Comparison of antiplatelet activity of microencapsulated aspirin 162.5 mg (Caspac XL), with enteric coated aspirin 75 mg and 150 mg in patients with atherosclerosis

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Aims A new formulation, low dose microencapsulated aspirin, permits slow absorption of aspirin and presystemic acetylation of platelet cyclo-oxygenase within the portal circulation, potentially avoiding deleterious effects on gastric and systemic prostaglandin synthesis. The objective of this study was to determine whether the administration of microencapsulated aspirin was as effective as enteric coated (EC) aspirin as an inhibitor of platelet function in patients with atherosclerosis.

Methods One hundred and four patients were enrolled and randomised after a run in period of at least 14 days on aspirin EC 75 mg (day 0), to receive either microencapsulated aspirin 162.5 mg ($n=34$), aspirin EC 150 mg ($n=36$) or continue on aspirin EC 75 mg ($n=34$) for 28 days. Serum thromboxane B₂ and collagen-induced platelet aggregation and release of 5-hydroxytryptamine (EC₅₀ values) were measured on days 0 and 28. Aggregation/release EC₅₀s were then repeated in the presence of a large dose of aspirin added *in vitro* to determine the EC₅₀ at the maximum level of platelet inhibition.

Results Median thromboxane B₂ levels were low after 14 days run-in therapy with aspirin EC 75 mg, but significant further reductions were seen on day 28 in patients randomised to microencapsulated aspirin 162.5 mg ($P=0.0368$) and aspirin EC 150 mg ($P=0.0004$) compared with those remaining on aspirin EC 75 mg. Median EC₅₀s on day 28 showed small but significant increases from baseline (day 0) in aggregation in patients randomised to microencapsulated aspirin 162.5 mg (0.62–0.85, $P=0.0482$) and in both aggregation and release in patients randomised to aspirin EC 150 mg (0.95–1.20, $P=0.0002$, 8.4–11.7, $P<0.0001$, respectively) signifying enhanced antiplatelet activity. No changes were seen in patients continuing on aspirin EC 75 mg. Results following addition of high dose aspirin *in vitro* suggest that mechanisms other than thromboxane synthesis may be operative in the long term effects of microencapsulated aspirin 162.5 mg and aspirin EC 150 mg over aspirin EC 75 mg.

Conclusions The results show good inhibition of thromboxane B₂ synthesis and subsequent platelet activity by all preparations of aspirin, although both microencapsulated aspirin 162.5 mg and aspirin EC 150 mg are slightly more effective than aspirin EC 75 mg. A randomised trial is now required to determine whether microencapsulated aspirin is associated with fewer gastric side-effects.

Keywords: enteric coated aspirin, low dose aspirin, microencapsulated aspirin, antiplatelet activity

Introduction

Acetylsalicylic acid (aspirin) is a powerful antiplatelet agent at low doses ≤ 325 mg day⁻¹ and its efficacy in

the prevention of thrombo-embolic events has been unequivocally established [1, 2]. However, low dose aspirin preparations are known to cause both irritation and injury to gastric mucosa with a significant increase in upper gastrointestinal bleeding [3] necessitating their withdrawal in some patients.

Mechanisms thought responsible for serious

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gastrointestinal side-effects include a local damaging effect of aspirin in the stomach and inhibition of prostaglandin synthesis mediated by aspirin present in the systemic circulation [4, 5]. The protective effect of gastric prostaglandins PGI₂ and PGE₂, which act to inhibit secretion of gastric acid and stimulate production of protective mucus, may thus be lost. Whilst both enteric coating and buffering agents may theoretically reduce irritation of gastric mucosa, blood levels are just as high as with conventional aspirin and recent work has shown similar rates of bleeding with these preparations [6].

A new formulation, low dose microencapsulated aspirin (Caspac XL) permits slow absorption of aspirin and presystemic acetylation of platelet cyclo-oxygenase within the portal circulation, potentially avoiding the deleterious effects on systemic prostaglandin synthesis. Platelets in the portal blood are irreversibly acetylated but as a result of almost 100% first pass metabolism only small amounts of aspirin pass through the liver and reach the systemic circulation. Previous studies using controlled-release preparations have confirmed the ability to selectively affect platelets in the portal circulation, suppressing thromboxane A₂ and preserving prostacyclin (PGI₂) synthesis in small numbers of normal volunteers [7–9] although a detailed comparison of antiplatelet activity in patients with vascular disease has not been performed.

The aim of this study was to determine whether administration of low dose microencapsulated aspirin 162.5 mg is as effective as enteric coated aspirin in reducing platelet function in patients with existing vascular disease and to compare the tolerability of the three treatments.

Methods

Subjects

Patients with a history of known atherosclerotic disease (ischaemic heart disease, stroke/transient ischaemic attack) of at least three months duration, aged 18 years or over and taking aspirin at a dose of ≤ 325 mg day⁻¹ for prevention of thromboembolism for a minimum of 1 month were eligible for inclusion. All patients were identified in the cardiology outpatient clinics at University Hospital, Nottingham between 10 December 1996 and 30 April 1997 and on admission to the study basic demographic data were recorded, a medical history taken, physical examination performed and blood taken for basic haematology and biochemistry. The local Ethics committee approved the study and all participants gave written informed consent prior to enrolment. Patients were excluded if they had active peptic ulceration or a history of such, were receiving any other antiplatelet agent or nonsteroidal anti-inflammatory agent, had a history of

easy bleeding or unexplained iron deficiency anaemia, were pregnant or lactating. Those with a history of sensitivity to aspirin or who were under follow up in any other study or receiving another investigational drug, had a history of alcohol or drug abuse or had serious intercurrent illness were ineligible for inclusion.

Study design

The study was a single-blind, parallel group comparison of the antiplatelet activity of low dose microencapsulated aspirin 162.5 mg, aspirin EC 75 mg and aspirin EC 150 mg taken once daily. Ethical considerations precluded withdrawal of aspirin from patients with established atherosclerotic disease and it was therefore not possible to obtain untreated baseline measures of platelet function to calculate percentage inhibition following aspirin therapy. Absolute values of thromboxane and platelet aggregation are thus used, all subjects receiving a standardized low dose of aspirin EC 75 mg during an open label run-in period of at least 14 days before randomization.

A total of 104 patients were enrolled during the run-in period and each subject was then randomised (day 0) to either microencapsulated aspirin 162.5 mg (Caspac XL), $n=34$ aspirin EC 150 mg, $n=36$ or maintained on aspirin EC 75 mg, $n=34$ for a period of 28 days. On days 0 and 28 a 20 ml blood sample was taken with a 19G needle for assessment of platelet function and measurement of thromboxane levels.

Thromboxane B₂ was measured in serum by radioimmunoassay using the Biotrax Thromboxane B₂ enzymeimmunoassay (Amersham Life Science). 10 ml⁻¹ whole blood was allowed to clot in a plain glass tube at 37° C for 30 min, centrifuged at 2500 g (3000 rev min⁻¹), the serum then aspirated and frozen in a polypropylene tube at -20° C prior to analysis.

Platelet aggregation and release of 5-hydroxytryptamine (5-HT) was measured in whole blood in response to a range of collagen (Horm Collagen, Nycomed UK) concentrations (0.5, 1, 2, 4 and 8 µg/ml⁻¹).

Platelet aggregation in whole blood

Aggregation was determined at 2 min following the addition of the agonist (collagen) by a cell counting technique using the Ultra-Flo 100 Whole Blood Platelet Counter [10]. The aggregation was measured as the fall in the number of single platelets as a percentage of the platelets in the sample before addition of agonist.

Platelet release reaction in whole blood

Whole blood was prelabelled with [¹⁴C]-5-HT (5-hydroxy[side-chain-2-¹⁴C]tryptamine creatinine sulphate

(Amersham); specific activity $2.07 \text{ GBq mmol}^{-1}$, 1.85 MBq ml^{-1}). Samples ($460 \mu\text{l}$) of whole blood were placed in polystyrene tubes with chlorimipramine ($20 \mu\text{l}$) and collagen or buffer as control ($20 \mu\text{l}$) was added and the samples stirred at $1000 \text{ rev min}^{-1}$ at 37°C . The amount of 5-HT released was measured at 4 min after addition of the agonist by counting ^{14}C -5-HT in cell-free supernatants and expressing this as a percentage of the amount of ^{14}C -5-HT taken up by the platelets during the original labelling procedure [11]. The collagen concentration producing 50% aggregation and release (EC_{50} values) was calculated. The dose response was repeated in the presence of added aspirin ($100 \mu\text{M}$) to determine the EC_{50} at the maximum level of platelet inhibition.

Statistical analysis

The sample size was based on one of the primary efficacy variables, serum thromboxane B_2 concentration. In a previous study the standard deviation between subjects in serum thromboxane concentrations after 14 and 28 days treatment with aspirin EC 300 mg was ≈ 1.25 natural logarithmic units. If the variability between subjects in this study was similar then it would have a 94% power to detect a three-fold difference in mean concentrations between two treatment groups and a 83% power to detect a 2.5-fold difference. EC_{50} values were calculated for aggregation and release using a curve fitting algorithm derived using the nonlinear regression procedure (PROC NLIN) in the SAS statistical package (Marquardt method).

Serum thromboxane levels and whole blood collagen induced aggregation and release reaction EC_{50} values were not normally distributed partly as a result of some large outliers. Results are thus expressed as medians and ranges and changes between day 0 and 28 assessed using the Kruskal-Wallis test (based on the ranks of the data or the Wilcoxon scores). Differences were considered significant at $P < 0.05$.

Results

Demographics and tolerability

Table 1 details patient demographics confirming that all three patient groups had a similar age and sex distribution. The majority of patients had a history of ischaemic heart disease. One hundred and four patients were admitted to the study. Six patients withdrew from the study during the run-in period and one was lost to follow up. A further two withdrew during the treatment phase, one in the microencapsulated aspirin 162.5 mg group (due to nausea) and one remaining on aspirin EC 75 mg (due to

loin pain). All three formulations were well tolerated and there were no significant safety issues.

Thromboxane B_2

Median thromboxane B_2 levels were always very low ($8.0\text{--}11.5 \text{ ng ml}^{-1}$) compared with nonaspirin taking controls (median 134 ng ml^{-1} , $n=12$ in determinations performed in the same laboratory). There were small but significant changes with lower thromboxane levels on day 28 in patients randomised to microencapsulated aspirin 162.5 mg and aspirin EC 150 mg when compared with those remaining on aspirin EC 75 mg ($P=0.0368$ and 0.004 , respectively, Figure 1).

EC_{50} values

In all the groups at day 0, having had a 14-day run-in on aspirin EC 75 mg, median EC_{50} s were indicative of marked inhibition of aggregation ($0.62\text{--}0.95 \mu\text{g/ml}^{-1}$) and release ($7.6\text{--}8.4 \mu\text{g ml}^{-1}$) when compared with nonaspirin taking controls (EC_{50} aggregation, median $<0.5 \mu\text{g ml}^{-1}$, $n=8$ and EC_{50} release, median $=2.4 \mu\text{g ml}^{-1}$, $n=8$ in determinations performed in the same laboratory). However, inhibition appeared incomplete as adding further aspirin *in vitro* increased EC_{50} values (Table 2). A baseline imbalance in aggregation data was seen with lower EC_{50} s ($P=0.0313$) implying more sensitive platelets in those subsequently prescribed microencapsulated aspirin.

Median EC_{50} values on Day 28 showed small but significant increases from Day 0 in those randomized to microencapsulated aspirin 162.5 mg (aggregation: $0.62\text{--}0.85$, $P=0.0482$) and in those randomized to aspirin EC 150 mg (aggregation: $0.95\text{--}1.20$, $P=0.0002$; release: $8.4\text{--}11.7$, $P<0.0001$) but not in those who remained on aspirin EC 75 mg. The between group comparisons of change reflected these trends (Table 2, release $P=0.0737$; aggregation $P=0.1674$).

However, there were also increases in EC_{50} from Day 0 to Day 28 when additional aspirin was added *in vitro* in those randomized to microencapsulated aspirin (aggregation: $0.85\text{--}0.96$, $P=0.0052$) and in those given aspirin EC 150 mg (release: $11.1\text{--}13.2$, $P=0.0189$). There were no changes from Day 0 to Day 28 in patients who remained on aspirin EC 75 mg (Table 2).

Individual EC_{50} values for aggregation and release varied widely (Table 2). Omitting some extreme outliers, values for aggregation range between 0.30 and 6.74 and values for release between 3.8 and 38.7 . Such wide range of values were evident in all patient groups irrespective of the type of aspirin administered and also after additional aspirin was added *in vitro*. This suggests there are factors

	Microencapsulated aspirin 162.5 mg	Aspirin EC 150 mg	Aspirin EC 75 mg
Patients enrolled	34	36	34
Completed study	31 (91%)	31 (86%)	33 (97%)
Mean age (years)	60.5	64.4	61.1
Male	26 (76%)	29 (81%)	27 (79%)
Diagnosis†			
Angina	18	18	25
Myocardial infarction	20	21	18
Coronary revascularisation	4	5	4
Hypertension	4	5	4
Stroke/transient ischaemic attack	0	4	1
Concomitant treatment			
β -adrenoceptor blocker	16	23	22
Calcium antagonist	12	14	12
Angiotensin converting enzyme inhibitor	10	4	6
Nitrate	23	24	27
Diuretic	9	13	15
Lipid lowering drug	9	7	1

†Diagnoses not mutually exclusive.

Figure 1 Median thromboxane levels at days 0 (■) and 28 (□) in patients randomised to microencapsulated aspirin 162.5mg, aspirin EC 150 mg and aspirin EC 75 mg. *P* values are for pairwise comparisons of change.

other than the capability for thromboxane synthesis that dictate platelet sensitivity to collagen.

Discussion

Microencapsulated aspirin 162.5 mg is a slow release formulation designed to affect platelets in the portal circulation, and to be degraded on first pass through the liver, thereby avoiding deleterious effects on systemic prostaglandins and hence gastric side-effects.

Previous meta-analysis has shown that doses of aspirin from 75 mg to 300 mg are effective in preventing stroke and myocardial infarction [1] and although no clinical differences between doses in this range have been seen, few formal comparisons have been undertaken.

Table 1 Characteristics of patients randomised to microencapsulated aspirin 162.5 mg and enteric coated aspirin 75 mg and 150 mg.

Previous work with microencapsulated aspirin 162.5 mg in a small number of normal individuals, has established that therapeutic antiplatelet effects can be seen within 6 h of administration although the maximum effect takes approximately one day longer than with conventional aspirin. Microencapsulated aspirin is not therefore intended to be used in situations where very rapid onset of action is required.

In view of the differing release characteristics and pharmacokinetic profile it was necessary to ensure that microencapsulated aspirin is as effective as conventional aspirin in its chronic effects on platelet activity. Patients with atherosclerosis were used instead of normal volunteers as they are older, take a variety of other cardiovascular drugs, might be expected to have different platelet reactivity and would ultimately form the target treatment group.

Thromboxane B₂ levels during the run-in phase on aspirin EC 75 mg were always very low compared to normal nonaspirin taking volunteers indicating a level of inhibition in excess of 90%. After 28 days a further small fall in thromboxane was seen in both the microencapsulated aspirin and aspirin EC 150 mg groups but not in the group on aspirin 75 mg demonstrating that the activity of the former two agents are similar and slightly greater than aspirin EC 75 mg.

Median EC₅₀ values for collagen induced release reaction revealed significant increases at day 28 in the group randomized to take aspirin EC 150 mg and for aggregation significant increases in both those randomised to microencapsulated aspirin 162.5 mg and aspirin EC 150 mg. The data were complicated by lower baseline EC₅₀s for collagen induced platelet aggregation in the

Table 2 Collagen induced platelet aggregation and release of 5-hydroxytryptamine (EC_{50} values in $\mu\text{g ml}^{-1}$) measured before and after the addition of 100 μM aspirin *in vitro*.

Group	Day 0	Day 28	Change (Day 28–day 0), median (range)	P value*
<i>Release reaction median EC_{50} values (range)</i>				
Microencapsulated aspirin 162.5mg	8.2 (5.2, 26.3)	9.5 (5.2, 38.7)	1.5 (–18.3, 32.7)	0.0773
Microencapsulated aspirin 162.5mg + aspirin 100 μM	11.8 (6.3, 31.9)	12.4 (8.4, 20.9)	0.4 (–18.6, 13.6)	0.6320
Aspirin EC 150 mg	8.4 (4.5, 18.5)	11.7 (5.3, 134)	2.0 (–6.4, 127)	<0.0001
Aspirin EC 150 mg + aspirin 100 μM	11.1 (7.6, 21.4)	13.2 (6.8, 943)	1.9 (–7.5, 926)	0.0189
Aspirin EC 75 mg	7.6 (5.2, 22.3)	8.7 (3.8, 144)	0.5 (–14.6, 129)	0.3416
Aspirin EC 75 mg + aspirin 100 μM	12.9 (7.5, 24.6)	11.9 (6.8, 532)	0.5 (–13.0, 517)	0.2691
Between group comparisons*				
Before addition of aspirin 100 μM			$P=0.0737$	
After addition of aspirin 100 μM			$P=0.2936$	
<i>Aggregation median EC_{50} values (range)</i>				
Microencapsulated aspirin 162.5mg	0.62 (0.30, 2.16)	0.85 (0.45, 2.93)	0.1 (–1.1, 2.3)	0.0482
Microencapsulated aspirin 162.5mg + aspirin 100 μM	0.85 (0.37, 2.15)	0.96 (0.58, 3.55)	0.2 (–0.6, 2.7)	0.0052
Aspirin EC 150 mg	0.95 (0.30, 4.91)	1.20 (0.43, 6.74)	0.3 (–2.4, 6.1)	0.0002
Aspirin EC 150 mg + aspirin 100 μM	0.93 (0.30, 5.43)	1.38 (0.37, 6.41)	0.1 (–2.2, 5.4)	0.0689
Aspirin EC 75 mg	0.91 (0.45, 3.39)	1.01 (0.40, 6.49)	0.0 (–2.0, 4.1)	0.2617
Aspirin EC 75 mg + aspirin 100 μM	1.02 (0.42, 3.74)	1.09 (0.45, 13.14)	0.1 (–1.9, 11.7)	0.2335
Between group comparisons*				
Before addition of aspirin 100 μM			$P=0.1674$	
After addition of aspirin 100 μM			$P=0.7338$	

*Non-parametric Kruskal–Wallis test based on ranked changes in EC_{50} values.

microencapsulated aspirin group implying more sensitive platelets in this group, nevertheless a significant increase is seen after 28 days consistent with an increased antiplatelet effect.

Whatever dose of aspirin was used, the inhibition of collagen induced platelet aggregation and release was incomplete, as adding further aspirin *in vitro* resulted in additional antiplatelet effects. For the groups randomised to microencapsulated aspirin 162.5 mg and aspirin EC 150 mg rather surprisingly an increase in EC_{50} s after the addition of further aspirin *in vitro* was seen at day 28, in aggregation for the former and release reaction for the latter—no change was seen in those remaining on aspirin EC 75 mg. The effects of a large dose of aspirin added to the blood *in vitro* should dwarf any effect of aspirin *in vivo* and consequently one would not expect an increase in any group between day 0 and 28. It is possible that chronic treatment with a higher dose of aspirin changes the sensitivity of platelets to collagen in a way other than by inhibition of cyclo-oxygenase. Formation of an active metabolite or an effect on platelets during development are plausible, although the latter is less likely as with microencapsulated aspirin systemic levels are very low. Further work is required to investigate these findings.

An additional finding in these experiments was that the amount of collagen required to stimulate platelet function in aspirin-taking patients varied widely indicating there are factors other than the capacity for thromboxane

synthesis that determine sensitivity of platelets to this aggregating agent. It is possible that those patients whose platelets remain sensitive to collagen stimulation postaspirin could benefit from additional antiplatelet agents with a different mechanism of action. One possibility could be the addition of an agent that limits the contribution of ADP.

In conclusion, the results overall reveal good inhibition of platelet activity by all the aspirin preparations tested and all three treatments were well tolerated. Microencapsulated aspirin 162.5 mg and aspirin EC 150 mg are slightly, but significantly more effective than aspirin EC 75 mg, with regards to antiplatelet activity, however, their additional effects (and the long-term effects of aspirin in general) may not be entirely due to their inhibition of thromboxane synthesis. A randomised trial is now required to address the issue of tolerability and gastric side-effects of microencapsulated aspirin compared to standard preparations.

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