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
of sampling and technique were carefully stand-
dardized 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 there-
after 9 ml were sufficient.

Platelet rich plasma (PRP) was prepared immedi-
ately by centrifugation at 150 g for 10 min at room
temperature. Aliquots of PRP were placed in dis-
posable polystyrene tubes and kept at room tem-
perature (17–22°C) until testing. All aggregation
studies were completed within 1 hr of venous sam-
pling. 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 turbidi-
metric 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 arith-
etical correction factor was applied to give com-
parable 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 Servo-
scribe 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 concen-
tration 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
mmol/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 prepara-
tion 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 sub-
sequently on the first, third and sixth days follow-
ing drug ingestion. In some subjects it was necessary
to take further samples before full restoration of plate-
let 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, aggrega-
tion 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
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).

### 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)

<table>
<thead>
<tr>
<th>Centre</th>
<th>Treatment</th>
<th>Dose</th>
<th>75 mg</th>
<th>150 mg</th>
<th>300 mg</th>
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<tr>
<td></td>
<td></td>
<td>Days</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Glasgow</td>
<td>Aspirin B.P.</td>
<td></td>
<td>9</td>
<td>7</td>
<td>4</td>
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<tr>
<td>9 Volunteers</td>
<td>Claradin</td>
<td></td>
<td>9</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Aloxiprin</td>
<td></td>
<td>8</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Slough</td>
<td>Aspirin B.P.</td>
<td></td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>8 Volunteers</td>
<td>Claradin</td>
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<td>5</td>
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<tr>
<td></td>
<td>Aloxiprin</td>
<td></td>
<td>2</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

### Figure 1. Inhibition of secondary platelet aggregation by aspirin B.P., ○—○; claradin, △—△; and aloxiprin, ■—■. 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

<table>
<thead>
<tr>
<th>Centre</th>
<th>Volunteer no.</th>
<th>Day</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<th>16</th>
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<tbody>
<tr>
<td>Slough</td>
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<td>Glasgow</td>
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<td>5</td>
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<td>+</td>
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</table>

+, 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, Rowell 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 number 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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