the ASA dose was 30 to 50 mg. Since dipyridamole alone did not decrease the TxB_2 production, the inhibitions caused by these combinations are most probably due to ASA. It is noteworthy that the inhibitions of platelet cyclo-oxygenase, as measured by decreases in TxB_2 production during clotting, are closely comparable with the direct data of Burch *et al.* (1978). Since platelets synthesize little or no new protein (Roth *et al.*, 1975; Roth & Majerus, 1975; Burch *et al.*, 1978), this cyclo-oxygenase inhibition lasts for as long as the platelets survive.

A number of controlled trials have suggested the potential usefulness of ASA treatment in the prevention of death after myocardial infarction (Elwood, Cochrane, Burr, Sweetnam, Williams, Welsby, Hughes & Renton, 1974; Boston Collaborative Drug Surveillance Group, 1974; The Coronary Drug Project Research Group, 1976; Elwood & Sweetnam, 1979). ASA also potentiates the anti-thrombotic effect of dipyridamole (Giromini *et al.*, 1972; Harker & Slichter, 1972; Moncada & Korbut, 1978). In our study, 2.6 or 5.7 mg/kg of ASA in combination with dipyridamole almost completely inhibited the platelet cyclo-oxygenase without changing the PGI_2 formation. However, the ischaemia-induced PGI_2 release may be more sensitive to ASA action, since 50% of it was inhibited by ASA doses of 4.9 mg/kg (Masotti, Galanti, Poggesi & Abbate, 1979). On the basis of these data, it seems justifiable to select an ASA dose in the order of 2.6 mg/kg for combinations with dipyridamole. Before the start of further clinical trials in the prevention of thrombosis, the effects of such combinations on PGI_2/TxA_2 balance over a more prolonged period of time should be evaluated.

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