Eur J Clin Pharmacol (1984) 27: 67–74

European Journal of Clinical Pharmacology

Inhibition of Platelet Function by a Controlled Release Acetylsalicylic Acid Formulation – Single and Chronic Dosing Studies

M. S. Roberts[1], L. J. McLeod[2], P. A. Cossum[1], and J. H. Vial[3]

[1]School of Pharmacy, [2]Department of Physiology, and
[3]Department of Medicine, University of Tasmania, G. P. O. Box 252C, Hobart, Tasmania, Australia, 7001

Summary. The extent to which a controlled release acetylsalicylic acid (ASA) formulation inhibited platelet function has been evaluated in single and chronic dosing studies. In the single dose study, the platelet inhibitory effect of the controlled release formulation was compared with that of an equivalent dose of soluble ASA and an equimolar dose of sodium salicylate (SA). In the chronic dosing study, ASA dose-response curves for platelet function, including cyclooxygenase activity, were determined for various doses (20–1300 mg) of the controlled release (enteric coated pellets) ASA formulation taken by volunteers daily for one week. Platelet function was assessed by the degree of inhibition of aggregation for several aggregating agents, and the degree of inhibition of activity of platelet cyclooxygenase quantified by the estimation of malondialdehyde (MDA) production. Plasma ASA and SA concentrations were also determined in each study. The controlled release product inhibited platelet function to the same extent as an equimolar dose of soluble ASA, but did so with much lower and sometimes undetectable peak systemic plasma ASA concentrations. SA, the direct metabolite of aspirin, did not have any effect on platelet function. The ASA dose-platelet function response curves obtained from chronic dosing with the controlled release formulation appeared to be similar to those reported previously for the soluble product. The inhibition of platelet function appeared to be unrelated to plasma ASA concentrations.

Key words: acetylsalicylic acid, platelet function; salicylate, controlled release formulation, single dosing, continuous dosing, healthy volunteers

Acetylsalicylic acid (ASA, aspirin) is being used clinically as an antithrombotic agent after transient cerebral ischaemic attacks in order to reduce the frequency of further attacks, to lower the incidence of stroke (Fields et al. 1977; Canadian Co-operative Study Group 1978), and to prevent reinfarction after myocardial infarction (Elwood and Sweetman 1979; Aspirin Myocardial Infarction Study Research Group 1980). The most suitable ASA dose and dosage interval for these uses is uncertain (Gallus 1981; Hanley et al. 1981). Ideally, the doses of ASA used should prevent platelet thromboxane A_2 (TXA_2) generation without interfering with vascular prostacyclin PGI_2 production (Moncada and Vane 1980). In most of the studies investigating optimal ASA dosage regimens for cardiovascular pathology the soluble dose form has been used either with a single dose or with chronic dosing (Masotti et al. 1979; Preston et al. 1981; Hanley et al. 1981; Hoogendijk and Ten Cate 1980; Patrignani et al. 1982; Fitzgerald et al. 1983; Burch et al. 1978; Baker and Pitney 1982; Nuotto et al. 1983).

Platelet function can be assessed by estimating the ability of platelet aggregators to induce ex vivo aggregation (Born 1962) and the platelet cyclooxygenase activity can be assessed from the production of TXA_2 or the ability of platelets to produce malondialdehyde (MDA), an end-product from arachidonic acid metabolism (Flower et al. 1973). The assessment of vessel wall cyclooxygenase activity is difficult, and previous methods based on the estimation of a metabolite of prostacyclin, 6-oxo-$PGF_{1\alpha}$, appear to be inadequate (Blair et al. 1982).

The bioavailability of ASA and SA from rapid and slow release formulations has recently been compared (Brantmark et al. 1982). As the bioavailability and peak concentrations of ASA was only rarely detected in systemic blood following the slow

release ASA, Brantmark et al. (1982) suggested that the rapid release formulations of ASA should be the preferred antiplatelet therapy. However, the relationships between pharmacokinetic data and platelet function have not been fully explored. In the first phase of the present study we compared the platelet aggregability, platelet cyclooxygenase activity, plasma ASA concentrations and plasma SA concentrations in blood samples serially taken from male volunteers after ingestion of either a 300 mg formulation of a controlled release ASA formulation containing enteric coated pellets, a soluble ASA formulation, or an equimolar dose of SA. In the second phase, we carried out a dose ranging evaluation of the effects of the controlled release product, given daily for one week, on the platelet function of male and female volunteers.

Materials and Methods

Materials

Preparations used in the study were: Aspro-Clear, Nicholas Pty. Ltd., Chadstone, Victoria; Astrix capsules containing enteric coated pellets (~1.2 mm) of ASA, F.H. Faulding & Co Ltd, Thebarton, South Australia; sodium salicylate mixture; Australian Pharmaceutical Formulary, 1984. Materials included (–)-adrenalin 1 mg/ml, David Bull Laboratories, Mulgrave, Victoria; equine collagen 1 mg/ml, Hormon-Chemie; arachidonic acid, Grade I porcine liver, Sigma Co; N-ethylmaleimide (NEM), Sigma Co.

Single Dose Study

After overnight fasting, 9 healthy male volunteers, aged between 19 an 24 years and weighing between 67 and 79 kg, received, with 200 ml water, equimolar oral doses of salicylate (230 mg) in the form of soluble ASA, sodium salicylate and a capsule formulation containing enteric coated pellets of ASA at different times. An interval of at least 2 weeks elapsed between the administration of each dose-form to the same subjects. Blood samples were collected immediately before ingestion of the dose and at fixed times thereafter.

Continuous Dose Study

Sixteen healthy volunteers (8 male and 8 female) were used whose ages ranged from 20 to 30 years (average 22.6 years) and with body weights ranging from 54 to 105 kg (average 68.8 kg). The study was conducted in a cross-over manner using 2 groups of 8 subjects, each subject ingesting 3 different doses of either 20, 50, 100, 200, 650 or 1300 mg ASA in the controlled release formulation. The appropriate amount of enteric coated pellets were weighed out and placed in gelatin capsules. Each group of 8 subjects consisted of 4 males and 4 females. A group of males (4) only, taking either 20, 50, 100 or 200 mg doses, and all subjects in the groups taking 650 and 1300 mg were used specifically to determine the degree of inhibition of platelet function induced by arachidonic acid (AA).

Control values of platelet aggregation and MDA production were estimated before the commencement of each dosing schedule. Subjects ingested one capsule of a given dose of the controlled release product each morning for 6 days. On the seventh morning, blood samples were taken for assessment of trough values of platelet function and plasma ASA concentrations. Subjects then ingested the last capsule and three hours later peak values of platelet function and plasma ASA concentrations were determined. This time was chosen to correspond with the time (3 h) to reach mean peak plasma ASA concentration determined in the single dose study (Fig. 2).

Subjects in both groups were not permitted to take any drugs for two weeks prior to the study or during the study. The experiments were performed according to the principles of the Declaration of Helsinki. Approval was obtained from the University of Tasmania Committee on ethical aspects of human biological research, and informed written consent was obtained from each subject. Unless otherwise stated the data are expressed as means ± SEM.

Blood Samples

Blood was collected into a plastic syringe either by venepuncture (18–19 G needles) or from an indwelling catheter (Jeltec 17–18 G) in a forearm vein. The blood was immediately distributed to tubes containing anticoagulants for the appropriate determination. Blood for platelet function tests was used within 3 h.

Plasma ASA and SA Concentrations

Plasma ASA and SA concentrations were determined by HPLC using the method developed in our laboratories (Rumble et al. 1980). Blood (5 ml) was added to ice-cold tubes containing 50 µl heparin (1000 µ/ml) and 50 µl of potassium fluoride (50% w/v). It was quickly mixed and centrifuged to obtain plasma which was frozen (−20 °C) until analysis within 3 days of collection. The procedure involves

injecting deproteinated plasma onto a C_{18} HPLC column. A mobile phase of 30% acetonitrile in 0.03% phosphoric acid pH 2.5, and a wavelength setting of 237 nm allows for the determination of ASA and SA. The minimum level of detection of ASA and SA is 0.1 μg/ml plasma with a coefficient of variation of 4.6% ($n = 10$) for ASA assays.

MDA Production

MDA production induced by stimulation of platelet rich plasma (PRP) with N-ethylmaleimide (NEM) was measured spectrophotometrically by a modified method of Catalano et al. (1981). PRP was obtained from 7.5 ml blood collected into 1 ml 3.8% w/v sodium edetate. Saline (1.5 ml) was added and the mixture centrifuged at $190 \times g$ for 15 min. Two ml of the resultant PRP were added to 5 ml saline and centrifuged at $2000 \times g$ for 10 min. The supernatant was decanted and the tube inverted and drained onto absorbent paper for 2 min. The platelet button so obtained was resuspended in 0.9 ml 14 mM Tris buffer (pH 7.4) and incubated for 15 min with 0.1 ml 50 mM NEM or 0.1 ml 2 mM arachidonic acid (AA). The reaction was stopped by the addition of 1 ml of 20% trichloracetic acid in 0.6 M HCl and cooled in ice for 5 min. The mixture was centrifuged at $2000 \times g$ for 15 min and 1.5 ml of the clear supernatant was mixed with 0.3 ml of 0.13 M thiobarbituric acid (TBA) reagent and heated in a water bath at 70 °C for 30 min using marbles as condensers. The TBA reagent was prepared by dissolving 1.73 g TBA in 0.25 M Tris buffer, adjusting the pH to 7 with 10 M HCl and filtering through a number 3 sintered glass funnel. The reaction mixture was cooled to room temperature and the absorbance of the pink chromagen was measured at 532 nm on a Pye Unicam SP6-550 spectrophotometer against a control from PRP without NEM or AA. Platelet counts were made on a Thromocounter C, Coulter Electronics Ltd., and MDA production was calculated as nanomoles per 10^9 platelets.

Platelet Aggregation

Platelets from 9 ml blood mixed with 1 ml 3.8% sodium acid citrate solution were obtained by centrifugation at $190 \times g$ for 15 min to produce PRP. Further centrifugation at $2000 \times g$ for 15 min gave platelet poor plasma (PPP). Platelet counts for PRP were obtained as before and PRP was diluted with PPP to give final platelet counts of $200–250 \times 10^9$/l. Platelet aggregation was measured by the method of Born (1962) using a Chronolog aggregometer. Aggregation of 450 μl PRP was initiated by adrenalin (2.5 μM) and collagen (2.5 μg). Arachidonic acid (AA) (0.87 mM) was also used in some experiments. Inhibition of platelet aggregation was measured as the decrease in light transmission and expressed as the percentage of control aggregation.

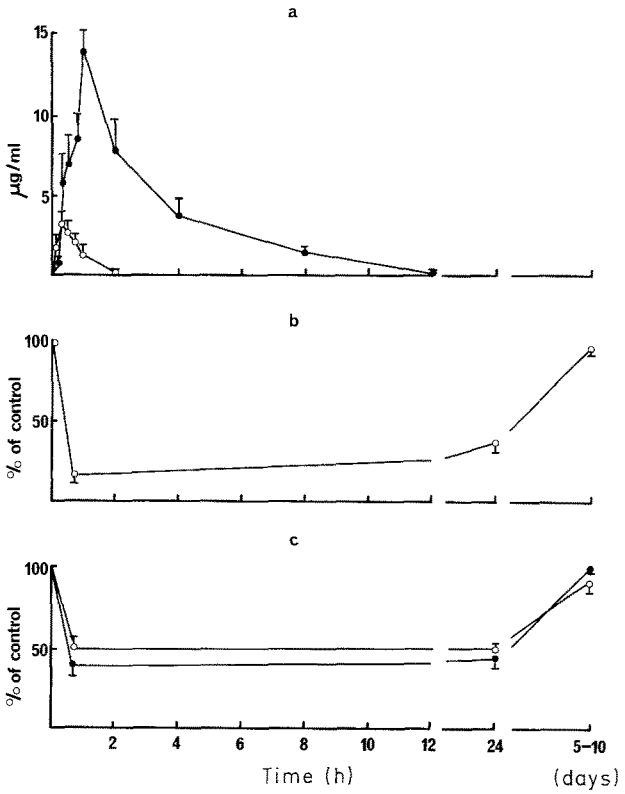

Fig. 1a–c. Plasma ASA/SA concentrations, platelet cyclooxygenase activity and platelet aggregability after ingestion of 300 mg soluble ASA; **a** Systemic plasma ASA (○) and SA (●) concentrations; **b** Platelet cyclooxygenase activity determined by MDA production induced by NEM; **c** Platelet aggregability after induction by collagen (●) and adrenalin (○) (mean ± SEM)

Results

Single Dose Study

Plasma ASA and SA Concentration. Figures 1a and 2a show the mean ASA and SA concentrations after ingestion of the soluble and the controlled release ASA. Fig. 3a shows the mean SA concentrations after ingestion of sodium salicylate mixture. ASA was absorbed much more slowly from the controlled release formulation than from the soluble product which is evident from the longer peak concentration times and lower plasma ASA concentrations for the controlled release product. The times to reach peak ASA and SA concentrations were 3.33 ± 0.55 and 4.38 ± 0.29 h for the controlled release formulation

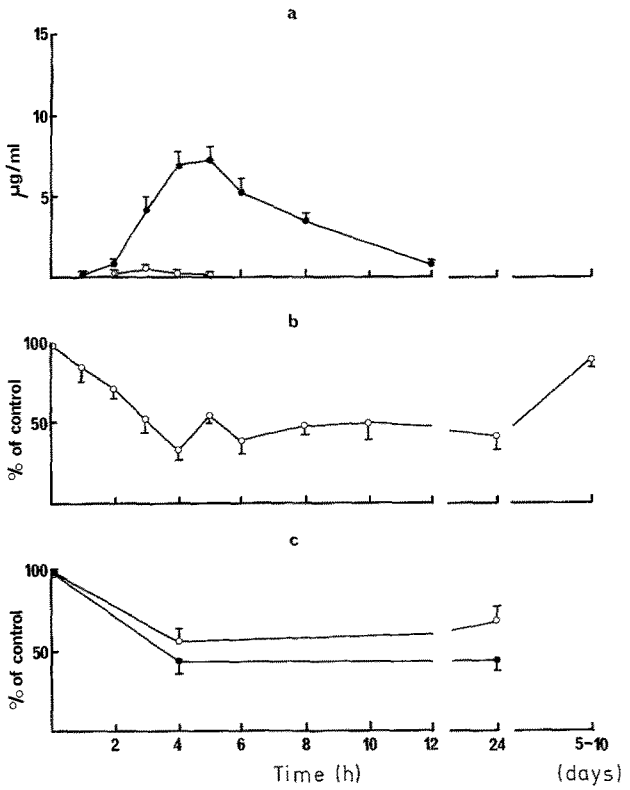

Fig. 2 a–c. Plasma ASA/SA concentrations, platelet cyclooxygenase activity and platelet aggregability after ingestion of 300 mg controlled-release ASA; **a** Systemic plasma ASA (○) and SA (●) concentrations; **b** Platelet cyclooxygenase activity determined by MDA production induced by NEM; **c** Platelet aggregability after induction by collagen (●) and adrenalin (○) (mean ± SEM)

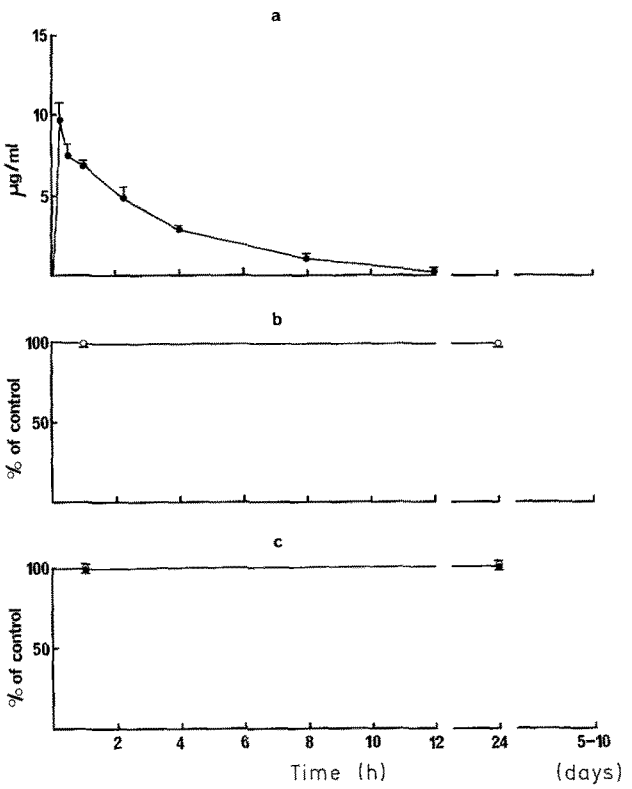

Fig. 3 a–c. Plasma SA concentrations, platelet cyclooxygenase activity and platelet aggregability after ingestion of sodium salicylate mixture containing 230 mg salicylate; **a** Systemic plasma concentrations; **b** Platelet cyclooxygenase activity determined by MDA production induced by NEM; **c** Platelet aggregability after induction by collagen (○) and adrenalin (●) (mean ± SEM)

and 0.38 ± 0.04 and 0.91 ± 0.16 h for the soluble formulation, respectively. The mean peak ASA concentration for the controlled release product (0.61 ± 0.22 μg/ml) was less than one tenth that achieved for the soluble form (7.65 ± 0.81 μg/ml). In two subjects taking the controlled release preparation, no ASA was detectable (< 0.1 μg/ml) in the plasma.

The area under the plasma ASA concentration-time curve was lower for the controlled release formulation (0.79 ± 0.26 $\mu g \cdot h \cdot ml^{-1}$) than for the soluble product (2.84 ± 0.40 $\mu g \cdot h \cdot ml^{-1}$). However the differences in area do not result from differences in absorption as the area under metabolite (salicylate) concentration-time curve was similar for the controlled release (39.45 ± 4.0 $\mu g \cdot h \cdot ml^{-1}$ and soluble (33.37 ± 5.13 $\mu g \cdot h \cdot ml^{-1}$) dose forms (Figs. 1a and 2a). A similar area (32.3 ± 2.9 $\mu g \cdot h \cdot ml^{-1}$) under the plasma SA concentration-time curve was also found after ingestion of the sodium salicylate mixture (Fig. 3).

Platelet Aggregability and MDA Production. The maximum inhibition by ASA of platelet aggregation induced by collagen and adrenalin was 40% and 70% respectively. Figs. 1b and 2b show mean inhibition of MDA production, and Figs. 1c and 2c show platelet aggregability after soluble and controlled release ASA respectively. The inhibition of platelet aggregability and MDA production was not significantly different (student's t-test $p > 0.05$) for each of the formulations. Both forms of ASA produced a prolonged inhibition of platelet function. Full recovery of platelet aggregability and MDA production occurred at 5–10 days in all subjects (Figs. 1b, c; 2b, c). An equimolar dose of sodium salicylate had no inhibitory effect either on MDA production or on platelet aggregation (Fig. 3b, c).

Figure 4 shows the relationships between the inhibition of MDA production in individual subjects and the corresponding peak ASA concentrations and area under the plasma ASA concentration-time profiles. It is apparent that the peak ASA concentrations and area under the plasma ASA concentration-time profiles vary considerably between subjects for both the soluble and controlled release formulations. No relationships are apparent between the inhibition

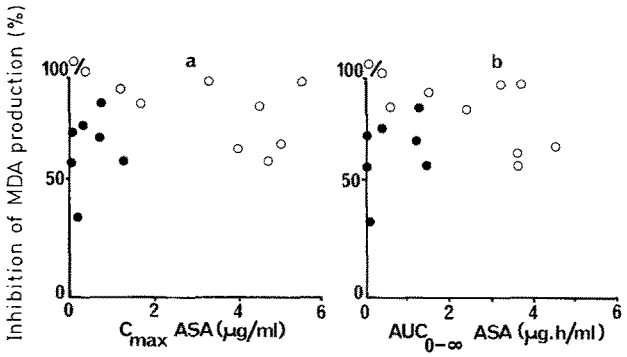

Fig. 4a and b. Platelet cyclooxygenase activity estimated as percentage inhibition of malondialdehyde (MDA) production in individual subjects after ingestion of 300 mg soluble and controlled-release ASA products: **a** Comparison between the inhibition of MDA production and the peak systemic plasma concentrations (C_{max}) of ASA for soluble (○) and controlled-release (●) dosage forms; **b** Inhibitions of MDA production compared with the area under the systemic plasma ASA concentration-time curve ($AUC_{0-\infty}$ ASA) for soluble (○) and controlled-release (●) dosage forms

Table 1. Plasma concentrations of aspirin (ASA) and salicylate (SA) 3 h after ingestion of the seventh daily dose of the controlled release formulation (Astrix) (mean ± SEM)

Dose [mg]	ASA concentration [μg/ml]	SA concentration [μg/ml]
20	< 0.1	0.37 ± 0.09
50	< 0.1	0.56 ± 0.21
100	0.15 ± 0.07	1.06 ± 0.45
200	0.21 ± 0.06	2.37 ± 0.86
650	0.68 ± 0.26	4.00 ± 1.28
1300	1.51 ± 0.50	17.56 ± 4.81

of MDA production, plasma ASA concentrations, and areas under the plasma ASA concentration-time curves.

Continuous Dose Study

Plasma ASA and SA Concentrations. Plasma ASA concentrations were not detectable in any blood samples taken immediately before the last ASA dose (trough sample). Corresponding plasma (trough) SA concentrations were only detectable in one subject taking 650 mg ASA daily and in four subjects taking 1300 mg ASA, the maximum SA concentration being 0.4 μg/ml. Table 1 shows the mean plasma ASA and SA concentrations in samples taken 3 h after (peak) the last ASA dose. ASA was not detectable in any subject after the ingestion of the 20 mg dose and in only 2 subjects given the 50 mg dose. Only one subject (taking 20 mg ASA) had undetectable levels of plasma SA. The detectable plasma concentrations of

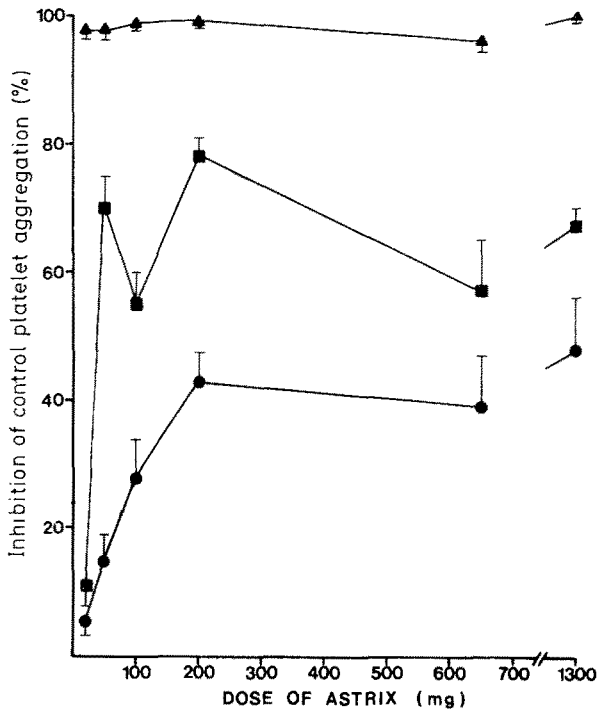

Fig. 5. The inhibitory effects of a controlled release formulation of ASA (Astrix) 20–1300 mg, on platelet aggregation induced by arachidonic acid (▲), adrenalin (■) and collagen (●) in healthy volunteers 3 h after ingestion of the seventh daily dose (mean ± SEM)

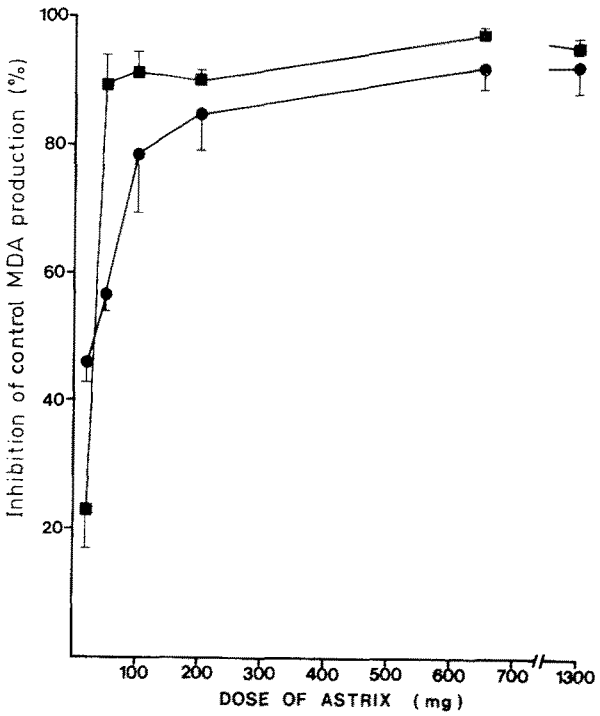

Fig. 6. The inhibitory effects of a controlled release formulation of ASA (Astrix), 20–1300 mg, on arachidonic acid (●) and N-ethylmaleimide (■) induced malondialdehyde production by the platelets of normal volunteers 3 h after ingestion of the seventh daily dose (mean ± SEM)

Table 2. Platelet function in blood samples taken immediately before the last dose (trough sample -T) and 3 h after the last dose (peak sample -P) in subjects ingesting the controlled release ASA formulation (Astrix) daily for one week

Dose [mg]	% Inhibition of control (mean ± SEM)					
	Adrenalin induced aggregation		Collagen induced aggregation		MDA synthesis	
	T	P	T	P	T	P
20	10.3± 3.6	11.2±3.8	6.7±2.5	5.6±1.9	41.5±4.8	23.0±6.0
50	63.5± 8.9	70.2±5.2	14.9±5.0	14.7±4.1	51.4±8.4	89.6±4.4
100	49.3±10.6	54.7±5.4	24.4±8.6	28.4±6.3	78.4±3.2	91.1±3.2
200	77.2± 3.4	78.0±3.7	37.6±3.7	43.4±5.1	83.4±5.5	89.4±1.2
650	53.2± 4.8	56.7±8.4	29.0±7.9	39.2±8.1	86.1±1.7	97.2±0.8
1300	65.7± 4.4	66.7±3.4	37.5±7.1	47.9±7.8	87.8±2.0	95.0±1.7

Table 3. Platelet function in males (M) and females (F) for the blood samples taken 3 h after the final daily dose of the controlled release ASA formulation (Astrix) taken for 1 week

Dose [mg]	% Inhibition of control (mean ± SEM)					
	Adrenalin induced aggregation		Collagen induced aggregation		MDA synthesis	
	M	F	M	F	M	F
20	11.0± 1.0	6.5± 3.7	3.2± 2.2	8.0± 3.0	28.3±12.3	16.0±9.9
50	74.5± 6.3	61.5± 7.5	9.7± 4.3	21.3± 6.3	86.0± 6.7	94.7±4.8
100	43.0±24.8	63.5± 1.5	33.5±12.2	23.3± 4.0	86.0± 4.9	96.2±2.5
200	76.7± 4.0	79.3± 6.9	41.0± 7.2	45.2± 7.9	84.5± 3.7	91.0±0.91
650	58.0± 9.0	52.5±14.6	46.0±12.4	32.5±10.8	97.0± 0.8	97.5±1.5
1300	62.0± 7.0	69.5± 6.6	50.5± 9.9	45.2±13.2	97.0± 1.4	92.0±4.0

ASA appeared to be directly related to the dose of the controlled release ASA formulation (Table 1).

Platelet Aggregability and MDA Production. Figure 5 shows the controlled release ASA formulation dose-response curves for platelet aggregation in samples taken 3 h after ingestion of the final ASA dose. With collagen the maximal inhibition of platelet aggregation by ASA was 40%. With adrenalin, ASA inhibited only the second phase of aggregation. The maximum inhibition of AA induced aggregation was 100% with all the doses of ASA used. However, to achieve maximal inhibition of collagen or adrenalin induced aggregation, doses of 100–200 mg ASA were required.

Fig. 6 shows the controlled release ASA formulation dose-response curves for AA and NEM induced MDA production by platelets in samples taken 3 h after ingestion of the final ASA dose. AA induced MDA production appeared to be maximally inhibited by 50 mg and above of the controlled release ASA product. Doses of 100 mg and above of the controlled release product were required for maximal inhibition of NEM induced MDA production. The extent of inhibition of MDA production and platelet aggregation was similar in blood samples taken immediately before the last dose and 3 h after the last dose (Table 2). No apparent differences in platelet function between male and females were found for any of the controlled release doses used (Table 3).

Discussion

The differences in bioavailability of ASA from single doses of the soluble and the controlled release formulations are consistent with that of Brantmark et al. (1982). However, the degree of inhibition of platelet function is similar for the two formulations despite marked differences in plasma ASA concentrations (Figs. 1 and 2). The magnitude of platelet function inhibition is unrelated to the peak ASA concentrations or to the area under the plasma ASA concentration-time curve (Fig. 4). A possible explanation for this lack of correlation is the irreversible and saturable acetylation of platelets (Roth et al. 1975). From the controlled release formulation the plasma ASA concentration were very low, and in two subjects were undetectable (<0.1 μg/ml). However, there was a corresponding marked inhibition of platelet function. It is apparent, therefore, that the platelets may be acetylated when plasma ASA concentration is very low. The lack of correlation between inhibition of platelet function and plasma ASA concentrations (Fig. 4) shows that plasma ASA concentrations cannot be used as an index of platelet inhibition pro-

duced by ASA. ASA, rather than SA, leads to platelet function inhibition because ingestion of an equimolar amount of sodium salicylate had no effect on platelet function (Fig. 3 b, c).

The present study shows that low doses of either a soluble or a controlled release formulation are adequate for the inhibition of platelet function as assessed in these experiments. Relatively high doses (1200–1300 mg) of soluble ASA were employed in the initial clinical trials evaluating the efficacy of ASA for transient ischaemic attacks (Fields et al. 1977; The Canadian Co-operative Study Group 1978). More recent studies have suggested that lower doses of soluble ASA may be preferred to the higher doses because the lower doses of ASA may inhibit platelet thromboxane A_2 (TXA_2) production without markedly interfering with PGI_2 production by vessel wall cyclooxygenase (Masotti et al. 1979; Preston et al. 1981; Hanley et al. 1981; Patrignani et al. 1982; Fitzgerald et al. 1983). Figures 5 and 6 show that low doses (< 200 mg) of the controlled release ASA formulation give maximal inhibition of platelet aggregation and MDA production.

The dose of the controlled release formulation required to produce 50% of maximal inhibition of platelet aggregation/MDA production was less than 50 mg for AA and adrenalin, and less than 100 mg for collagen. This dose is comparable with doses reported for faster releasing ASA formulations. Burch et al. (1978) found that repeated daily administration of 20, 80 and 325 mg ASA for 5–7 days produced, 61, 86 and > 95% inactivation of ASA susceptible cyclooxygenase, respectively. Lorenz et al. (1981) found that repeated daily administration of 100 mg ASA almost totally inhibited aggregation and TBX_2 formation after induction by AA, adenosine diphosphate and low collagen concentrations. Larger collagen concentrations (of the order used in this study and in that of Masotti et al. 1979) resulted in reduced platelet aggregation at 100 mg ASA per day but with complete suppression of TBX_2 formation (Patrona et al. 1980). The incomplete inhibition by ASA of higher concentrations of collagen induced aggregation (Fig. 1) probably results from collagen inducing aggregation by other mechanisms independent of TXA_2 synthesis (Emms et al. 1982). As the ASA dose-platelet function response curves appear to be similar for the controlled release and a conventional soluble ASA formulation, the relative extent to which these formulations affect vessel wall cyclooxygenase is of clinical significance. The effect of conventional (fast release) ASA formulations on PGI_2 synthesis in vivo has been evaluated by several workers. Masotti et al. (1979) suggested that a single dose of approximately 175 mg (2.5 mg/kg body weight) had no effect on PGI_2 synthesis induced by forearm ischaemia, whereas larger doses (3.5 to 10 mg/kg body weight) significantly inhibited PGI_2 synthesis with full recovery by 48 h. Preston et al. (1981) found that 150 and 300 mg ASA substantially depressed the production of 6-oxo-$PGF_{1\alpha}$ from autologous human venous biopsies. Hanley et al. (1981) found 81 mg ASA taken 14 h preoperatively resulted in approximately 60% depression of the release of PGI_2-like material from autologous postoperative venous biopsies. However a single dose of 40 mg had no effect on PGI_2 synthesis by venous tissue.

The effects of ASA on endothelial cell wall PGI_2 production are difficult to estimate because PGI_2 is a local hormone present only in very low concentrations in systemic plasma (Haslam and McGlenanghan 1981; Blair et al. 1982). It has been suggested that under normal physiological conditions the concentrations of PGI_2 present in plasma are too low to have effect on platelet function (Steer et al. 1980). Recently, the metabolites of prostacyclin have been quantified (particularly 6-oxo-$PGF_{1\alpha}$) in the urine of subjects taking soluble ASA (Fitzgerald et al. 1983; Patrignani et al. 1982). Patrignani et al. (1982) suggested that 0.45 mg ASA/kg (i.e. about 30 mg) may be a suitable dose since platelet activity was impaired while renal production of PGI_2 was not during one month of therapy in healthy subjects. However, Fitzgerald et al. (1983) using urine levels of metabolites of TxA_2 and PGI_2 as indices of platelet and vessel wall cyclooxygenase activity found that even doses as low as 20 mg per day for 8 d had a marginal effect on vessel wall cyclooxygenase, the degree of selectivity decreasing with increasing dose. Figures 1, 2, 5 and 6 show that the controlled release preparation in doses in excess of 100 mg produce maximum inhibition of platelet aggregation as measured by ex vivo methods. The extent to which vessel wall cyclooxygenase inhibition is dependent on the dose of controlled release ASA is unknown. However, it is possible that the lower plasma ASA concentrations found for the controlled release formulation are of clinical significance because the lower peak ASA concentrations in the systemic circulation could cause less inhibition of endothelial cell prostacyclin production. A controlled release formulation may result in selective inhibition of platelet cyclooxygenase in the portal circulation with little systemic ASA available from the controlled release form for the inhibition of vascular endothelial cyclooxagenase (Ali et al. 1980; McLeod et al. 1981). It has also been suggested that the major metabolite of ASA, salicylic acid (SA), may protect the vessel wall more than platelets from the effects of ASA (Cerletti et al. 1981). The peak plasma ASA/SA concentration ratio is about 0.1 for

the controlled release formulation (Fig. 1). In contrast, peak plasma ASA/SA concentration ratio for soluble ASA products is about 0.5 (Rowland et al. 1972). Although the above data may support a more selective inhibition of platelet cyclooxygenase by controlled release ASA formulations, than by soluble products, this hypothesis can only be tested by quantification of PGI_2 production. Being enteric coated, the formulation of controlled release aspirin may be preferable to a soluble product because it would cause less gastric irritation and/or bleeding.

In conclusion, although similar ASA dose-platelet function response curves appear to exist for the controlled release and a conventional ASA formulation, the possibility exists that the controlled release formulation may have only a relatively small effect on vessel wall cyclooxygenase because of the much smaller systemic plasma levels of ASA produced.

Acknowledgements. We would like to thank F. H. Faulding & Co. Ltd., Thebarton, South Australia for the supply of Astrix, and for their generous financial support.

References

1. Ali M, McDonald JWD, Thiessen JJ, Coates PE (1980) Plasma acetylsalicylate and salicylate and platelet cyclooxygenase activity following plain and enteric-coated aspirin. Stroke 11: 9–13
2. Aspirin Myocardial Infarction Study Research Group (1980) A randomized controlled trial of aspirin in persons recovered from myocardial infarction. J Am Med Assoc 243: 661–672
3. Baker RI, Pitney WK (1982) How much aspirin to inhibit platelet function? Med J Aust 2: 376–377
4. Blair IA, Barrow SE, Waddell KA, Lewis PJ, Dollery CT (1982) Prostacyclin is not a circulating hormone in man. Prostaglandins 23: 579–589
5. Born GVR (1962) Aggregation of platelets by adenosine diphosphate and its reversal. Nature 194: 927–929
6. Brantmark B, Wåhlin-Boll E, Melander A (1982) Bioavailability of acetylsalicylic acid and salicylic acid from rapid and slow release formulations, and in combination with dipyridamol. Eur J Clin Pharmacol 22: 309–314
7. Burch JW, Stanford N, Majerus PW (1978) Inhibition of platelet prostaglandin synthesis by oral aspirin. J Clin Invest 61: 314–319
8. Catalano PM, Smith JB, Murphy S (1981). Platelet recovery from aspirin *in vivo;* differing patterns under various assay conditions. Blood 57: 99–105.
9. Cerletti C, Livio M, DeGaetano G (1981). Non-steroidal anti-inflammatory drugs react with two sites on platelet cyclooxygenase. Evidence from *in vivo* drug interaction studies in rats. Biochem Biophys Acta 714: 122–126.
10. Elwood PC, Sweetman PM (1979). Aspirin and secondary mortality after myocardial infarction. Lancet 2: 1313–1315.
11. Emms H, Lewis CP, Watts IS (1982). The role of thromboxane A_2 (TxA_2) and ADP in collagen-induced platelet aggregation. Br J Pharmacol 75: 167P
12. Fields WS, Lemak NA, Frankowski RF, Hardy RJ (1977). Controlled trial of aspirin in cerebral ischaemia. Stroke 8: 301–314
13. Fitzgerald GA, Oates JA, Hawiger J, Mass RL, Roberts LJ, Lawson JA, Brash AR (1983). Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man. J Clin Invest 71: 676–688
14. Flower RJ, Cheung HS, Cushman DW (1973). Quantitative determination of prostaglandins and malondialdehyde formed by the arachidonate oxygenase (prostaglandin synthetase) system of bovine seminal vesicles. Prostaglandins 4: 325–341
15. Gallus AS (1981) Prostacyclin and blood coagulation? What therapeutic implications? Curr Ther 22 [8]: 19–22
16. Hanley SP, Bevan J, Cockbill SR, Heptinstall S (1981) Differential inhibition by low-dose aspirin of human venous prostacyclin synthesis and platelet thromboxane synthesis. Lancet 1: 969–971
17. Haslam RJ, McGlenaghan MD (1981) Measurement of circulating prostacyclin. Nature 292: 364–366
18. Hoogendijk EMG, Ten Cate JW (1980). Aspirin and platelets. Lancet 1: 93–94
19. Lorenz R, Siess W, Weber PC (1981) Effects of very low versus standard dose acetylsalicylic acid, dipyridamole and sulfinpyrazone on platelet function and thromboxane formation in man. Eur J Pharmacol 70: 511–515
20. McLeod LJ, Cossum PA, Roberts MA, Vial JH (1981) The effects of sodium salicylate, soluble aspirin and enteric coated aspirin on platelet activity. Aust J Pharm Sci 10: 51
21. Masotti G, Poggesi L, Galanti G, Abbate R, Neri Serneri GG (1979) Differential inhibition of prostacyclin production and platelet aggregation. Lancet 2: 1213–1216
22. Moncada S, Vane JR (1980) Biological significance and therapeutic potential of prostacyclin. J Med Chem 23 [6]: 591–593
23. Nuotto E, Gordin A, Paasonen MK, Metsä-Ketelä T, Lamminsivu U (1983) Effects of acetylsalicylic acid on plasma thromboxane B_2 and platelet aggregation in man. Eur J Clin Pharmacol 25: 313–317
24. Patrignani P, Filabozzi P, Patrona C (1982) Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. J Clin Invest 69: 1366–1372
25. Preston PE, Whipps S, Jackson CA, French AJ, Wyld PJ, Stoddard CJ (1981) Inhibition of prostacyclin and platelet thromboxane A_2 after low dose aspirin. N Engl J Med 304: 76–79
26. Roth GJ, Stanford N, Majerus PW (1975) Acetylation of prostaglandin synthetase by aspirin. Proc Natl Acad Sci 73: 3073–3076
27. Rowland M, Riegleman S, Harris PA, Sholkoff SD (1972) Absorption kinetics of aspirin in man following oral administration of an aqueous solution. J Pharm Sci 61: 379–385
28. Rumble RH, Roberts MS, Wanwimolruk S (1981) Determination of aspirin and its major metabolites in plasma by high performance liquid chromatography without solvent extraction. J Chromatogr Biomed Appl 225: 252–268
29. Steer ML, MacIntyre DE, Levine L, Salzman EW (1980) Is prostacyclin a physiologically important circulating antiplatelet agent? Nature 283: 194–195
30. The Canadian Co-operative Study Group (1978) A randomized trial New Engl J Med 229: 53–59

Received: September 17, 1983
accepted in revised form: April 17, 1984

M. S. Roberts, Ph. D.
The University of Tasmania
School of Pharmacy
Box 252C
Hobart, Tasmania, Australia 7001