Dose- and time-dependent antiplatelet effects of aspirin

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Summary

Aspirin is widely used, but dosages in different clinical situations and the possible importance of “aspirin resistance” are debated. We performed an open cross-over study comparing no treatment (baseline) with three aspirin dosage regimens – 37.5 mg/day for 10 days, 320 mg/day for 7 days, and, finally, a single 640 mg dose (cumulative dose 960 mg) – in 15 healthy male volunteers. Platelet aggregability was assessed in whole blood (WB) and platelet rich plasma (PRP). The urinary excretions of stable thromboxane (TxM) and prostacyclin (PGI-M) metabolites, and bleeding time were also measured. Platelet COX inhibition was nearly complete already at 37.5 mg aspirin daily, as evidenced by >98% suppression of serum thromboxane B2 and almost abolished arachidonic acid (AA) induced aggregation in PRP 2–6 h after dosing. Bleeding time was similarly prolonged by all dosages of aspirin. Once daily dosing was associated with considerable recovery of AA induced platelet aggregation in WB after 24 hours, even after 960 mg aspirin. Collagen induced aggregation in WB with normal extracellular calcium levels (hirudin anticoagulated) was inhibited <40% at all dosages. TxM excretion was completely suppressed, and increased <24 hours after the cumulative 960 mg dose. Aspirin treatment reduced PGI-M already at the lowest dosage (by ~25%), but PGI-M excretion and platelet aggregability were not correlated. Antiplatelet effects of aspirin are limited in WB with normal calcium levels. Since recovery of COX-dependent platelet aggregation occurred within 24 hours, once daily dosing of aspirin might be insufficient in patients with increased platelet turnover.

Keywords

Platelet function, thromboxane, prostacyclin, acetylsalicylic acid, dosage

Introduction

Acetylsalicylic acid, commonly referred to as aspirin, is a widely used and very well documented antiplatelet drug that lowers cardiovascular morbidity and mortality (1, 2). The antiplatelet effects of aspirin are related to irreversible inhibition of platelet cyclooxygenase (COX), and reduced production of the potent vasoconstrictor and platelet activator thromboxane A2 (TxA2) (2). However, aspirin has limitations as an antithrombotic agent, and there is still debate about the optimal dosages in different clinical situations. Aspirin has variable antiplatelet activity when assessed by different methods, and the poorly defined phenomenon of “aspirin resistance” is much discussed. Using various test methods, 5–57% of patients in different studies were judged not to have optimal antiplatelet effects during aspirin treatment (3), and several authors have reported an increased risk of suffering major cardiovascular events in post hoc analyses of studies among such patients (2–4). However, prospective studies validating the clinical utility of identifying biochemical or functional “aspirin resistance” are lacking.

TxA2 generated by activated platelets forms a positive feedback loop which accelerates the local platelet activation (5). Less than 5% residual capacity to generate TxA2 is enough to fully sustain thromboxane dependent platelet aggregation (6, 7), and full aggregation was achieved in vitro with only 2.5% of the platelet population being aspirin free (7). Thus, nearly complete irreversible inhibition of platelet COX is required for effective antithrombotic aspirin therapy. Interestingly, recovery of TxB2 was found already 4 hours after ingestion of 650 mg aspirin (7).

Chronic daily administration of as little as 0.45 mg/kg aspirin provides sufficient platelet COX inhibition, defined as a virtually complete inhibition of serum TxB2, in healthy volunteers (8). In clinical trials, however, daily aspirin dosages of 75–150 mg appear to be most effective in patients with atherosclerotic vas-
cular disease, and the clinical efficacy of aspirin is reduced or lost at doses below 75 mg/day (1, 2). A recent retrospective analysis of two GPlIb/IIIa inhibitor trials suggested that a higher dose of aspirin may afford better protection against myocardial infarction (9), but a similar analysis from the CURE study indicated that ≤100 mg/day was more effective than ≥200 mg/day (10). Prospective, placebo-controlled trials have shown excellent protection with 75 mg aspirin daily in patients with stable (11) or unstable (12) angina pectoris.

The effect of repeated daily low doses of aspirin is cumulative, and steady state with regard to platelet COX inhibition is reached within 7 days (8). After a single full dose of aspirin, COX activity recovers by some 10% per day, as a function of platelet turnover (2), which may vary both within and between patients. Phasic increases in thromboxane synthesis were observed in patients with unstable angina, indicating that platelet activation may occur during spontaneous ischemia (13). Furthermore, diabetes, preeclampsia and advanced coronary artery disease are associated with increased platelet turnover (14–16). Thus, the requirements for dosages and dosing intervals with aspirin treatment may differ in different clinical situations.

At low dosages, first pass hepatic metabolism of aspirin limits the systemic exposure of the patients to COX inhibition, thus preserving the vasodilating and antiplatelet effects of endothelial prostacyclin (17). Higher doses of aspirin may, however, attenuate this potentially antithrombotic mechanism (2, 18). Experiments in knock-out mice support an antithrombotic role for endogenous prostacyclin (19). The importance of prostacyclin in thrombotic processes in humans is not completely understood, but several studies have recently demonstrated unfavourable cardiovascular outcomes in patients treated with COX-2 inhibitors (20), and this may be related to suppression of prostacyclin production (21).

In the present study we compared the effects of different aspirin dosages, from 37.5 mg daily to a cumulative single dose of 960 mg, on various aspects of platelet function in healthy volunteers. We studied platelet aggregation in platelet rich plasma and in whole blood, and we measured the urinary excretion of thromboxane and prostacyclin metabolites, as well as TxB2 generation in cloting whole blood. We used hirudinized blood to avoid the artefactual enhancement of the thromboxane dependence of platelet aggregation (and thus the efficacy of aspirin) which is seen when citrate is used as anticoagulant and extracellular calcium levels are low (22–24). We also investigated the time dependence of the effects of different aspirin dosages to clarify if there is significant recovery of COX-dependent platelet function during the normal dosing interval of 24 hours.

**Study design**

The study was an open cross-over study with measurements at baseline (untreated) and during treatment with three different dosages of aspirin. Measurements were performed at baseline, and after aspirin treatment with 37.5 mg/day for 10 days (half a tablet Trombly® 75 mg, Pharmacia Sverige AB), and 320 mg/day for 7 days (one tablet Alka Seltzer®, Bayer, Germany). Finally, they took a single dose of 640 mg aspirin (two tablets of Alka Seltzer® 1–2h after the last 320 mg dose (cumulative dose 960 mg on the last day). There was a 10–14 day wash-out period between the low dose and the intermediate/high dose investigations. The volunteers were instructed to refrain from caffeine, tobacco or food intake >12 hours before each visit.

Blood sampling was performed after 30 min rest in the supine position on all occasions. Between samplings the volunteers were free to move about, but were instructed not to perform rigorous physical activity. At baseline, the sampling was performed three times during 24 hours (9 AM, 1 PM, and again at 9 AM) before the treatment was initiated. Thereafter, the subjects received 37.5 mg aspirin daily (low dose) for 10 days, and on the 10th day blood sampling was performed 1.5–2 hours (9 AM), =6 hours (1 PM), and 24 hours after the last dose of aspirin. The same procedure was performed after the last ingested dose of 320 mg aspirin; i.e. on the 7th day of treatment. However, after the 9 AM sampling (1.5–2h after drug ingestion) the volunteers took an additional single 640 mg aspirin dose to ensure complete platelet COX-1 inhibition. Sampling was then repeated 4 hours and 24 hours after this high bolus dose.

Each subject brought his morning urine to the laboratory before the first blood sampling and when he returned the next day for 24 h sampling. Urine samples were also collected at 1 PM on days of experiment. These 1 PM samples reflect urinary excretions of analytes <4h (960 mg) or <6h (37.5 mg) after drug intake; diurnal variability was examined by 1 PM measurements also at baseline. Baseline values were the means of the two nocturnal samples before treatment.

**Blood sampling**

Venous blood was sampled without stasis from an antecubital vein through a 19 G butterfly needle (Abbott Ltd, Sligo, Ireland) and was anticoagulated with recombinant desulphathohirudin (CGP 39393; 11 700 ATU/mg; Ciba-Geigy, Basle, Switzerland; diluted in physiological saline, final concentration 20 µg/ml). Two centrifugations at 190 x g and 1,400 x g for 10 minutes were used to prepare platelet rich plasma (PRP) and platelet poor plasma (PPP) immediately after blood sampling. For analyses using the PFA-100 system (see below), blood was anticoagulated with sodium citrate (final concentration 0.38 % w/v) according to instructions from the manufacturer.

**Whole blood aggregometry**

Platelet aggregation in whole blood was studied using an impedance aggregometer (Chrono-log model 570-VS Four Sample; Chrono-log Corp, Haverton, PA, USA). The blood was anticoagulated with hirudin and diluted 1:1 with physiological saline. Samples were preincubated at 37°C for 5 minutes, after which agonists were added. Agonists used were collagen type 1 (Horm, Nycomed Arzneimittel, Munich, Germany) at final con-
centrations of 1, 3 or 5 µM and arachidonic acid (AA) dissolved in ethanol (Sigma Chemical Co., St. Louis, MO, USA) at final concentrations of 0.2, 0.5 or 1 mM. Each experiment included a test to verify that the final ethanol concentration in the sample had no effect per se. The amplitude of aggregation was measured after 8 minutes.

**Aggregometry in platelet rich plasma (PRP)**

A four-channel platelet aggregation profiler (PAP-4, Bio-Data Corporation, Hatboro, PA, USA) was used to study platelet aggregation in PRP. Adenosine diphosphate (ADP, Sigma Chemical Co.) was diluted in Tris buffer and the EC50 for ADP (i.e. the concentration required for half-maximal aggregation) was determined by a dose-response procedure in which the extent of aggregation after 4 minutes was measured; for details see (24). Platelet COX inhibiting effects of aspirin treatment were determined as the ability of AA (final concentrations 0.5 and 1 mM) to induce aggregation in PRP.

**Bleeding time**

Standardized transverse incisions were made on the lateral volar side of the forearm, at a constant venous pressure of 40 mmHg, using a disposable device (Surgicutt, Ortho Diagnostics, Raritan, NJ, USA). Each incision was made 10 mm distally to the previous one. Total bleeding time was measured by collecting blood on filter papers at 15 s intervals, according to a technique previously shown to reveal aspirin effects in our laboratory (25).

**Thromboxane related measurements**

Thromboxane B2 (TxB2) is the chemically stable and inactive hydration product of TXA2, and measurements of TxB2 in serum reflect platelet COX inhibition. 11-dehydro-TxB2 is the major metabolite of TxB2 which is excreted in man (26).

**Urinary 11-dehydro-TXB2 (TxM)** was determined by enzyme immunoassay (Cayman Chemicals, Ann Arbor, MI, USA) using a sample work-up procedure previously described and validated by us (27). Two ml of centrifuged urine was diluted 1:2 with 63 mM ammonium bicarbonate buffer pH 8.6 and was incubated for 3 hours to convert 11-dehydro-TxB2 to its open ring form before extraction with Bond-elute Certify-II columns (Varian). The analyte was eluted with 2% formic acid in methanol. The eluate was evaporated in a vacuum centrifuge, reconstituted in buffer (pH 8.6), and incubated 6 hours before analysis. Urinary TxB2 is expressed in relation to urinary creatinine. Data obtained with this modified assay correlate well with results obtained with GC-MS analysis of 11-dehydro-TxB2 (27).

**Serum TxB2** was determined using commercially available kits for measurements of TxB2 (Cayman Chemicals). Sample preparation and analysis were according to instructions from the manufacturer. Serum samples were only obtained from 9 individuals, as the assay was established and included in the study at a later stage.

**Urinary 2,3-dinor-6-keto prostaglandin F1α (PGI-M)**

Analysis of the 2,3-dinor-6-keto-prostaglandin F1α metabolite of prostacyclin in urine seems to be the most accurate method for assessment of systemic (extrarenal) prostacyclin biosynthesis (28). However, influences of physical activity, day to day variation and large interindividual variation have to be taken into consideration.

Urinary 2,3-dinor-6-keto prostaglandin F1α (PGI-M) was determined by LC/MS-MS (Rooney C, Harhen B, Fitzgerald D, Treumann A, manuscript in preparation). Briefly, 2 ml of urine spiked with 4 ng of the internal standard 2,3-dinor-6-keto-prostaglandin F1α-D3 (Biomol, PA, USA) was adjusted to pH 3 and centrifuged at 950 x g for 5 minutes before being applied to Empore solid phase extraction disc cartridges (3M, USA) from which the PGI-M was eluted with 1 ml of 1% methanol in ethyl acetate. Following liquid-liquid extraction with 50 mM sodium borate (pH 8.0) and derivatization with methoxyamine (Sigma, UK), the purified and derivatized organic extract was loaded onto an omni- sphere C18 reversed phase column (3 µm x 2.1 x 100 mm, Varian, UK) and eluted with a linear gradient from 5% to 45% acetonitrile in 10 mM ammonium acetate. The effluent was analyzed by a triple quadrupole tandem mass spectrometer (API 3000, Applied Biosystems). The transition of the ion at m/z 370 to 150.2 was monitored for the analyte and that of the ion at m/z 373 to 150.2 was monitored for the internal standard, using an optimized collision energy of 28 V. Concentrations of PGI-M were determined by calculating the peak height ratios for sample and internal standard.

Data from two out of nine samples in each of two individuals are missing due to technical problems with the analysis. PGI-M data from one individual were completely excluded due to variability related to poor compliance with the restrictions of physical activity during the experiment. Thus, the PGI-M data presented are from 12–14 individuals.

**Platelet function test (PFA-100) and platelet counts**

The PFA-100 analyzer (Dade Behring, Germany) was used for measurements of platelet-related primary hemostasis capacity of citrate-anticoagulated whole blood under high shear conditions (29). Closure time (CT) is the time needed to form a platelet plug occluding the aperture cut in a collagen/epinephrine coated membrane. Measurements could only be performed in 6 individuals. Platelets were counted by a semiautomatic cell counter (Cellanalyzer 460, Medonic Solna).

**Statistics**

Data are presented as mean values ± SEM. Normally distributed variables were compared with paired t-tests, whereas bleeding time data were compared with the Wilcoxon test and Spearman’s rank correlation. Effects of aspirin treatment were analyzed by 2-factor repeated measures ANOVA:s for overall effects, followed by appropriate after-testing. The software used was Statistics, version 5.5 for PC. A p value <0.05 was considered significant.

**Results**

Aspirin treatment did not change platelet or leukocyte counts or mean platelet volume.

**Bleeding time**

There was no diurnal variation of the bleeding time without treatment (6.3±0.4 to 6.1±0.5 min). Ingestion of 37.5 mg aspirin for 10 days increased the bleeding time to 10.4±1.0 min (p<0.001)
1.5–2 h after the last dose with no significant variation 24 h thereafter. The bleeding time was 11.7±1.3 min (p<0.001) 1.5–2 h after 320 mg aspirin. It was 12.2±2.3 min 4 h after 960 mg aspirin (p<0.001), and similar after 24 hours (12.3±1.4 min). Eight subjects had <60% increments of bleeding time after 37.5 mg aspirin, but similarly low increments after 320 mg. Bleeding time responses to 37.5 and 320 mg aspirin were correlated (r=0.53; p=0.041; Spearman test).

Whole blood aggregometry

There was no dose-response relationship for platelet aggregation induced by 0.2–1 mM AA in whole blood, and no significant diurnal variability without aspirin treatment. However, the response became time- and dose-dependent during aspirin treatment (Fig. 1). Thus, 37.5 mg aspirin suppressed aggregation mainly at low concentrations of AA; the inhibitory effects of low-dose aspirin were significantly attenuated after 24 h compared to 6 h (p<0.01) (Fig. 1). When using the lowest concentration of AA (0.2 mM), platelet aggregation was almost completely inhibited after 320 and 960 mg aspirin (p<0.001 for both), but there was less complete inhibition with higher concentrations of AA, and significant recovery 24 h after high-dose aspirin (Fig. 1). In fact, the aggregatory response to 1 mM AA was almost normalized 24 h after 960 mg aspirin (p<0.001 compared to 4 h value).

Treatment with 37.5 mg aspirin slightly attenuated platelet aggregation in hirudinized whole blood induced by 1 µM collagen, as the impedance decreased by 12.5±5.1% (from 19.8±0.9 to 17.1±1.2 Ohm; p<0.05). The inhibition was more pronounced after 320 mg aspirin (36.4±5.4%; p<0.001), but increasing the dose to 960 mg caused no further inhibition. Higher collagen concentrations (3 and 5µM) yielded nearly maximal platelet aggregation in hirudinized whole blood with all aspirin dosages.

Aggregometry in platelet rich plasma (PRP)

AA (1.0 mM) yielded 89±13% aggregation in hirudinized PRP before treatment. All doses of aspirin (37.5 mg, 320 mg and 960 mg) inhibited AA-induced platelet aggregation almost completely (to less than 1%), indicating good compliance and effective platelet COX inhibition. Three individuals had small aggregatory responses (3–5 %) to AA after 37.5 mg aspirin, which were abolished after 320 and 960 mg.

The platelet sensitivity to ADP decreased after 37.5 mg aspirin, as the EC₅₀ for ADP increased by 65±11% (from 1.26±0.21 to 1.97±0.31 µM; p=0.01). Higher dosages decreased the responsiveness to ADP less (EC₅₀: increased by 23±9% and 26±13% after 320 and 960 mg, respectively). One individual showed extreme increases in the EC₅₀ for ADP (e.g. from 1.3 to 275 µM after 960 mg), and was excluded from the analysis. There was no significant time dependence of aspirin effects on ADP induced aggregation in PRP.

Serum thromboxane B₂ and urinary 11-dehydrothromboxane B₂ (TXM)

There was no significant diurnal variability of the urinary TXM excretion. The nocturnal TXM excretion was reduced by 74.0±2.0% (p<0.001) after 37.5 mg aspirin, and there was no difference at <6 compared to <24 h after the last dose. After 320 mg aspirin the degree of inhibition was 82.0±1.8% (p<0.001 vs. baseline, and 37.5 mg). Four hours after 960 mg aspirin the TXM excretion was reduced by 83.9±2.8% (p<0.001 vs. baseline, and 37.5 mg), but there was significant recovery after <24 hours to 78.5±1.4% inhibition (TXM excretion increased from 9.4±1.1 to 14.8±1.5 ng/mmol creatinine; p<0.001) (Fig. 2A).

The TXB₂ production in serum was inhibited by 98.5±0.7% (n=8) after 37.5 mg aspirin, and by 98.9±0.5% (n=9) after 320 mg aspirin.
Urinary 2,3-dinor-6-keto prostaglandin F$_{1\alpha}$ (PGI-M)

The urinary PGI-M excretion was 16.5±1.6 and 14.6±1.0 ng/mmol creatinine in nocturnal and 1 PM samples, respectively, without treatment. The nocturnal PGI-M excretion decreased by 25±7% (p<0.05) after 37.5 mg aspirin, and by 28±14% (p=0.066) after 320 mg (Fig. 2B). PGI-M excretion during the day tended to decrease (by 18±8%; p=0.055) with 37.5 mg aspirin, and was reduced by 26±10% (p=0.05) 4 h after 960 mg aspirin, but recovered (from 10.6±1.5 to 13.9±2.0 ng/mmol creatinine; p<0.05) in the ensuing nocturnal sample (Fig. 2B).

Closure time, PFA-100

The closure time with the collagen/epinephrine cartridge tended to increase after 10 days of treatment with 37.5 mg aspirin (from 99.3±10.4 to 153.6±32 s; ns), and was significantly prolonged (to 179.3±32.2 s; p<0.05) after 320 mg aspirin. There was a significant correlation between closure time and bleeding time after aspirin (r=0.78; p<0.01).

Comparison of methods and correlations

Figure 3 compares the inhibitory effects of aspirin treatment at various dosages on platelet related variables in the study. It may be seen that the parameter most sensitive to inhibition by aspirin was AA induced aggregation in PRP, followed by serum TxB$_2$. TxM excretion was less markedly, but dose-dependently reduced by aspirin treatment. A dose related but weak inhibition of collagen induced aggregation in hirudinized whole blood was also found with the lowest collagen concentration, and some inhibition of PGI-M excretion.

Changes in PGI-M excretion were not correlated to changes in the platelet related variables tested in this study (bleeding time, platelet aggregation in PRP or whole blood, or TxM excretion) during aspirin treatment at any dose level. Platelet aggregation in whole blood stimulated by low AA concentrations was correlated to serum TxB$_2$ levels after 37.5 mg aspirin (e.g. r=0.73, p<0.05, at 0.2 mM AA); correlations disappeared with higher AA concentrations and aspirin dosages.

Discussion

The present study shows that once daily dosing of aspirin may be insufficient even though there is nearly complete inhibition of platelet COX activity early after dosing. Aspirin treatment suppressed TxB$_2$ in serum by more than 98% and abolished AA-induced platelet aggregation in PRP already at a very low dose level (37.5 mg daily), but there was significant recovery of platelet function 24 hours after dosing even with high doses of aspirin. Thus there was incomplete suppression of TxM excretion in urine, and AA-induced platelet aggregation could still occur in whole blood. Furthermore, we found that treatment with 37.5 mg aspirin decreased the sensitivity to stimulation by ADP in platelet rich plasma, but that this effect was less pronounced (not significant) after higher doses of aspirin, in accordance with our earlier results (30). Bleeding time increased similarly after all dosages of aspirin, and was not related to changes in the urinary excretion of PGI-M. Thus, the effectiveness of aspirin is highly dependent on the method used to evaluate its antplatelet effect.
When platelet function is investigated in vitro, the anticoagulant used may artifactually modify platelet responses. Sodium citrate is the most commonly used anticoagulant, which lowers extracellular calcium and enhances the antiplatelet effects of aspirin (22–24). We used hirudin (a highly selective thrombin inhibitor) to preserve normal ionized calcium levels, and found limited inhibition by aspirin of collagen- and AA-induced platelet aggregation. Thus, AA-induced platelet aggregation occurred even after 960 mg aspirin if the AA concentration was high enough. During low-dose aspirin treatment (37.5 mg/day) platelet aggregation in hirudinized whole blood was virtually unaffected when stimulated by 1.0 mM AA. Collagen induced platelet aggregation in hirudinized whole blood was minimally affected by aspirin treatment.

A problem with in vitro studies of platelet function in PRP is that centrifugation of the blood may remove the largest and most active platelets along with the red and white blood cells, which are known to influence platelet behaviour (31, 32). Responses to antplatelet agents like aspirin may also be influenced by the removal of other blood cells. For example, the antplatelet efficacy of low-dose aspirin (50 mg/day) is attenuated by the presence of erythrocytes (31). Leukocytes can, via transcellular prostanoid metabolism, influence platelets even if the platelet COX is inhibited (33, 34), and leukocyte-platelet cross-talk may contribute to platelet responsiveness in whole blood (32). The less efficient antiaggregatory effect of aspirin treatment in whole blood compared to PRP may thus reflect erythrocyte enhancement, extraplatelet sources of PGH₂, and/or transcellular formation of TXA₂ despite efficient inhibition of COX in platelets.

This study illustrates the importance of distinguishing between inhibition of thromboxane synthesis and inhibition of platelet activation, as reflected by, e.g., platelet aggregation, which may be more clinically relevant. Thus, we found that the very low 37.5 mg dose of aspirin, which appears to be subtherapeutic (1, 2), effectively inhibited platelet-dependent thromboxane formation with very modest effects on aggregation in whole blood. Nucleated cells are capable of regenerating the COX-1 enzyme after a couple of hours and provide PGH₂ to platelets to bypass the inhibition of platelet COX-1 (34, 35). There was less pronounced inhibition of TXM excretion compared to serum TXB₂ generation, and partial recovery of TXM excretion at the end of the dosing interval, indicating that aspirin-insensitive (presumably non-platelet) thromboxane synthesis had occurred in vivo.

Several studies have shown that aspirin effects may be limited, and that some patients at risk may not benefit from aspirin protection against cardiovascular events. There are large inter-individual differences in various responses to aspirin treatment (3, 4). However, the phenomenon which is often named “aspirin resistance” should not be confused with “treatment failure”, which may be related to aspirin insensitive disease mechanisms and poor compliance rather than “true” or “biochemical” aspirin resistance (2–4). Eikelboom, et al. found that incomplete suppression of thromboxane generation (measured as urinary TXM) was associated with an increased risk of suffering MI, stroke or cardiovascular death (36). The aspirin dosages prescribed were 80–325 mg/day, but the timing of sampling in relation to dosing, and patient compliance are not known. We found that 960 mg aspirin resulted in more pronounced inhibition of urinary TXM than 37.5 mg some 5 hours after ingestion, but that this difference had decreased 24 hours after ingestion. Chamorro, et al. showed that non-responders to 300 mg aspirin (defined as patients with recurrent stroke within 12 months despite aspirin treatment) had less effectively inhibited AA dependent platelet aggregation than patients without events (37). However, a higher dose of aspirin (600 mg/day) resulted in similar inhibition of responses to AA in the two groups (37). This is in accordance with our results showing more effective inhibition of AA-induced platelet aggregation in whole blood with higher doses of aspirin, and that aggregation was correlated to serum TXB₂ levels at a low aspirin dosage. Thus, the ability of aspirin to block platelet COX-1 (“true aspirin resistance”) should be clearly distinguished from treatment failure when discussing “aspirin resistance” (4), and the time-dependence of inhibitory effects should be considered in this context.

We found slight reductions of PGI-M excretion after aspirin treatment at all dose levels. After 960 mg aspirin there was a further reduction of PGI-M excretion <4 h after dosing, but a significant recovery after <24 hours. Vesterqvist, et al. showed that the PGI-M excretion in urine was reduced during only three hours after ingestion of high-dose aspirin (38). Others have found different degrees of inhibition of PGI-M excretion after aspirin treatment (17, 39). Thus, findings differ between studies, but it appears that aspirin treatment may reduce prostacyclin production even at low dosages. We found no relationship between changes in PGI-M excretion and any platelet function variable.

The relationship between surrogate markers for platelet function and clinical events is a critical issue determining dosing regimens. There is no single or accepted platelet function test that is optimal for the monitoring of aspirin treatment. Using urinary TXM and whole blood aggregometry in combination may be the most suitable approach for the evaluation of in vivo effects of aspirin treatment, but AA-induced aggregation in PRP may provide the best assessment of platelet COX inhibition. However, these approaches have not been evaluated in relation to clinical outcomes during aspirin treatment. Furthermore, the presently observed recovery of COX-dependent platelet aggregation between doses might need consideration regarding the dosage interval; perhaps twice daily treatment might be more efficient in patients with increased platelet turnover. Our findings of dose- and time-dependent recovery of platelet function within 24 h after dosing of aspirin are, e.g., of relevance for the inability of 100 mg aspirin every other day to reduce cardiac events in the Womens Health Study (40), as such a dose regimen may provide incomplete COX-1 inhibition during at least part of the dosing interval.

In conclusion, this study illustrates the complexity involved in evaluating the effectiveness of aspirin therapy, as different effect parameters yield very different results. Importantly, aspirin provides only weak inhibition of platelet aggregation in whole blood with normal calcium levels, despite effective platelet COX inhibition. Furthermore, there is recovery of platelet function within a normal 24 h dosing interval. The optimal dose of aspirin has been discussed for several years. Defining the optimal as-
pirin dose (and the dosing interval) for each patient group, and establishing well documented means of monitoring individual patients may further improve the secondary prevention of atherothrombotic disease by aspirin treatment.

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