The effects of varying doses of aspirin on human platelet activation induced by PAF, collagen and arachidonic acid

M. L. TAYLOR¹, N. L. A. MISSO¹, G. A. STEWART² & P. J. THOMPSON¹

¹Department of Medicine, University of Western Australia, Queen Elizabeth II Medical Centre, Nedlands, W.A. 6009 and ²The Western Australian Research Institute for Child Health, Princess Margaret Hospital for Children, Subiaco, W.A. 6008, Australia

- 1 The effect of increasing doses of orally administered aspirin (30–900 mg) on platelet aggregation and ATP release induced by arachidonic acid (AA), collagen and platelet activating factor (PAF) was assessed in 12 normal volunteers.
- 2 Aspirin ingestion was associated with a significant increase in EC_{50} for AA ($P < 0.0001$) and collagen ($P < 0.0001$) but not for PAF ($P > 0.495$) although the normal biphasic aggregation response for the latter was abolished. Maximum ATP release was reduced by aspirin for all three agonists.
- 3 The mean maximum degrees of inhibition of platelet aggregation induced by aspirin for AA, collagen and PAF were 100%, 48% and 21% of baseline, respectively. The corresponding mean maximum inhibition of ATP release was 100%, 63% and 57%. The minimum cumulative doses of aspirin producing these effects were 240, 240 and 90 mg for AA, collagen and PAF respectively. For collagen alone, there was a significant decrease in the degree of inhibition of aggregation between the last dose on day 1 (150 mg) and the baseline measurement on day 2.
- 4 Platelets from female subjects were more sensitive to collagen ($P < 0.05$) and AA ($P < 0.01$) stimulation compared with males. However, prior to aspirin ingestion, PAF produced a greater maximum response in platelets from females ($P < 0.02$) while following aspirin ingestion PAF-induced activation was inhibited to a greater degree in females ($P < 0.02$).
- 5 These results indicate that collagen- and PAF-induced platelet activation are only partially dependent on cyclo-oxygenase and for PAF this seems related only to the second phase of aggregation. The data also indicates that for platelet aggregation, the agonist and the sex of the subjects influence the degree of inhibition obtained with aspirin and these factors may be important when determining the optimum dose of aspirin for clinical use.

Keywords aspirin platelet activating factor arachidonic acid collagen platelet aggregation

Introduction

Aspirin (acetylsalicylic acid) has been used as a therapeutic agent for many decades and a wide range of doses have been recommended for a variety of medical conditions (Gallus, 1985). In many of these, its clinical efficacy has been associated with its ability to inhibit platelet aggregation and subsequent mediator release (Parkin, 1987). As such platelets aggregate following exposure to a variety of stimuli including arachidonic acid (AA), ADP, adrenaline, ATP, collagen and platelet activating factor (PAF) and it is often assumed that aspirin inhibits aggregation and mediator release,

irrespective of the stimulus, via inhibition of cyclo-oxygenase (Parkin, 1987).

Many studies have demonstrated inhibition of AA- and collagen-induced platelet activation by aspirin (Bye *et al.*, 1979; Herd *et al.*, 1987; Kuster & Frolich, 1986; McLeod *et al.*, 1986; Siebert *et al.*, 1983) but in many of these only a limited range of aspirin doses and stimulus concentrations have been used. Aspirin has also been shown to partially inhibit *ex vivo* PAF-induced platelet aggregation and ATP release (Lecrubier *et al.*, 1983; Rao *et al.*, 1982) although some investigators have been

unable to demonstrate such effects (Kuster *et al.*, 1986). Dose dependent inhibition at low but not high concentrations of PAF has also been reported (Cattaneo *et al.*, 1985; Fouque & Vargaftig, 1984; Macconi *et al.*, 1985). As a result, the effect of aspirin on PAF-induced platelet aggregation remains unclear.

There have been no comparative studies of the relative potency of aspirin with respect to inhibiting different stimuli of platelet activation and it is possible aspirin may have actions unrelated to cyclo-oxygenase inhibition (Eynard *et al.*, 1986). Clinically, a wide range of aspirin doses are given (Bochner & Lloyd, 1986) and whether aspirin is equally effective against all agonists for a range of doses is unclear. This study was therefore designed to investigate the *ex vivo* effect of a wide range of aspirin doses on human platelet aggregation and ATP release induced by three potent agonists namely AA, collagen and PAF.

Methods

Subjects and study design

Twelve healthy volunteers without a history of aspirin sensitivity were studied and comprised six men (mean age $\pm$ s.d., 33.0 ± 7.6 years) and six women (mean age $\pm$ s.d., 31.0 ± 6.9 years). All subjects gave informed consent and the study was approved by the institutional Ethics Committee. Subjects were not allowed to take aspirin or other NSAIDs during the 14 days prior to the study. Each subject was studied over a 2 day period and given three doses of aspirin on each day. Incremental oral intake of aspirin occurred at 2 hourly intervals to allow adequate time for absorption. The doses of aspirin administered were 30, 60 and 150 mg on day 1, and 300, 600 and 900 mg on day 2. At the beginning of each test day and 2 h after each dose of aspirin, platelet aggregation curves and ATP release, in response to varying concentrations of AA, collagen and PAF, were determined.

Materials

AA, collagen, PAF (1-0-hexadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine), luciferase-luciferin, human serum albumin and ATP were obtained from Sigma Chemical Co. (St Louis, MO, USA). AA was dissolved in phosphate-buffered saline (PBS) whereas collagen and luciferase-luciferin were dissolved in sterile, deionised water. PAF was dissolved in PBS containing 0.25% (w/v) human serum albumin.

Platelet aggregation and ATP release assays

Whole blood (40 ml) was collected from each volunteer by venepuncture and transferred to 5 ml siliconized glass tubes containing 0.5 ml of 3.8% (w/v) sodium citrate. Aspirin ingestion and blood collection occurred at the same time each day, to avoid any possible effects of diurnal variation. Platelet rich plasma (PRP) was obtained by centrifugation at 150 g for 15 min. The PRP was removed and the residual red cell fraction

centrifuged again at 500 g for 10 min to obtain platelet poor plasma (PPP). To minimise pH changes, the PRP was stored under 5% CO₂. A SEAC platelet counter (Firenze, Italy) was used to determine the plateletcrit and the number of platelets in all blood and PRP samples and haematocrit values were obtained in seven subjects. Platelet aggregation and ATP release were measured simultaneously in a Chronolog lumi-aggregometer (Havertown, PA, USA) at 37° C with constant stirring at 900 rev min⁻¹ and data were normalised for the platelet count for each individual by instrument calibration. For each aggregation, the required concentration of agonist was added to a 500 μ l aliquot of PRP in a siliconized glass cuvette. Luciferase-luciferin (25 μ l) was added in order to measure ATP release and the amount of ATP released was determined by calibrating the results against the response to a known amount of ATP in a separate aliquot of PRP.

Complete concentration-response curves for PAF, AA and collagen were generated by adding increasing concentrations of agonist to successive aliquots of PRP. The concentrations of the agonists required to generate the response curves ranged from 7–725 nmol l⁻¹ for PAF, 0.025–12 mmol l⁻¹ for AA and 3.7–734 μ g ml⁻¹ for collagen, depending on the subject and aspirin dose. Aggregations were always carried out in the same sequence, namely, PAF, AA and collagen to minimise the effect of time-dependent variations in aggregation (Misso & Thompson, 1990). The amplitude of the aggregation response was measured 4 min after addition of the agonist and expressed as a percentage of the light transmission through autologous PPP.

Data analyses and statistics

For each volunteer, the concentration-response curves generated before aspirin administration on day 1 were used to determine both the EC₅₀ for each agonist (the concentration producing 50% of maximum aggregation response) and the minimum concentration which produced a maximum response (MCMR). For each agonist and each dose of aspirin, separate concentration-response curves for aggregation were plotted. From these, the EC₅₀ and MCMR of the agonist was derived for each dose of aspirin together with the maximum % aggregation achieved (Max Agg). The maximum ATP release (Max ATP) was also calculated for each aspirin dose.

Using the MCMR of each agonist for each individual (as determined at baseline on day 1), the inhibitory effect of aspirin on a single concentration of agonist was also determined. The degree of inhibition of aggregation and ATP release, expressed as a percentage of the response at baseline on day 1, was plotted against aspirin dose and the areas under the inhibition curve (AUC) were determined for each subject.

To facilitate comparisons of the relative inhibitory effect of aspirin on the different agonists, both the EC₅₀ and MCMR obtained after 900 mg aspirin were expressed as a ratio of their respective baseline values. Similar ratios were also determined for Max ATP release. Comparisons between agonists were also made by determining the ratios for the area under the

percentage inhibitory dose-response curves when the MCMR was used as the standard agonist concentration.

Simple descriptive statistics were used to generate mean ($\pm$ s.e. mean) data for all variables. In addition, the data for aggregation and ATP release were compared with each other using regression analysis, while the effects of increasing aspirin doses on mean platelet responses were compared by repeated measures analysis of variance. In cases where a significant aspirin dose effect was observed, Duncan's test at a significance level of $P = 0.05$ was used to compare differences among the means. Other statistical comparisons utilised Student's paired and unpaired t -tests. The statistical analyses were performed using the SPSS and SAS programs for personal computers.

Results

Platelet responses before aspirin ingestion

Prior to the ingestion of aspirin on day 1 the mean ($\pm$ s.e. mean) whole blood platelet count was $284 (\pm 38) \times 10^9 \text{ l}^{-1}$ while in PRP it was $432 (\pm 52) \times 10^9 \text{ l}^{-1}$. Analysis of variance showed that neither in whole blood nor in PRP were there significant variations in platelet count following the administration of increasing doses of aspirin. Similarly for the mean plateletcrit for whole blood and PRP the values did not change significantly with the values at baseline being 0.25% and 0.28% for males and 0.24% and 0.27% for females respectively. The mean haematocrit for three males was 42.0% and for four of the females 41.0%.

Concentration-response curves for each agonist were generated before the administration of aspirin on day 1 of the study. From these baseline (B1) curves, the EC_{50} , the MCMR, the Max Agg and the Max ATP were determined for each agonist (Table 1). These results were in keeping with those observed in previous studies of normal volunteers (Misso & Thompson, 1990).

Figure 1 Effects of aspirin on the EC_{50} for platelet aggregation induced by a) arachidonic acid, b) collagen and c) PAF. Values are means ($\pm$ s.e. mean) of data from 12 subjects. B2 represents the mean value on day 2 prior to aspirin dosing.

* Denotes value is significantly different to the baseline mean B1 by analysis of variance and Duncan's test ($P = 0.05$).

Table 1 Baseline platelet responses to AA, collagen and PAF

Agonist	Parameter determined at baseline (B1) on Day 1*			
	EC_{50}^{**}	MCMR [†]	Max Agg [#] (%)	Max ATP ^{##} ($\mu\text{mol l}^{-1}$)
AA	0.22(0.04) mmol l^{-1}	0.31(0.06) mmol l^{-1}	88.5(1.7)	7.2(1.2)
Collagen	26.1(5.4) $\mu\text{g ml}^{-1}$	64.2(10.8) $\mu\text{g ml}^{-1}$	89.4(1.7)	5.5(1.0)
PAF	17.9(2.8) nmol l^{-1}	66.8(9.7) nmol l^{-1}	87.7(2.2)	4.9(1.2)

* Results are means ($\pm$ s.e. mean) of data from 12 subjects.

** Concentration of agonist producing 50% of maximum aggregation response.

† Minimum concentration of agonist producing a maximum aggregation response.

Maximum aggregation as % of light transmission through autologous PPP.

Maximum ATP release.

Effects of aspirin dose on platelet aggregation and ATP release

Analysis of the mean data from the donors showed that all doses of aspirin greater than 30 mg significantly increased the EC_{50} and MCMR for AA ($P < 0.0001$; Figure 1a) and Max ATP was significantly reduced for 150–900 mg ($P < 0.0001$; Figure 2a). Aspirin doses of 150–900 mg also caused the mean EC_{50} and MCMR for collagen to be significantly increased compared with B1 ($P < 0.0001$; Figure 1b) and Max ATP was significantly reduced following aspirin doses of 300–900 mg ($P < 0.0004$; Figure 2b).

Aspirin doses up to 900 mg did not significantly alter the EC_{50} for PAF ($P > 0.495$; Figure 1c) while the MCMR for PAF was significantly increased, but only for the highest dose of aspirin, when compared with B1 ($P < 0.0005$). The Max Agg for PAF was significantly reduced following aspirin doses of 60–900 mg when

Figure 2 Effects of aspirin on maximum ATP release induced by a) arachidonic acid, b) collagen and c) PAF. Values are means ($\pm$ s.e. mean) of data from 12 subjects. B2 represents the mean value on day 2 prior to aspirin dosing. * Denotes value is significantly different to the baseline mean B1 by analysis of variance and Duncan's test ($P = 0.05$).

compared with B1. The Max Agg on day 2, prior to further aspirin dosing (B2) was equivalent to the 150 mg response on day 1. Aspirin doses of 60–900 mg caused significant falls in Max ATP release compared with B1 ($P < 0.013$; Figure 2c).

The ratios for EC_{50} and MCMR values for platelet aggregation after 900 mg of aspirin compared to B1 (Table 2) showed PAF-induced aggregation was least sensitive to the effects of aspirin while AA-induced aggregation was most affected, with the effects on collagen being intermediate. The corresponding ratios for maximum ATP release indicated aspirin inhibited PAF- and collagen-induced responses to the same degree whereas there was a significantly greater inhibition of AA-induced ATP release.

Effects of increasing aspirin doses on platelet responses to a single concentration of agonist (MCMR)

With AA as the agonist, complete inhibition of both platelet aggregation and ATP release was observed following administration of a cumulative aspirin dose of 240 mg (Figure 3a) and there was a significant

Figure 3 Effects of aspirin on platelet aggregation (●—●) and ATP release (▲—▲) induced by minimum concentrations of a) arachidonic acid, b) collagen and c) PAF producing a maximum response for each subject. The values are means ($\pm$ s.e. mean) of data from 12 subjects and are expressed as % inhibition of response relative to the response at baseline on day 1 (B1).

* Denotes values are significantly different by analysis of variance and Duncan's test ($P = 0.05$).

Table 2 The ratios of the EC_{50} , MCMR and Max ATP values after 900 mg of aspirin to those at baseline on day 1 (B1)

Agonist	Ratio EC_{50} * 900:B1	Ratio MCMR** 900:B1	Ratio Max ATP† 900:B1
AA	7.5	34.7	5.1
Collagen	3.5	4.9	2.3
PAF	1.3	2.0	2.6

* Ratio of concentration of agonist producing 50% of maximum aggregation response after 900 mg aspirin to that producing the same response at baseline on day 1.

** Ratio of the minimum concentration of agonist producing a maximum aggregation response after 900 mg aspirin to that producing the same response at baseline on day 1.

† Ratio of maximum ATP release after 900 mg aspirin to that at baseline on day 1.

correlation between the inhibition of the two responses ($r = 0.99$, $P < 0.0001$). Analysis of the areas under the percentage inhibitory dose-response curves for each subject showed there were no apparent differences in the degree of the inhibitory effect of aspirin on aggregation when compared with ATP release.

With collagen as the agonist, a cumulative aspirin dose of 240 mg was associated with a maximum inhibition of aggregation of 48% and a maximum inhibition of ATP release of 63% (Figure 3b). Again, there was a significant correlation between the inhibition of aggregation and ATP release ($r = 0.79$; $P < 0.0001$) with the area under the curve analysis showing ATP release (mean AUC, 349.7 ± 38.5) was significantly more inhibited than aggregation (mean AUC 207.3 ± 21.7 ; $P < 0.0009$). A significant decrease in the degree of inhibition of collagen induced aggregation during the 18 h period between the last dose on day 1 and the baseline aggregation on day 2 was observed (48.1% vs 27.8%; Duncan's test, $P = 0.05$). There was a corresponding decrease in the inhibition of ATP release (63.0% vs 52.2%) but this did not reach statistical significance. This phenomenon was not observed with either PAF or AA.

A cumulative aspirin dose of 90 mg produced only a 21% inhibition of platelet aggregation and a 57% inhibition of ATP release when PAF was the agonist. Subsequent increasing doses of aspirin (up to 900 mg) did not induce any greater inhibition of aggregation or ATP release (Figure 3c). Regression analysis of the percentage inhibition of aggregation against percentage inhibition of ATP release showed that the two were correlated ($r = 0.68$; $P < 0.00001$). Analysis of the areas under the percentage inhibitory dose-response curves showed platelet ATP release (mean AUC, 356.1 ± 54.4) was significantly more inhibited than aggregation (mean AUC 114.2 ± 16.7 ; $P < 0.0002$). Significantly, aspirin abolished the second wave of aggregation in all 12 subjects.

The ratios of the areas under the mean percentage inhibition of aggregation dose-response curves for AA, collagen and PAF were 5:1.8:1 (Figure 3) and analysis of variance followed by Duncan's test ($P = 0.05$) showed AA-induced aggregation was significantly more inhibited than collagen-induced aggregation with both AA and collagen being significantly more inhibited than PAF. The corresponding ratios for the areas under the mean percentage inhibitory dose-response curves for ATP release were 1.6:1:1 (Figure 3). There was no significant difference between PAF and collagen for the inhibitory effect of aspirin on ATP release but AA-induced ATP release was significantly more inhibited by aspirin than that induced by the other two agonists.

Sex differences in platelet responses before aspirin ingestion

Comparison of the data for the six males and six females in the study showed that prior to aspirin ingestion on day 1, the Max Agg for PAF was significantly higher in females than in males (92.7% vs 82.7%, $P < 0.017$). With collagen as the agonist the female subjects had a lower EC_{50} (15.2 vs 37.0 $\mu\text{g ml}^{-1}$, $P < 0.036$) and a lower MCMR (43.1 vs 85.3 $\mu\text{g ml}^{-1}$, $P < 0.044$). With AA as

the agonist the female subjects also had a lower MCMR (0.16 vs 0.45 mmol l^{-1} , $P < 0.006$).

Gender differences and the effects of aspirin on platelet responses

In female, but not in male, subjects the ingestion of 900 mg aspirin resulted in the MCMR for PAF being significantly increased compared with the response at B1 (189 vs 82.5 nmol l^{-1} , $P < 0.002$). Similarly in females, but not in males, the Max Agg and Max ATP for PAF were significantly inhibited at all aspirin doses ($P < 0.0001$ and $P < 0.0012$ respectively). Analysis of the areas under the inhibitory dose-response curves also showed that inhibition of platelet responses to PAF by aspirin was greater in females than in males ($P < 0.013$ for aggregation and 0.003 for ATP release). However, for both collagen and AA as agonists, the EC_{50} , MCMR and Max ATP data as well as analysis of the areas under the inhibitory dose-response curves showed that there were no significant differences between the sexes with respect to the inhibitory effects of aspirin on platelet aggregation and ATP release.

Discussion

The results reported in this study suggest the inhibitory effects of aspirin on platelet activation depend both upon the dose of aspirin used and the nature of the agonist. In this regard, aspirin was shown to be a potent inhibitor of AA-induced aggregation and release but was less effective with respect to collagen- or PAF-induced platelet responses.

Aspirin irreversibly acetylates the enzyme cyclo-oxygenase (Burch *et al.*, 1978) and thereby inhibits the production of prostaglandins and thromboxane A_2 from AA (Smith & Willis, 1971). Since thromboxane A_2 is an important mediator of platelet aggregation it is not surprising that, in this study, a cumulative dose of 240 mg aspirin was able to inhibit totally platelet aggregation and ATP release induced by exogenous AA. These results are in agreement with many others (Bye *et al.*, 1979; Herd *et al.*, 1987; McLeod *et al.*, 1986; Wilson *et al.*, 1990) and also with current recommendations for the use of low-dose aspirin in the prophylaxis of cerebrovascular and cardiovascular disease (Bochner & Lloyd, 1986).

However, this study has shown that aspirin did not completely inhibit platelet aggregation induced by collagen and PAF. It has been suggested that products of the cyclo-oxygenase pathway play an important role in platelet responses to collagen (Siess *et al.*, 1983) but the results of the present study and others (Kuster & Frolich, 1986; McCulloch *et al.*, 1989; McLeod *et al.*, 1986) in which collagen-induced platelet activation was only partially inhibited by aspirin are more in keeping with the concept that at least two independent mechanisms contribute to collagen-induced aggregation and secretion and that only one of these is inhibited by aspirin (Best *et al.*, 1980; Siess, 1989; Zucker & Peterson, 1970).

A significant recovery of platelet response to

collagen but not to PAF or AA was observed between the first and second days of the study. A similar recovery of platelet aggregation to collagen 24 h after a single aspirin dose of 600 mg has been noted in one previous study (Siebert *et al.*, 1983). These investigators suggested entry of new platelets into the circulation during the 24 h period was the most likely explanation since a small percentage of platelets unaffected by aspirin may cause considerable platelet aggregation (Ali *et al.*, 1980; Cerskus *et al.*, 1980; Di Minno *et al.*, 1983). However, since this effect was seen only for collagen and not for AA or PAF, it is feasible that aspirin may also be acting via a mechanism independent of cyclo-oxygenase inhibition which, as yet, remains to be identified. In this context, it is unclear whether aspirin has any modulatory role with respect to the expression or function of surface adhesion receptors such as GPIIb-IIIa and GMP-140 (Parmentier *et al.*, 1990).

Platelet aggregation induced by PAF was least affected by aspirin with a maximum of 21% inhibition of aggregation being achieved. However, a cumulative aspirin dose of only 90 mg was sufficient to achieve this degree of inhibition. PAF-induced ATP release was significantly inhibited and to a greater degree, reaching a maximum of 57% at the same cumulative aspirin dose. Previous studies of the effects of aspirin on PAF-induced platelet activation have produced conflicting results. In one study in which five healthy volunteers took 500 mg of aspirin daily for five days, there was no inhibition of PAF-induced aggregation (Kuster *et al.*, 1986). However, other investigators have shown aspirin inhibits aggregation induced by low concentrations of PAF but not at higher concentrations (Cattaneo *et al.*, 1985; Fouque & Vargaftig, 1984; Macconi *et al.*, 1985) and a recent study by McCulloch *et al.* (1989) has suggested low dose aspirin can inhibit both platelet aggregation and release.

The results obtained in the present study confirm that low aspirin doses are sufficient to inhibit both PAF-induced aggregation and release and that higher doses of aspirin did not increase the degree of inhibition obtained. In all subjects, the second phase of PAF-

induced aggregation and the release reaction which accompanied it were readily inhibited by aspirin while the first phase of aggregation remained unaffected. Therefore, the initial stages of PAF-induced platelet aggregation are seemingly independent of the cyclo-oxygenase pathway with the second phase possibly being due to amplification of the primary response by thromboxane A₂ (Lauri *et al.*, 1985).

This study has also demonstrated a significant difference between the sexes in the platelet responses to all three agonists prior to aspirin ingestion. The lower EC₅₀ and MCMR values at baseline indicate platelets from females were more sensitive to collagen and AA than cells from males with PAF producing a greater maximum aggregation response as well. Other studies using either PRP or whole blood have noted similar gender associated increases in platelet aggregation using ADP, collagen or PAF (Coppe *et al.*, 1981; Meade *et al.*, 1985; Zwierzina *et al.*, 1987). The explanation for these sex differences remains uncertain but it has been suggested lower haematocrit values for women may account for this observation (Kelton *et al.*, 1980). However, other substantive studies and our own data have failed to demonstrate such an association (Coppe *et al.*, 1981; Zwierzina *et al.*, 1987). It has also been demonstrated that androgens and/or other sex-linked (hormonal) factors, may underlie the differences in platelet aggregability (Zwierzina *et al.*, 1987). Of interest, after aspirin ingestion, gender-associated effects were restricted to platelet responses to PAF alone, with aspirin having a greater effect in females than in males.

The differential inhibitory effect of aspirin on platelet aggregation to different stimuli is consistent with the concept that platelet aggregation agonists have varying degrees of cyclo-oxygenase dependence. Factors such as gender and the stimulus for platelet aggregation strongly influence the degree of inhibition seen with aspirin. In order to achieve a more complete inhibition of platelet aggregation in clinical settings in which arterial occlusion is of concern these factors will need to be considered and possibly a re-evaluation of recommended doses may be required.

References

- Ali, M., McDonald, J. W. D., Thiessen, J. J. & Coates, P. E. (1980). Plasma acetylsalicylate and salicylate and platelet cyclooxygenase activity following plain and enteric coated aspirin. *Stroke*, **11**, 9–13.
- Best, L. C., Holland, T. K., Jones, P. B. B. & Russell, R. G. G. (1980). The interrelationship between thromboxane biosynthesis, aggregation and 5-hydroxytryptamine secretion in human platelets *in vitro*. *Thromb. Haemost.*, **43**, 38–40.
- Bochner, F. & Lloyd, J. (1986). Is there an optimal dose and formulation of aspirin to prevent arterial thromboembolism in man? *Clin. Sci.*, **71**, 625–631.
- Burch, J. W., Stanford, N. & Majerus, P. W. (1978). Inhibition of platelet prostaglandin synthetase by oral aspirin. *J. clin. Invest.*, **61**, 314–319.
- Bye, A., Lewis, Y. & O'Grady, J. (1979). Effect of a single oral dose of aspirin on the platelet aggregation response to arachidonic acid. *Br. J. clin. Pharmacol.*, **7**, 283–286.
- Cattaneo, M., Canciani, M. T. & Mannucci, P. M. (1985). Human platelet aggregation and release reaction induced by platelet activating factor (PAF-acether)—effects of acetylsalicylic acid and external ionized calcium. *Thromb. Haemost.*, **53**, 221–224.
- Cerskus, A. L., Ali, M., Davies, B. J. & McDonald, J. W. D. (1980). Possible significance of small numbers of functional platelets in a population of aspirin-treated platelets *in vitro* and *in vivo*. *Thromb. Res.*, **18**, 389–397.
- Coppe, D., Wessinger, S. J., Ransil, B. J., Harris, W. & Salzman, E. (1981). Sex differences in the platelet response to aspirin. *Thromb. Res.*, **23**, 1–21.

- Di Minno, G., Silver, M. J. & Murphy, S. (1983). Monitoring the entry of new platelets into the circulation after ingestion of aspirin. *Blood*, **61**, 1081–1085.
- Eynard, A. R., Galli, G., Tremoli, E., Maderna, P., Magni, F. & Paoletti, R. (1986). Aspirin inhibits platelet 12-hydroxy-eicosatetraenoic acid formation. *J. lab. clin. Med.*, **107**, 73–78.
- Fouque, F. & Vargaftig, B. B. (1984). Triggering by Paf-acether and adrenaline of cyclo-oxygenase-independent platelet aggregation. *Br. J. Pharmac.*, **83**, 625–633.
- Gallus, A. S. (1985). Aspirin and other platelet-aggregation inhibiting drugs. *Med. J. Aust.*, **142**, 41–47.
- Herd, C. M., Rodgers, S. E., Lloyd, J. V., Bochner, F., Duncan, E. M. & Tunbridge, L. J. (1987). A dose-ranging study of the antiplatelet effect of enteric coated aspirin in man. *Aust. N.Z. J. Med.*, **17**, 195–200.
- Kelton, J. G., Powers, P., Julian, J., Boland, V., Carter, C. J., Gent, M. & Hirsh, J. (1980). Sex-related differences in platelet aggregation: influence of the hematocrit. *Blood*, **56**, 38–41.
- Kuster, L. J., Filep, J. & Frolich, J. C. (1986). Mechanism of PAF-induced platelet aggregation in man. *Thromb. Res.*, **43**, 425–433.
- Kuster, L. J. & Frolich, J. C. (1986). Platelet aggregation and thromboxane release induced by arachidonic acid, collagen, ADP and platelet-activating factor following low dose acetylsalicylic acid in man. *Prostaglandins*, **32**, 415–423.
- Lauri, D., Cerletti, C. & De Gaetano, G. (1985). Amplification of primary response of human platelets to platelet-activating factor: aspirin-sensitive and aspirin-insensitive pathways. *J. lab. clin. Med.*, **105**, 653–658.
- Lecrubier, C., Conard, J., Horellou, M. H. & Samama, M. (1983). Study of platelet aggregation induced by platelet activating factor (PAF) after administration of ticlopidine or aspirin. *Agents and Actions*, **13**, 77–80.
- Macconi, D., Morzenti, G., Livio, M., Morelli, C., Cassina, G. & Remuzzi, G. (1985). Acetyl glycerylphosphorylcholine aggregates human platelets through two distinct pathways, both dependent on arachidonic acid metabolism. *Lab. Invest.*, **52**, 159–168.
- McCulloch, R. K., Summers, J., Vandongen, R. & Rouse, I. L. (1989). Role of thromboxane A₂ as a mediator of platelet-activating-factor-induced aggregation of human platelets. *Clin. Sci.*, **77**, 99–103.
- McLeod, L. J., Roberts, M. S., Cossum, P. A. & Vial, J. H. (1986). The effects of different doses of some acetylsalicylic acid formulations on platelet function and bleeding times in healthy subjects. *Scand. J. Haematol.*, **36**, 379–384.
- Meade, T. W., Vickers, M. V., Thompson, S. G., Stirling, Y., Haines, A. P. & Miller, G. J. (1985). Epidemiological characteristics of platelet aggregability. *Br. med. J.*, **290**, 428–432.
- Misso, N. L. A. & Thompson, P. J. (1990). The human platelet aggregation model for assessing antiasthma drugs and PAF antagonists. In *Platelet-activating factor in endotoxin and immune diseases*, eds Handley, D.A., Saunders, R. N., Houlihan, W. J. & Tomesch, J. C., pp 245–256. New York: Marcel Dekker Inc.
- Parkin, J. D. (1987). The enigma of aspirin. *Aust. N.Z. J. Med.*, **17**, 192–194.
- Parmentier, S., Kaplan, C., Catimel, B. & McGregor, J. L. (1990). New families of adhesion molecules play a vital role in platelet functions. *Immunol. Today*, **11**, 225–227.
- Rao, G. H. R., Schmid, H. H. O., Reddy, K. R. & White, J. G. (1982). Human platelet activation by an alkylacetyl analogue of phosphatidylcholine. *Biochim. Biophys. Acta*, **715**, 205–214.
- Siebert, D. J., Bochner, F., Imhoff, D. M., Watts, S., Lloyd, J. V., Field, J. & Gabb, B. W. (1983). Aspirin kinetics and platelet aggregation in man. *Clin. Pharmac. Ther.*, **33**, 367–374.
- Siess, W. (1989). Molecular mechanisms of platelet activation. *Physiol. Rev.*, **69**, 58–178.
- Siess, W., Cuatrecasas, P. & Lapetina, E. G. (1983). A role for cyclo-oxygenase products in the formation of phosphatidic acid in stimulated human platelets. *J. biol. Chem.*, **258**, 4683–4686.
- Smith, J. B. & Willis, A. L. (1971). Aspirin selectively inhibits prostaglandin production in human platelets. *Nature (London) New Biol.*, **231**, 235–237.
- Wilson, K. M., Siebert, D. M., Duncan, E. M., Somogyi, A. A., Lloyd, J. V. & Bochner, F. (1990). Effect of aspirin infusions on platelet function in humans. *Clin. Sci.*, **79**, 37–42.
- Zucker, M. B. & Peterson, J. (1970). Effect of acetylsalicylic acid, other non-steroidal anti-inflammatory agents, and dipyridamole on human blood platelets. *J. lab. clin. Med.*, **76**, 66–75.
- Zwierzina, W. D., Kunz, F., Kogelnig, R. & Herold, M. (1987). Sex-related differences in platelet aggregation in native whole blood. *Thromb. Res.*, **48**, 161–171.

(Received 3 April 1991
accepted 8 August 1991)