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Fast Platelet Suppression by Lysine Acetylsalicylate in Chronic Stable Coronary Patients. Potential Clinical Impact over Regular Aspirin for Coronary Syndromes

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Summary

Background: The rapid utilization of fibrinolytics following Q-wave myocardial infarction has clearly modified the evolution of this disease. However, it is still not known whether the immediate inhibition of platelet aggregation (PA) during the coronary event improves outcomes.

Hypothesis: The present study was designed to test, in patients with known coronary artery disease (chronic stable angina), whether the particular kinetic pattern of lysine acetylsalicylate (LA) compared with aspirin may affect the time to onset of inhibition of platelet aggregation.

Methods: Ten patients suffering from chronic stable angina participated in this study to compare the efficacy and speed of the inhibition of PA with 320 mg of LA versus 320 mg of aspirin. All patients discontinued the use of aspirin and any other anti-inflammatory agents for 15 days prior to the beginning of the study. They were randomly assigned to LA or aspirin. Blood specimens were obtained to measure the PA at admission, and 5, 10, 20, 30, and 60 min after ingestion. Patients continued to take the assigned drug once a day for the following 4 days. On Day 5, a new blood sample was taken. After this, patients underwent a 15-day wash-out period, and then crossed over to the opposite drug. The samples were analyzed immediately using platelet-rich plasma stimulated with adenosine diphosphate (ADP) 2 μmol/l, collagen 1 μg/ml, epinephrine 20 μmol/l, and sodium arachidonate acid 0.75 mm/l.

Results: The same level of PA inhibition after 30 and 60 min of aspirin administration can be obtained with LA 5 min following ingestion (sodium arachidonate acid: LA: 16.3 ± 25.9 vs. aspirin 57.6 ± 8.2; p = 0.00014; collagen: LA 18.9 ± 20.1 vs. aspirin 47.2 ± 10.5; p = 0.00092; ADP: LA 27.3 ± 18.4 vs. aspirin 39.7 ± 21.8, p = 0.18; epinephrine: LA 22.0 ± 9.9 vs. aspirin 55.4 ± 10.9, p = 0.00002.

Conclusions: Platelet aggregation inhibition immediately following LA may have significant clinical implications for the treatment of coronary syndromes.

Key words: aspirin, lysine acetylsalicylate, angina, atherosclerosis, platelet inhibition, thrombosis

Introduction

The large-scale randomized thrombolytic trials have contributed greatly to the understanding of myocardial reperfusion therapy.[1,2] These clinical studies proved that thrombolytic therapy saves lives, nearly 20 of 1,000 patients treated. This reduction in mortality seems to be linked to early and complete infarct vessel patency. In this sense, there is a striking benefit in the first 60 min: an approximate 50% reduction of mortality, declining after 1 h, probably tied to the lesser opportunity for myocardial salvage.[3] In addition to this, the Second International Study of Infarct Survival (ISIS-2) trial,[4] which enrolled more than 17,000 patients, demonstrated the additive effect of aspirin (160 mg, and continued daily) for survival benefit. However, it is still not known whether the immediate inhibition of platelet aggregation using aspirin during the coronary event improves outcomes.

We have learned that although regular and enteric-coated aspirins produce equivalent antiplatelet effects,[5] the time to on-

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set of antiplatelet effect is longer after enteric-coated aspirin.[6] As a consequence, a much longer time to antiplatelet effect may be ineffective during the acute phase of coronary syndromes when rapid onset is needed.

Recently, lysine acetylsalicylate (LA), a powerful antiplatelet compound with fewer gastrointestinal side effects than regular and enteric-coated aspirins, proved to be effective in reducing platelet beta thromboglobulin and platelet factor 4, considered to be an index of platelet activation.[7] Lysine acetylsalicylate is a soluble salt that, soon after being administered, is converted to acetylsalicylic acid which is metabolized in the liver to salicylic acid,[8,9] the active component of salicylates.

The present study was designed to test, in patients with known coronary artery disease (chronic stable angina), whether the particular kinetic pattern of LA compared with aspirin may affect the time to onset of inhibition of platelet aggregation.

Methods

The present study was designed as a crossover, operator-blind, randomized trial. It included 10 patients (7 men and 3 women, aged 47 to 60 years), suffering from stable coronary artery disease; they were nonsmokers, had taken no aspirin or related drugs during the 2 weeks before the study, and had given informed consent. The protocol had been previously approved by an independent ethics committee. Patients on aspirin or other inhibitors of platelet aggregation, steroids, migraine relievers, histamine 2 (H2) receptor antagonists, and gastric proton pump inhibitors were excluded. Pregnant or childbearing women, patients with a history of gastritis, gastroduodenal ulcer, adverse reactions while taking nonsteroidal anti-inflammatory drugs (NSAIDs), or allergic diseases were also excluded. Patients suffering from nausea, vomiting, diarrhea, inflammatory bowel disease, malabsorption disorders, ileal bypass, blood discrasia, renal failure, hepatic diseases, or stage III-IV New York Heart Association (NYHA) heart failure were excluded from the study.

Patients were assigned to receive, in random sequence, either LA (Corplus, Laboratorios Bagó SA, Argentina) 320 mg (two 160 mg oral suspension) as initial 1-day dose followed by 160 mg daily for 4–6 additional days and, after the wash-out period, aspirin (Desenfriolito, Labor-atorios Essex, Argentina) 320 mg (four 80 mg pills) initial 1-day dose followed by 160 mg daily for 4–6 consecutive days, or aspirin and then LA on the same schedule as mentioned before. During 15 days before and throughout the study period, volunteers were required not to take any drug capable of affecting platelet function except for the drug under investigation.

On Day 1, a basal whole blood sample was obtained. Thereafter, patients received the assigned medication, and blood samples were collected at 5, 10, 20, 30, and 60 min, and 4–6 days later. Patients remained at rest until the last sample was drawn. On Day 5, 6, or 7, depending on the schedule, an additional single fasting blood sample was collected 12 h after the last dose. The same procedures were followed while taking the other drug under investigation after a 2-week washout period.

Blood Sample Collection

Blood samples were obtained without stasis from an antecubital vein using an 18-gauge needle (butterfly). A 10 ml whole blood sample was collected in a plastic syringe containing 0.1 mmol/l of sodium citrate solution (1:10 v/v) for platelet count and aggregation studies.

Platelet Aggregation Analysis

Platelet-rich plasma (PRP) was obtained by centrifuging whole blood at 900 × g for 10 min and platelet-poor plasma (PPP) by centrifugation of PRP at 8200 × g for 2 min at room temperature. Platelet-rich plasma was adjusted to a platelet count of 300,000/ml with autologous PPP. If contamination of PRP with other blood cells was observed by light microscopy, a second centrifugation at 900 × g for 5 min was used to minimize the number of these blood cells. Plastic syringes, tubes, and pipettes were used for all platelet functional tests.

Platelet aggregation in PRP were measured photometrically in a double-channel Lumi-Aggregometer (Chrono-log Corp., Havertown, Penna.) The light transmittance was set at 10% for PRP and 90% for PPP. The aggregating agent (1–10 µl) was added to the PRP in an aggregometer at 37°C with constant stirring (1,000 rpm). Adenosine diphosphate (ADP) (Sigma Chemical Co., St. Louis, Mo.) 2 µmol/l, collagen (collagen reagent Horn, Nycomed Arzneimittel, GmbH, Munich, Germany) 1 mmol/l, epinephrine (Sigma Chemical Co.) 20 µmol/l, and sodium arachidonate (Sigma Chemical Co.) 0.75 mmol/l were used as inductor agents.

Aggregation was measured as the maximal percentage light transmittance 1 min (initial response) and 4 min (strength of reaction) after addition of the agonist.

Statistical Analysis

Data are expressed as mean ± standard error and evaluated statistically by a nonparametric analysis of variance (ANOVA) for repeated measures. Differences between measures were assessed by post hoc paired *t*-test with Bonferroni's correction. A p value of < 0.05 was considered significant.

Results

Ten patients suffering from chronic stable angina (7 men and 3 women, aged 47 to 60 years, mean: 58 years) entered the study. Their disease had lasted between 5 to18 years; two patients had undergone coronary bypass surgery and three prior percutaneous transluminal coronary angioplasty (PTCA); every patient was being treated with antianginal drugs. Three men and two women started taking LA and then were changed to aspirin, while four men and one woman started taking regular aspirin and then crossed over to LA.

The time elapsed between the start of the study and the last blood sample drawn while on the same treatment was 5.8 ± 0.8 days for the first and 6.7 ± 0.82 for the second treatment schedule.

TABLE I Platelet aggregation studies. Platelet-rich plasma stimulated with epinephrine

	LA	Aspirin	p Value
Basal	60.0 ± 8.9	60.1 ± 15.0	0.98
5 min	22.0 ± 9.9	55.4 ± 10.9	0.00002
10 min	23.4 ± 10.2	46.6 ± 20.4	0.004
20 min	20.2 ± 10.9	33.8 ± 24.9	0.13
30 min	17.7 ± 11.9	21.3 ± 15.7	0.56
60 min	1.0 ± 9.0	18.4 ± 13.0	0.62
Day 4 sample	19.4 ± 10.6	30.2 ± 31.5	0.30

Abbreviation: LA = lysine acetylsalicylate.

TABLE II Platelet aggregation studies. Platelet -rich plasma stimulated with collagen

	LA	Aspirin	p Value
Basal	59.6 ± 8.9	53.1 ± 9.7	0.12
5 min	18.9 ± 20.1	47.2 ± 10.5	0.00092
10 min	8.7 ± 10	42.9 ± 15.1	0.00002
20 min	8.1 ± 11.6	24.7 ± 23.7	0.06
30 min	5.7 ± 8.3	7.5 ± 1.9	0.50
60 min	5.0 ± 9.0	3.7 ± 4.7	0.68
Day 4 sample	4.9 ± 7.7	5.4 ± 6.0	0.86

Abbreviation as in Table I.

TABLE III Platelet aggregation studies. Platelet-rich plasma stimulated with sodium arachidonate

	LA	Aspirin	p Value
Basal	63.5 ± 9.6	63.8 ± 15.2	0.96
5 min	16.3 ± 25.9	57.6 ± 8.2	0.00014
10 min	5.3 ± 4.7	58.5 ± 11.1	0.00001
20 min	6.2 ± 7.9	41.2 ± 28.3	0.014
30 min	5.0 ± 7.0	22.2 ± 23.1	0.03
60 min	4.6 ± 4.8	5.75 ± 9.4	0.74
Day 4 sample	4.4 ± 4.9	5.0 ± 5.6	0.80

Abbreviation as in Table I.

Typical results of platelet aggregation tested ex vivo after adding three major inductors are shown in Tables I, II, and III.

We observed a steady inhibition of platelet aggregation in both groups reaching the lower point 5 min following LA ingestion. At that time, LA almost completely inhibited platelet aggregation; meanwhile similar results were obtained not before 30 min following ingestion of regular aspirin (Fig. 1).

Discussion

The present findings demonstrated that soluble LA in a dose of 320 mg inhibits platelet aggregation in response to arachidonate acid, collagen, and epinephrine 5 min following ingestion. Aspirin used at a similar dose achieved a level of platelet inhibition similar to that of LA after 30 and 60 min of intake. The aggregation response to ADP was equivalent in both LA and aspirin.

Prior clinical studies that tested the effects of aspirin were conducted, particularly in healthy volunteers.[10] Most of these studies found a great variability of platelet aggregation measurements, and very few studies have attempted to correlate changes in platelet functional responses with variable degrees of aspirin dosage.[11]

In other studies conducted in patients post myocardial infarction, no statistically significant differences were observed in platelet aggregation among different doses of aspirin.[12, 13] The secondary wave of ADP-induced aggregation, epinephrine, and arachidonate-induced platelet aggregation, was maximally suppressed with respect to both basal and placebo measurements.

Because of the fact that most frequent aspirin users worldwide are patients with coronary artery disease, the safety and efficacy of these compounds are crucial. In this sense, it has been accepted for theoretical and practical reasons to choose the lowest dose of aspirin in the clinical field.

The daily administration of a low dose of this compound is associated with biochemical changes that are similar to those achieved with high doses. Thus, the research is moving to focus on the rapidity of onset of activity of those antithrombotic compounds.

In a previous study, when an enteric-coated form of aspirin is chewed, a more rapid inhibition of the platelet aggregation and thromboxane A2 production within 15 min was documented, transforming this aspirin into soluble form.[14] In the present study, soluble LA increased the speed of inhibition on platelet aggregation compared with oral aspirin in the same group of chronic coronary patients who served as their own control group at the same time. The inhibitory effect was clearly sustained during the following 6 days of continued administration, before starting the wash-out period.

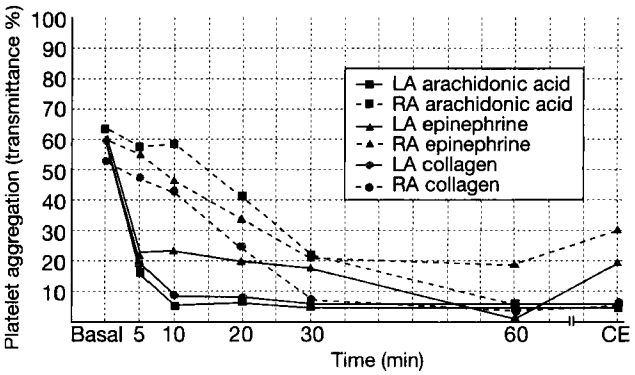

FIG. 1 Platelet aggregation. Epinephrine, collagen, and arachidonic acid studies. LA = lysine acetylsalicylate, RA = regular aspirin, CE = chronic effect.

A potential limitation of this study could be attributed to the fact that the patients receiving soluble LA were not matched against a chewed enteric-coated form of aspirin. However, Gatti *et al.*[15] attempted to resolve this question in a previous work. They analyzed the pharmacokinetics of LA vs. acetylsalicylate, vs. effervescent-buffered aspirin in a comparable dose, all three in a soluble formulation, for the purpose of avoiding the time necessary for tablet disintegration and dissolution. No statistically significant differences were detected in this study. In this respect, the absorption profile is equivalent, suggesting that other unknown properties may play a role in the rapid onset of action achieved.

Pedersen and FitzGerald[16] showed that the fractional systemic biovailability of aspirin is constant over a wide dose range. In this study, using a very low dose of aspirin (20 and 40 mg), a significant decrease of thromboxane B2 levels 5 min following ingestion preceded detection of the drug in the systemic circulation, suggesting that the presystemic acetylation of cyclooxygenase in the antiplatelet action of low-dose aspirin plays a significant role.

Conclusions

The inhibition of platelet aggregation immediately following soluble lysine acetylsalicylate administration may have significant clinical implications for the treatment of coronary patients, particularly during the acute phase of this syndrome. Further clinical trials may elucidate this question.

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