Inhibition of platelet release reaction by acetylsalicylic acid

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Summary

The inhibitory potency and duration of action of single doses of aspirin B.P., claradin (a low sodium effervescent preparation of acetylsalicylic acid) and aloxiprin (an aluminium co-polymer of acetylsalicylic acid) on platelet release reaction induced by adenosine diphosphate (ADP) were studied in seventeen volunteers. Aspirin B.P. and claradin at 300 mg and 150 mg inhibited release reaction in all subjects within 24 hr; 75 mg was effective only in some subjects. Aloxiprin gave less marked response and a dose of 300 mg was required to inhibit the effect in all volunteers. Where occurring, inhibition of release reaction persisted for three days after treatment with all preparations and restoration to normal occurred in most subjects by the sixth day.

A daily dose of 50 mg claradin for 12-15 days in five volunteers produced complete inhibition of release reaction for most of the treatment period. Inhibition of release reaction took up to 3 days to occur. Normal aggregation returned within 3 days of discontinuing treatment in all subjects. A daily dose of 25 mg claradin gave inconsistent results.

It is suggested that if a trial of acetylsalicylic acid be undertaken for the prevention of arterial thrombosis based on its ability to inhibit platelet release reaction then a daily dose of 50 mg would be sufficient.

Introduction

During the past decade considerable research activity has centred on drugs which inhibit platelet function. Mustard and Packham (1970) have comprehensively reviewed all aspects of platelet adhesion and aggregation and their clinical implications. It has long been suggested that pharmacological agents which inhibit platelet adhesion and aggregation may

be clinically useful in the prevention of thrombotic incidents (McDonald and Edgill, 1959; Breddin, 1968; Bygdeman and Wells, 1969; Renaud and Godu, 1970; Weiss, Danese and Valeti, 1970; Wood, 1972; Breddin, 1973). Inhibitors of platelet function, both naturally occurring substances and drugs, may be divided into three categories, namely (a), selective inhibitors of specific aggregating agents; (b), general inhibitors of primary aggregation; (c) specific inhibitors of secondary aggregation (Mills, 1972). This last group includes acetylsalicylic acid (ASA), a drug which appears promising as an effective and safe anti-aggregant (O'Brien, 1968a; Weiss, Aledort and Kochwa, 1968; Renaud and Godu, 1970; Stuart, 1970; Weiss *et al.*, 1970).

Clearly, a therapeutic trial of salicylates is justified in individuals at risk from arterial thrombosis and it has even been suggested that such a trial be extended to all men over the age of 20 years and all women over the age of 40 years on a long term basis (Wood, 1972). Exceptions to this would be persons suffering from haemostatic defects, allergy to salicylates, and a previous history of bleeding from the gastrointestinal tract or other system.

It is the purpose of this paper to report studies designed to assess the potency and duration of inhibition of platelet release reaction produced by various pharmaceutical formulations of ASA over a spectrum of dosage levels in normal volunteers. Furthermore, since gastro-intestinal haemorrhage is a well recognized complication of prolonged salicylate ingestion, it becomes mandatory to define the minimum effective drug dosage (Andrássy *et al.*, 1973).

Subjects and methods

For each of the studies, volunteers were obtained from two centres, Glasgow and Slough. All aspects

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of sampling and technique were carefully standardized between the two centres. Venous sampling was carried out at a fixed time of day for each individual. Using disposable plastic syringes, blood was obtained by clean venepuncture and mixed with 3.8% trisodium citrate in a ratio of 9 V of venous blood to 1 V citrate, the blood being taken either into siliconized glass bottles capped with parafilm or into plastic containers. An initial 18 ml sample of blood was required for base-line studies, but thereafter 9 ml were sufficient.

Platelet rich plasma (PRP) was prepared immediately by centrifugation at 150 g for 10 min at room temperature. Aliquots of PRP were placed in disposable polystyrene tubes and kept at room temperature (17–22°C) until testing. All aggregation studies were completed within 1 hr of venous sampling. PRP platelet counts were performed on the Coulter Thrombocounter C (Rowan, 1973) to ensure that the samples contained between 250,000 and 400,000 platelets/ml.

Platelet aggregation was measured by the turbidimetric method of Born (1962) and O'Brien (1962) using either a Bryston aggregometer (Glasgow) or an Eel Model 169 aggregometer (Slough). Both instruments measure changes in optical density of PRP at 37°C as platelets aggregate. Since different volumes of PRP were used in the two instruments, an arithmetical correction factor was applied to give comparable results. After pre-warming at 37°C for 2 min, the PRP to be tested was added to the reaction cuvette of the aggregometer and there continuously agitated by a magnetic stirrer at 1100 rev/min. Changes in optical density were recorded on a Servoscribe single channel recorder which had previously been adjusted to give a maximal optical density reading on the subject's PRP and a full-scale deflection (1 mV) with the corresponding platelet poor plasma.

The sodium salt of ADP (Sigma) was dissolved in distilled water to a concentration of 1 mmol/l. This stock solution was stored in aliquots at –20°C and working solutions were prepared on the day of use by further dilution in barbitol-buffered saline at pH 7.35. In each volunteer, the minimum concentration of ADP which produced a secondary wave of platelet aggregation was taken as the base-line for all subsequent studies.

All volunteers selected demonstrated reproducible biphasic platelet aggregation in citrated PRP in response to exogenous ADP. There were 140 normal subjects who had previously been studied and the lowest concentration of ADP required to induce secondary platelet aggregation ranged from 0.5–1.8 µmol/l, levels which were in broad agreement with those reported by Hardisty *et al.* (1970).

A check list of ASA-containing preparations and

other platelet inhibiting drugs was prepared and circulated to all volunteers to ensure that medication which might interfere with the study would be avoided.

The aim of the first study was to monitor the duration of inhibition of secondary aggregation following single doses of ASA. Three different preparations were used, aspirin B.P., aloxiprin, which is an aluminium co-polymer of ASA, and claradin which is a low sodium effervescent preparation of ASA. Each drug was given in doses of 300, 150, and 75 mg. Seventeen healthy volunteers (eleven male and six female) of age range 18–45 years were studied initially. Each volunteer was subjected to nine cycles of investigation, drug and dose being randomly allocated. The previous medical history of all volunteers had been carefully scrutinized and present health status was assessed. No pregnant subject was accepted, nor was anyone with a history suggestive of allergy to ASA. Any person with a history of regular dyspepsia which might predispose to gastro-intestinal haemorrhage was excluded. Platelet aggregation studies were performed before each dose of ASA to confirm the existence of base-line release reaction and subsequently on the first, third and sixth days following drug ingestion. In some subjects it was necessary to take further samples before full restoration of platelet function became evident.

The second study involved five healthy volunteers who were given 50 mg claradin daily for up to 14 days. The same stringent criteria were applied in the selection of volunteers for this part of the study. Aggregation studies were performed immediately before therapy started on day 1 to establish base-line values. Subsequently, specimens were examined daily until several days after inhibition of biphasic response occurred. Thereafter specimens were examined at intervals until dosage ceased. At the end of the test period, when treatment was discontinued, aggregation studies were performed daily until a return to base-line biphasic response occurred.

A third study was carried out on four volunteers using 25 mg claradin daily. Two subjects took this preparation after an overnight fast; the remainder following a light breakfast. Aggregation studies were performed daily until inhibition of platelet release reaction was achieved or to a maximum of 6 days.

Results

First study

The results are summarized in Table 1 in terms of the number of times secondary aggregation did not occur on the first, third and sixth days after ASA ingestion for each preparation at each dose level. In the Glasgow volunteers no significant difference

TABLE 1. Number of times secondary aggregations not achieved on each ASA preparation after 1, 3, and 6 days (i.e. the number of times the effect of ASA was noted)

Centre	Treatment	Dose Days	75 mg			150 mg			300 mg		
			1	3	6	1	3	6	1	3	6
Glasgow 9 Volunteers	Aspirin B.P.		9	7	4	9	9	6	9	9	7
	Claradin		9	8	1	9	9	6	9	9	3
	Aloxiprin		8	5	1	9	9	5	9	9	4
Slough 8 Volunteers	Aspirin B.P.		5	3	0	8	6	2	8	6	0
	Claradin		7	5	2	8	5	1	8	5	2
	Aloxiprin		2	6	0	5	3	2	8	4	1

was noted between subjects or treatments. The responses, however, at 75 mg were significantly less than those of 150 mg and 300 mg ($P < 0.05$); there was evidence of a levelling of dose response at 150 mg.

In the Slough volunteers the results were more variable and large differences were noted between the subjects and treatments. The treatment differences arose mainly from the reduced response to aloxiprin. As in the Glasgow results, the responses after 75 mg were significantly less ($P < 0.05$) than at the other two doses and there was an indication of levelling in the dose response curve at 150 mg. All subjects taking aspirin B.P. and claradin at the 300 mg and 150 mg dose levels demonstrated inhibition of secondary aggregation after 24 hr. The duration of inhibition differed in individuals but this was presumed to be due to variation in platelet turnover. Some subjects demonstrated an effect for 10 days. Aspirin B.P. produced inhibition of platelet aggregation in excess of 72 hr in sixteen of seventeen subjects studied and claradin in fifteen of seventeen subjects. With aloxiprin, while all volunteers demonstrated inhibition of secondary aggregation at 300 mg, persistence of biphasic reaction occurred in two subjects at 150 mg and in three subjects at 75 mg. All Glasgow volunteers, if showing inhibition, did so 24 hr after commencing treatment. The situation differed with the Slough volunteers in that on seven occasions (aloxiprin 75 mg $\times$ 5, aloxiprin 150 mg $\times$ 1, claradin 75 mg $\times$ 1) secondary aggregation persisted at 24 hr but was inhibited at 72 hr (Fig. 1).

FIG. 1. Inhibition of secondary platelet aggregation by aspirin B.P., $\circ$ — $\circ$; claradin, $\blacktriangle$ — $\blacktriangle$; and aloxiprin, $\blacksquare$ — $\blacksquare$. A, Glasgow and B, Slough volunteers.

Second study

Using claradin in a dose of 50 mg daily, all subjects show inhibition of secondary aggregation but induction occurred at different rates, Slough volunteers taking longer than Glasgow volunteers (Table 2).

TABLE 2. Duration of inhibition of secondary platelet aggregation produced by claradin 50 mg/day

Centre	Volunteer no.	Day																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Slough	1	+	+	+	—	—	—	—	—	—	—	—	()	—	—	+	+		
	2	+	+	—	—	—	—	—	—	—	—	—	()	—	+	+			
	3	+	+	—	—	—	—	—	—	—	—	()	—	—	+	+			
Glasgow	4	+	—	—	—	—	—	—	—	—	—	—	—	(—)	—	—	+		
	5	+	—	—	—	—	—	—	—	—	—	—	(—)	—	—	—	+		

+, Secondary aggregation occurred; —, secondary aggregation inhibited; (), indicates that this was the last day of dosage.

Third study

The four volunteers taking claradin 25 mg daily failed to show consistent results whether ingestion occurred in the fasting or the non-fasting state. This level of dosage was considered unsatisfactory and not pursued.

Discussion

Evidence continues to accumulate implicating the platelet in the initiation of arterial thrombus formation. Platelets rapidly adhere to exposed collagen in the arterial tree and subsequently undergo release action (Mustard, Rowsell and Murphy, 1966). The ADP liberated causes other platelets to aggregate which in turn sets up a chain reaction. Thus, drugs which interfere with this process may well prevent thrombus formation.

Many common drugs impair platelet aggregation *in vitro* (Mustard *et al.*, 1966; Evans *et al.*, 1967; Mills and Roberts, 1967; Weiss *et al.*, 1968) but ASA has been the most widely studied. During these studies, a wide range of doses has been used varying from 150 to 3600 mg daily (O'Brien, 1968a, b; Weiss *et al.*, 1968; Zucker and Peterson, 1968; Stuart, 1970; Hirsch *et al.*, 1973). However, above 150 mg, the degree of inhibition of platelet aggregation does not increase (Bjornson and Eika, 1973).

The mechanism whereby ASA affects platelet function is incompletely defined. The observation that platelets can remain abnormal for several days after the ingestion of a single dose of ASA suggests that platelets are permanently affected by the drug and that restoration to normal probably represents the appearance of a new platelet population. Rosenberg *et al.* (1971) have demonstrated that platelets are acetylated by ASA probably via a mechanism involving the membrane protein. This will affect the physico-chemical properties of the platelet membrane protein which could account for the inhibition of secondary aggregation. An alternative or additional explanation is afforded by the work of Vargaftig and Zirinis (1973) who found that ASA and other anti-inflammatory drugs inhibit the production of a cyclic endoperoxide intermediate formed during the biosynthesis of prostaglandins from arachidonic acid in human and rabbit platelets. They suggest that this intermediate is responsible for platelet aggregation induced by collagen and that ADP released during the process plays an essential role.

ASA is absorbed from all parts of the gastrointestinal tract including the stomach. While initial absorption of ASA takes place across the gastric mucosa, the major site of absorption appears to be the proximal portion of the small intestine (Levy, 1961). This is the result of the interaction of a num-

ber of factors including the pH of the stomach and small intestine, the rate of stomach emptying, the rate of dissolution and the degree in dissociation of the ASA molecule. The pharmaceutical formulation can influence all of these and is therefore of importance.

In the first study, aspirin B.P. and claradin at the 300 and 150 mg dose level produced uniform inhibition of secondary platelet aggregation. The overall performance with these preparations at the 75 mg level was good but not completely predictable. Aloxiprin, on the other hand, gave less satisfactory results, particularly at doses of 75 and 150 mg. This difference is presumably related to the variation in pharmaceutical formulation. The rates of dissolution of aspirin B.P. and claradin are much greater than that of aloxiprin and consequently the rates of absorption of the former preparations are more rapid, resulting in earlier and higher peak levels. A study comparing serum salicylate concentrations after single doses of claradin and aspirin B.P. showed that the former resulted in a peak level 10–20 min after ingestion whereas the latter did not show a definite maximum during the 60-min period of study (Cummings, Martin and Renton, 1971). Furthermore, the serum salicylate level achieved with claradin was substantially higher than that achieved by aspirin B.P. at all times. For this reason claradin was preferred to aspirin B.P. during the subsequent part of the present investigation. The slower absorption of aspirin B.P. implies longer contact with gastro-intestinal mucosa and could therefore increase the risk of side effects.

Claradin in daily doses of 50 mg effectively inhibits platelet release reaction in healthy volunteers. There would, however, appear to be a population difference at this dose level. Glasgow volunteers invariably showed inhibition within 24 hr of starting treatment, whereas volunteers from Slough required 48–72 hr for protection. The reason for this is not apparent. However, once inhibition occurs with 50 mg claradin daily the effect persists until some days after discontinuing treatment.

A daily dose of 25 mg claradin produced an unpredictable response in terms of platelet aggregation and no difference was noted in relation to food ingestion.

Evidence is now accumulating to support a therapeutic trial of ASA for the prevention of arterial thrombosis. If platelet aggregation studies accurately reflect *in vivo* platelet release phenomena, the results of the present study suggest that a daily dose of 50 mg ASA would be suitable for such a trial. Furthermore, it is of interest to note that these studies have revealed that inhibition of platelet release reaction may be the most potent action of ASA in man.

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