

# No Effect of Acetylsalicylic Acid on B-Thromboglobulin And Platelet Factor 4 Plasma Levels In Patients With Transient Ischaemic Attacks

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**SUMMARY** We studied the effect of acetylsalicylic acid (ASA) versus placebo on B-thromboglobulin (B-TG) and platelet factor 4 (PF4) plasma levels and ADP-induced platelet aggregation in 25 male patients with transient ischaemic attacks (TIA). The patients were allocated randomly to two groups: 14 patients received oral treatment with ASA 500 mg b.i.d. for 14 days, 11 patients placebo b.i.d. for the same period. B-TG and PF4 plasma levels and ADP-induced platelet aggregation were determined in basal conditions, and two hours, and seven and fourteen days after starting with ASA or placebo. In addition, the same parameters were studied in a group of 20 healthy males of matched age. Basal levels of plasma B-TG and PF4 and the maximal amplitude of ADP-induced platelet aggregation were abnormally high in TIA patients. ASA caused a significant reduction of B-TG plasma levels in TIA patients 2 hours after the first administration, but no effect was observed at the 7th and 14th day of treatment. PF4 plasma levels were unaffected by ASA treatment. It is concluded that ASA, at the dose conventionally used in clinical trials, does not affect the release of two alpha-granule proteins.

Stroke Vol 17, No 6, 1986

ACETYLSALICYLIC ACID (ASA) has been used in prospective trials aimed at preventing cerebrovascular disease, however with conflicting results.<sup>1-4</sup> Moreover, the optimal dosage of ASA required to prevent arterial thrombosis has not yet been established. The daily dose of 1-1.3 g ASA used in the clinical trials inhibits vascular cyclo-oxygenase activity, as well as platelet activity, and it has been suggested that lower doses of ASA would inhibit platelet cyclo-oxygenase without affecting the enzyme in the vascular endothelium.<sup>5-7</sup>

B-thromboglobulin (B-TG) and platelet factor 4 (PF4) are platelet specific proteins released from alpha-granules during the release reaction. Increased levels of these proteins, in particular of B-TG, have been reported in deep venous thrombosis,<sup>8</sup> in peripheral vascular disease,<sup>9</sup> in patients with prosthetic heart valves,<sup>10, 11</sup> in diabetes mellitus,<sup>12, 13</sup> in acute myocardial infarction<sup>14-16</sup> and in cerebrovascular disease.<sup>17-20</sup> A T<sub>1/2</sub> of 100 min has been reported for the clearance of B-TG, while the clearance of PF4 could not be determined because of its very fast clearance.<sup>21</sup> The more rapid clearance of PF4 could be due to its binding to endothelial cells.<sup>22</sup> Patients with chronic renal failure show elevated levels of B-TG, which is metabolized by the kidney, but not of PF4, and the increase of B-TG is highly correlated with creatinine clearance.<sup>23, 24</sup> The determination of plasma levels of B-TG and PF4 is considered as a marker of platelet function *in vivo*.<sup>25-27</sup>

The aim of this study was to evaluate the effect of ASA, at the dosage conventionally used in clinical trials, on platelet function in patients with transient ischaemic attacks (TIA).

## Materials and Methods

The study comprised 25 males, aged 47 to 68 years ( $m \pm SD = 57.7 \pm 7.0$ ), with a history of one or more TIA in the carotid or the vertebrobasilar territory. The last acute event occurred 4 days to 1 month before the study. None had smoked or taken drugs that could inhibit platelet aggregation for at least 10 days before the treatment period, and no drugs were given during the study. Patients with arterial hypertension, diabetes, chronic renal failure or cardiac vascular disease were considered ineligible. The design of the investigation was single-blind. The patients were allocated randomly to two groups: 14 patients, aged 47 to 66 years ( $m \pm SD = 57.2 \pm 6.6$ ), received oral treatment with ASA 500 mg b.i.d. for 14 days, 11 patients, aged 47 to 68 years ( $m \pm SD = 58.4 \pm 7.7$ ), placebo b.i.d. for the same period. B-TG and PF4 plasma levels and ADP-induced platelet aggregation were determined under basal conditions, two hours after the ingestion of a single dose of ASA or placebo, and at the 7th and 14th day of treatment two hours after the morning dose. In addition, the same parameters were determined in a group of 20 healthy males of matched age who had not smoked or taken any drugs for at least 10 days prior to the study. Informed consent was obtained from all participants.

For B-TG and PF4 assays, blood samples were collected without stasis, using a 21-gauge needle, into tubes containing the original mixture of EDTA, theophylline and PGE<sub>1</sub>,<sup>28</sup> and placed immediately in ice water. The plasma was separated by centrifugation at 2000 g at 4°C for 20 minutes. The top 0.5 ml was withdrawn and stored at -70°C for B-TG and PF4 determination. All the assays were carried out simultaneously by the same operator, who was unaware of the group to which the patient belonged. B-TG and PF4 plasma levels were measured in the duplicate using the Amersham and the Abbott radioimmunoassays, respectively.

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TABLE 1 B-TG and PF4 Plasma Levels and ADP-induced Platelet Aggregation (as maximal amplitude) in Combined Group of TIA Patients and Healthy Volunteers

	N	Age	B-TG (ng/ml)	PF4 (ng/ml)	Maximal amplitude (%)
TIA patients	25	56.8 ± 6.8	73.8 ± 45.3†	18.0 ± 13.8†	79.4 ± 10.6*
Healthy volunteers	20	54.6 ± 7.3	27.1 ± 14.7	6.1 ± 4.0	70.8 ± 9.8

m ± SD.

\* $p < 0.05$ , † $p < 0.01$ , compared with healthy volunteers.

Platelet aggregation was measured by the turbidimetric method of Born and Cross,<sup>29</sup> using an Elvi 840 aggregometer (Logos, Milan, Italy), equipped with a linear recorder. Blood samples for platelet aggregation were collected into siliconized test tubes containing 3.8% sodium citrate and immediately centrifuged (160 g) at room temperature for 10 minutes to obtain platelet-rich plasma. ADP at final concentration of 2  $\mu$ M was used as an aggregant agent. Results of aggregation studies are indicated as the maximal amplitude, i.e., the maximal deflection of the curve observed during the recording, expressed as percent of the maximal theoretical aggregation.

The statistical evaluation between the ASA group and the placebo group was performed with analysis of variance; when this was significant we applied Dunnett's or Student's *t* tests to the average values of each treatment. A Student's *t* test was used for the comparison between healthy controls and combined TIA group in basal conditions.

### Results

The mean of B-TG and PF4 plasma levels were significantly higher in the combined group of 25 TIA patients than in controls. A significant difference was also observed in the maximal amplitude of ADP-induced platelet aggregation between patients and controls (table 1).

In the ASA group a significant reduction of B-TG plasma levels was observed 2 hours after the first ASA administration, but not at the 7th and 14th day of treatment. There was no significant difference in PF4 plasma levels at any time with respect to basal values.

ADP-induced platelet aggregation was already reduced in all patients after two hours and it remained constant throughout the treatment period. The mean values of maximal amplitude were significantly decreased at the various experimental times (table 2).

No significant differences in B-TG and PF4 levels were observed in the placebo group (table 2). The differences between the treated group and the placebo group were significant, both for maximal amplitude and for B-TG plasma levels at 2 hours (table 2).

Both in controls and in TIA patients B-TG levels were always higher than PF4 levels; the difference between the mean of B-TG and PF4 plasma levels was significant ( $p < 0.001$ ).

### Discussion

The increase in B-TG plasma levels and in ADP-induced platelet aggregation shows that there is platelet activation in TIA patients, in agreement with the findings of earlier studies.<sup>17-20</sup>

ADP-induced platelet aggregation was inhibited by ASA administration in all patients. This finding confirms the drug compliance. ASA failed to reduce B-TG and PF4 plasma levels, which in agreement with previous reports on healthy subjects.<sup>30, 31</sup>

However, a decrease in the plasma levels of B-TG has been reported in myeloproliferative disorders<sup>32</sup> and in ischaemic cerebrovascular disease<sup>18</sup> after ASA treatment.

B-TG and PF4 are found in similar amounts in platelets.<sup>33</sup> *In vitro* studies have shown that release of B-TG equals that of PF4.<sup>30</sup> However, *in vivo*, because of the lower clearance of B-TG from blood, the average plas-

TABLE 2 B-TG and PF4 Plasma Levels and ADP-induced Platelet Aggregation (as maximal amplitude) in TIA Patients before and during ASA or Placebo Treatment

	Before	During treatment		
		After 2 hours	After 7 days	After 14 days
ASA group (14)				
B-TG (ng/ml)	73.7 ± 52.6	51.5 ± 29.7*‡	67.0 ± 33.5	64.6 ± 43.7
PF4 (ng/ml)	18.3 ± 15.2	14.8 ± 10.4	15.4 ± 9.6	15.8 ± 10.2
Maximal amplitude (%)	78.9 ± 10.8	50.3 ± 7.6†§	52.6 ± 10.7†§	51.6 ± 9.8†§
Placebo group (11)				
B-TG (ng/ml)	73.1 ± 36.0	74.5 ± 35.0	69.1 ± 26.9	65.6 ± 29.1
PF4 (ng/ml)	17.6 ± 12.5	17.2 ± 9.9	15.7 ± 8.2	14.5 ± 7.7
Maximal amplitude (%)	80.9 ± 11.8	79.2 ± 12.5	75.7 ± 9.1	74.1 ± 9.5

m ± SD.

In parentheses number of cases.

\* $p < 0.05$ , † $p < 0.01$ , versus basal values.

‡ $p < 0.05$ , § $p < 0.01$ , ASA versus placebo.

ma level of B-TG is much higher than that of PF4. No such difference would be expected when release occurs *in vitro*.<sup>27</sup> Our results of a statistically significance between B-TG and PF4 levels support an *in vivo* release of the two proteins.

The *in vitro* release of B-TG and PF4 induced by ADP, epinephrine and arachidonic acid is totally inhibited in plasma from volunteers treated with ASA, which indicates that cyclo-oxygenase is essential for the release of the two proteins.<sup>30</sup> The *in vivo* situation appears to be different. In fact, we found that *in vivo* ASA does not inhibit the release of the two proteins.

A probable explanation of our findings is that only a small fraction of plasma B-TG and PF4 arises from a cyclooxygenase-dependent pathway, and that the majority results from a process other than arachidonic acid metabolism.<sup>15</sup>

In conclusion, although ASA inhibits ADP-induced platelet aggregation *in vitro*, it appears that at a dose of 500 mg b.i.d. it does not affect the release of two alpha-granule proteins *in vivo*.

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