Effect of enteric coating on antiplatelet activity of low-dose aspirin in healthy volunteers.

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Effect of Enteric Coating on Antiplatelet Activity of Low-Dose Aspirin in Healthy Volunteers

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Background and Purpose—Aspirin resistance may be relatively common and associated with adverse outcome. Meta-analysis has clearly shown that 75 mg plain aspirin is the lowest effective dose; however, it is not known whether the recent increased use of enteric-coated aspirin could account for aspirin resistance. This study was designed to determine whether enteric-coated aspirin is as effective as plain aspirin in healthy volunteers.

Methods—Seventy-one healthy volunteers were enrolled in 3 separate bioequivalence studies. Using a crossover design, each volunteer took 2 different aspirin preparations. Five aspirin preparations were evaluated, 3 different enteric-coated 75-mg aspirins, dispersible aspirin 75 mg and asasantin (25-mg standard release aspirin plus 200-mg modified-release dipyridamole given twice daily). Serum thromboxane (TX) B2 levels and arachidonic acid–induced platelet aggregation were measured before and after 14 days of treatment.

Results—All other aspirin preparations tested were inferior to dispersible aspirin (P<0.001) in their effect on serum TXB2 level. Treatment failure (<95% inhibition serum TXB2 formation) occurred in 14 subjects, none of whom were taking dispersible aspirin. Mean weight for those demonstrating treatment failure was greater than those with complete TXB2 (>99%) inhibition (P<0.001). Using logistic regression analysis an 80-kg subject had a 20% probability of treatment failure. Asasantin was the most potent preparation in terms of inhibition of platelet aggregation.

Conclusions—Equivalent doses of the enteric-coated aspirin were not as effective as plain aspirin. Lower bioavailability of these preparations and poor absorption from the higher pH environment of the small intestine may result in inadequate platelet inhibition, particularly in heavier subjects. (Stroke. 2006;37:2153-2158.)

Key Words: antiplatelet drugs • aspirin • cyclooxygenase • platelet inhibitors

Aspirin (acetylsalicylic acid) is widely used as an antiplatelet agent among patients with cardiovascular disease.1 It inactivates cyclooxygenase (COX), the enzyme responsible for generation of a potent platelet activator, thromboxane (TX) A2.2 As a consequence, aspirin suppresses TXB2 formation (the stable metabolite of TXA2) in serum. Aspirin also inhibits platelet aggregation to arachidonic acid, the substrate for TXA2, and to weak agonists such as low-dose ADP, but not stronger agonists like thrombin. Aspirin irreversibly inhibits COX; thus, its effect on the anucleate platelet, which lacks transcriptional ability, is prolonged for the lifetime of the cell.3 As a result, complete suppression of platelet TXA2 formation is possible with chronic aspirin administration at doses as low as 30 mg daily.4 Because COX is found in many tissues and especially the stomach, where inhibition predisposes to gastric ulceration, a strategy of low-dose aspirin administration is used to target the platelet as unlike the anucleate platelet other tissues can regenerate COX.5

In contrast to the steep dose-response curve observed with suppression of platelet TXA2 formation, inhibition of prostacyclin generation in humans increases progressively as aspirin dose exceeds that required for an antiplatelet effect. Prostacyclin is the major COX product of endothelial cells and a potent platelet inhibitor. Disruption of the prostacyclin receptor (IP) in the mouse aggravates the response to vascular injury attributable in part to the unopposed effects of TXA2.6 The balance between prostacyclin and TXA2 may be equally important in patients, because selective inhibition of COX-2, the principal source of prostacyclin in human, has been associated with an increased risk of myocardial infarction.7,8

Low-dose aspirin is also used to minimize gastrointestinal injury, which increases progressively as the dose exceeds that required for an antiplatelet effect.9 The Antithrombotic Trialists’ Collaboration, which analyzed results from a large number of clinical trials in patients with vascular disease, concluded that low doses of aspirin, in the range of 75 to 150 mg/d, were as effective as higher doses.1 In an attempt to further prevent adverse gastric effects, enteric coating is often used to prevent release of aspirin into the stomach. Thus, 75 mg/d aspirin preparations, many of which today are entericoated, are being prescribed more frequently.10
low-dose preparations may achieve the desired effect on the population; however, drug pharmacokinetics may vary among individuals. At least 95% inhibition of serum TXB₂ formation by aspirin is required to prevent thromboxane-mediated platelet activation. Here, we compare the ability of 5 low-dose aspirin preparations to achieve this treatment threshold.

Materials and Methods

Data

The data in this study were drawn from 3 bioequivalence studies, carried out by our department. Each crossover study compared 2 aspirin preparations. Table 1 summarizes the preparations and participants.

Drugs

Three different 75-mg enteric-coated aspirin preparations were used: Nu-Seals (Lilly; enteric 2a and 2b), Caprin (Sinclair Pharmaceuticals; enteric 1) and Protek (Antigen Pharmaceuticals; enteric 3). We also studied Asasantin Retard (Aggrenox; Boehringer Ingelheim), which contains 25-mg aspirin in combination with 200-mg modified-release dipyridamole and is administered twice daily, and 75-mg dispersible aspirin (Lowasa, Central Laboratories Ltd).

Study Design

Three separate studies were approved by the Irish Medicines Board and the Ethics Committee of Beaumont Hospital, Dublin. These studies were designed as regulatory studies for the approval of new low-dose aspirin preparations. Each study was designed as a bioequivalence study using an approved product as the comparator. Each was performed on healthy volunteers who provided written, informed consent (see Table 1). Volunteers, aged between 20 and 50 years, were not on medication, had no chronic illnesses and had normal clinical examinations. A randomized, open-label, cross-over design was used in each case and the initial treatment was randomly selected. Volunteers (22 to 25 as determined by European Agency for the Evaluation of Medicinal Products (EMEA) regulatory requirements, see Table 1) received drug for 14 days, followed by a 14-day wash-out period before administration of the second treatment. Aspirin is an irreversible inhibitor of COX and the mean platelet lifespan is 10 days; therefore, a wash-out period of 14 days was considered adequate to ensure complete elimination of aspirin before the next treatment arm. Comparison of the pretreatment serum TXB₂ levels from each arm using paired t test showed no significant difference and thus confirmed the effectiveness of the wash-out. Blood samples were taken for analysis on the first day predose and on day 14. In the case of Nu-Seals, Protek and Caprin, 2 tablets were chewed on the first day. Asasantin tablets were administered twice daily. Compliance was confirmed by tablet-counting.

Pharmacokinetic studies are not feasible with low-dose aspirin because the drug is unstable and much of the platelet inhibition occurs in the presystemic circulation. Therefore, bioequivalence analysis was based on pharmacodynamic measurements. The primary end point of the study was defined as percentage inhibition of TXB₂ on day 14. This assay provides an estimate of platelet COX inhibition by aspirin. We defined <95% inhibition as treatment failure, 95% to 99% inhibition as incomplete inhibition and >99% inhibition as successful treatment. A secondary end point was inhibition of arachidonic acid-induced platelet aggregation.

TABLE 1. Profile of Volunteers in the 3 Studies Showing mean±SD

<table>
<thead>
<tr>
<th>Study</th>
<th>Drug</th>
<th>Comparator</th>
<th>n</th>
<th>Age</th>
<th>Weight, kg</th>
<th>M/F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Caprin (enteric 1)</td>
<td>Nu-Seals (enteric 2a)</td>
<td>22</td>
<td>28.2±5.4</td>
<td>67.5±10.6</td>
<td>11/11</td>
</tr>
<tr>
<td>2</td>
<td>Protek (enteric 3)</td>
<td>Nu-Seals (enteric 2b)</td>
<td>24</td>
<td>32.0±6.9</td>
<td>71.5±9.8</td>
<td>11/13</td>
</tr>
<tr>
<td>3</td>
<td>Asasantin</td>
<td>Dispersible aspirin</td>
<td>25</td>
<td>27.7±4.7</td>
<td>76.2±17.7</td>
<td>16/9</td>
</tr>
</tbody>
</table>

Serum TXB₂

Whole blood was collected in nonsiliconized glass tubes and allowed to clot at 37°C for 1 hour. The tubes were centrifuged at 900g for 5 minutes and the serum removed and stored at −80°C. For analysis, samples were thawed, diluted 1 in 500 and assayed by ELISA according to the manufacturers instructions (R&D Systems).

Platelet Aggregation

Whole blood was collected into 3.8% sodium citrate and centrifuged at 190g for 2 minutes. The platelet-rich plasma was removed and the remaining blood centrifuged at 900g for 2 minutes to obtain platelet-poor plasma. Platelet aggregation studies were performed by optical aggregometry in a platelet aggregometer (PAP-4, BioData) using arachidonic acid (1.5 mmol/L; BioData).

Statistical Analysis

Data were initially analyzed by ANOVA (nonparametric Kruskal-Wallis test). Subsequently, binomial regression (Stata Release 8) was used to model predictors of outcome. Binomial regression is a specialized case of the generalized linear model family. It differs from logistic regression in using a log-link function rather than the logistic-link function. Its advantage is that the model estimates the risk ratio, rather than the odds ratio, making interpretation of the results more intuitive. However, unlike logistic regression models, binomial regression models with the log-link function may fail to converge, and, where this occurred, the relationship was instead modeled using logistic regression and the effect reported as an odds ratio.

Comparisons of TXB₂ values between different aspirin preparations were performed after log-transforming TXB₂ values, resulting in a good fit to the normal distribution. In the case of study 3 (asasantin versus asasantin) the TXB₂ assay used was unable to detect values of <1 ng/mL. Regression models were therefore based on interval regression, implemented in Stata’s –intreg- procedure, to account for the censoring of values introduced.

The clustering of values attributable to repeated measurements on the same patients was dealt with by using Huber-White robust variance estimates. Regression dummy terms for each preparation were used to compare preparations individually with dispersible aspirin. Wald post hoc tests were used to compare preparations with each other.

Results

Treatment Effectiveness

The mean serum TXB₂ levels differed significantly between each treatment group (ANOVA; P<0.001; see Figure 1). To further investigate the differences, the 2 Nu-Seals studies were pooled (enteric 2) and all of the preparations were compared with dispersible aspirin using interval regression. Dispersible aspirin (Geometric mean 0.28 ng/mL, 95% CI: 0.091 to 0.87) was more potent than any other preparation (P=0.001). enteric 2 (2.75 ng/mL, 95% CI: 2.09 to 3.63) was more effective than enteric 3 (5.50 ng/mL, 95% CI: 3.72 to 7.94; P=0.0016) and similar to enteric 1 (2.24 ng/mL, 95% CI: 1.62 to 3.09) and asasantin (1.86 ng/mL, 95% CI: 1.07 to 3.24). Nu-Seals were administered to 2 separate groups and produced very similar effects on thromboxane levels (4.9 ng/mL, 95% CI: 1.7 to 8.1 and 4.1 ng/mL, 95% CI: 2.4 to 5.8,
Treatment failure (<95% inhibition of thromboxane) did not occur with dispersible aspirin. Asasantin had an 8% failure rate, and the enteric-coated aspirin preparations had a 13% failure rate. When all preparations were compared using a $\chi^2$ test for their ability to produce either <95% or >95% inhibition of serum thromboxane levels there was a statistically significant difference ($P=0.011$) between preparations. Incomplete TXB$_2$ inhibition (<99% inhibition) rose from 8% in the aspirin group to 54.3% among those on enteric-coated aspirin and inclusion of this in the $\chi^2$ test was highly significant ($P=0.0004$). The number of subjects achieving predefined levels of inhibition of serum TXB$_2$ is compared for the different preparations in Figure 2 and Table 2.

The levels of platelet aggregation induced by arachidonic acid were compared and aspirin preparations differed significantly (ANOVA; $P=0.001$; Figure 3). Asaasantin was the most effective inhibitor (4% aggregation, 95% CI: 2.5 to 5.4) and enteric 3 was the least effective (12.8% aggregation, 95% CI: 3.4 to 22).

**Effect of Weight**

Table 3 shows the effect of weight on risk of treatment failure for asasantin and enteric-coated aspirin. Weight was significantly associated with both risk of treatment failure (<95% inhibition of TXB$_2$) and incomplete TXB$_2$ inhibition (<99%). A 10-kg (22-lb) increase in weight was associated with an approximate doubling of risk of treatment failure (relative risk 1.9 [95% CI: 1.3 to 2.7] for asasantin and 2.2 [95% CI: 1.7 to 3.0] for enteric-coated aspirin). The binomial model for weight as a predictor of incomplete inhibition among participants taking asasantin failed to converge, and the effect was estimated using logistic regression, and therefore Asasantin data are presented as odds ratios rather than risk ratios. Figure 4 graphically represents the relationship between weight and treatment outcome for participants taking enteric-coated as-

![Figure 1. Serum TXB$_2$ levels in (a) volunteers before starting aspirin therapy and (b) 14 days after aspirin therapy. Enteric 2a and 2b are 2 separate studies with the same product. The horizontal line in the center of the box represents the 50th percentile, the ends of the box represent the 25th and 75th percentiles, the tips of the whiskers represent the 5th and 95th percentiles and the closed circles indicate individual outliers.](image-url)
pirin. Predicted probability of incomplete treatment response rose from 40% at 60 kg (132 lb) to 90% at 100 kg (220 lb), whereas predicted probability of treatment failure rose from 10% at 70 kg (154 lb) to over 50% at 90 kg (198 lb).

Discussion

We performed 3 bioequivalence studies on new low-dose aspirin preparations according to EMEA requirements. Surprisingly, each study showed that the test product was either significantly inferior or superior to the comparator. Thus, we decided to combine all of the studies to determine whether we could identify factors which determined efficacy of the different preparations. In particular we addressed the effect of enteric-coating on aspirin bioavailability.

All aspirin preparations studied produced marked inhibition of serum TXB2 production that ranged from 96.4% inhibition for enteric 3 to 99.5% inhibition for plain aspirin. These results are comparable with a recent study designed to test the bioavailability of enteric-coated aspirin (81 mg) that showed 97.4% inhibition of serum thromboxane production among 12 subjects15 although no comparison with plain aspirin was performed. There was significant variability in the levels of inhibition of thromboxane production achieved with the different preparations with 75-mg dispersible aspirin producing the highest levels of inhibition with the lowest variability. These results are consistent with a previous report of inter- and intrasubject variability in low-dose aspirin response among healthy volunteers.16 Variability was also found when 2 enteric-coated preparations of aspirin 500 mg were compared with uncoated aspirin.17 Studies that focus on mean inhibition of serum thromboxane generation may overlook the significant number of patients with lower levels of inhibition.

All preparations showed strong inhibition of arachidonic acid–induced platelet aggregation; however, there was significant variation between preparations. The most effective preparation was asasantin despite the fact that it delivers the lowest aspirin dose (50 mg) which may reflect the role of dipyridamole in the asasantin preparation. Dipyridamole inhibits platelet aggregation by inhibition of both adenosine uptake and phosphodiesterase activity.18 Two volunteers showed sustained platelet aggregation despite treatment for 14 days with Protek. Platelet aggregation assays were repeated but remained high. Compliance was confirmed by direct enquiry, tablet counting and by the high suppression of thromboxane synthesis. However, these volunteers could not be classed as aspirin resistant because they showed complete inhibition of platelet aggregation on the comparator enteric preparation.

The clinical relevance of incomplete inhibition of platelet function by aspirin has been evaluated in a number of studies.

### TABLE 2. Outcome of Treatment With Three Classes of Preparation

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of Participants</th>
<th>Treatment Failure* (95% CI)</th>
<th>Incomplete Inhibition** (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>25</td>
<td>0% (0%–13.3%)</td>
<td>8% (2.1%–30.5%)</td>
</tr>
<tr>
<td>Asasantin</td>
<td>25</td>
<td>8.0% (1.9%–27.7%)</td>
<td>36.0% (21.3%–60.9%)</td>
</tr>
<tr>
<td>Enteric-coated aspirin</td>
<td>46</td>
<td>13.0% (7.8%–21.0%)</td>
<td>54.3% (44.2%–66.9%)</td>
</tr>
</tbody>
</table>

*The results are expressed as percentages and their confidence intervals.

*Treatment failure: <95% inhibition of thromboxane; **Incomplete inhibition: <99% inhibition of thromboxane.
Persistent thromboxane production has been associated with poor outcome, and there is also evidence of poorer outcome in patients defined as aspirin resistant by platelet aggregometry. In our study 14 subjects failed to achieve an adequate inhibition on an alternative aspirin preparation. In all cases, each subject showed adequate inhibition on an alternative aspirin preparation, suggesting that differences were in bioavailability rather than drug response. Subjects who failed to achieve >95% inhibition of serum TXB$_2$ were heavier, a finding which supports a mechanism of reduced bioavailability. Thus, enteric-coated preparations may deliver a lower dose of aspirin than equivalent doses of plain aspirin. Reduced aspirin delivery may be more evident in heavier subjects who have a larger volume of distribution.

It is not unreasonable to expect a drug to achieve its intended pharmacological effect, which in the case of aspirin is inhibition of platelet COX. Thus, aspirin efficacy is best determined by assays directly dependent on COX function such as TXB$_2$ generation in serum and arachidonic acid–induced platelet aggregation. Complete suppression of platelet COX and thromboxane formation appears to be necessary for optimal clinical benefit. Mechanisms of aspirin resistance may be broadly divided into pharmacokinetic, attributable to inadequate aspirin absorption and pharmacodynamic, where insufficient COX inhibition occurs despite normal absorption. Our study suggests that reduced bioavailability of aspirin from low-dose, enteric-coated preparations may explain some cases of aspirin resistance (pharmacokinetic resistance). Aspirin is deacetylated to inactive salicylate at a number of sites, including the gut; thus, its bioavailability is about 50%. Plain aspirin is absorbed from the stomach, where the low pH protects aspirin against deacetylation and maintains aspirin in a nonionized form which encourages absorption. In contrast, enteric-coated preparations release aspirin into the upper small intestine, which has a near-neutral pH, and therefore more of the aspirin may be inactivated. Also enteric preparations may differ in their rate of dissolution at the intestinal pH (pH 6). Consequently, the dose of aspirin delivered to the circulation (bioavailability) may be less for enteric-coated preparations.

The above referenced secondary prevention studies suggest that aspirin resistance is common among patients with cardiovascular disease including those who received higher doses of aspirin. Our data, based on healthy volunteers, demonstrated no case of pharmacodynamic resistance and supports a pharmacokinetic mechanism for suboptimal aspirin response. Therefore, in addition to reduced bioavailability, other factors may determine aspirin response in patients with cardiovascular disease. Generation of aspirin-resistant thromboxane has been demonstrated in patients with unstable angina and is thought to be attributable to the formation of thromboxane or a precursor in cells other than the platelet. Peroxidation of arachidonic acid may lead to enhanced formation of aspirin-resistant isoprostanes and platelet activation or COX-2–mediated production of thromboxane by circulating monocytes may occur.

There is no doubt that our study was performed in a different population (healthy volunteers) to the target patient population. However, in a recent study of stable cardiovascular patients on enteric-coated aspirin we also found a weight-dependent effect of aspirin on the suppression of TXA$_2$. Compliance is also an issue; however, our population was hospital staff and tablet counting was used to check compliance. If anything, compliance would be higher than in a patient group. If stable plasma levels of drug cannot be obtained in highly motivated healthy volunteers under close scrutiny it is unlikely to be achieved in patients on multiple medications who have coexisting pathology which predisposes to aspirin resistance.

Increasingly, lower doses of aspirin are being used in an effort to avoid adverse effects and preserve prostacyclin formation. However, current aspirin doses are reaching the limit of efficacy and in some instances low-dose aspirin is no-dose aspirin. Current American Heart Association guidelines recommend the use of 75 to 160 mg aspirin daily for primary prevention of stroke and myocardial infarction. Our data would suggest that patients who use low-dose enteric-coated aspirin preparations are less likely to attain full benefit of aspirin because enteric-coated preparations deliver a dose equivalent to 50 mg plain aspirin. The use of a higher dose of aspirin would solve the problem; however, this leads to increased bleeding events. Evidence for increased gastrointestinal bleeding with low-dose plain aspirin relative to equivalent dose enteric-coated preparations is unconvincing, and therefore perhaps reversion to the original drug preparation, if tolerated, may be the best strategy. In conclusion, the use of enteric-coated 75-mg aspirin preparations in heavier individuals may predispose to incomplete inhibition of COX compared with plain aspirin (75 mg). To ensure adequate inhibition of thromboxane production plain (75 mg) aspirin or a bioequivalent dose of enteric aspirin (estimated to be around 100 mg) should be used.

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Disclosures
None.

References


