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Ticlopidine, Alka-Seltzer®, or a Combination of Citric Acid With Aspirin: Effects on Platelet Aggregation in Individuals With an Insufficient Response to Aspirin Alone

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Summary: Aspirin (ASA) does not effectively lower platelet aggregation in all people. The platelet aggregation (PA) score is an easily used clinical method for measuring the effect in individuals of antiplatelet medications. Fifteen apparently healthy subjects (2 men and 13 women), selected for their resistance to ASA's antiaggregation effect, completed a sequential trial of ticlopidine, Alka-Seltzer®, and ASA + citric acid (CTA). Ticlopidine was the strongest aggregation inhibitor and the ASA + CTA combination was more inhibitory than Alka-Seltzer. It was determined that measuring antiaggregation effects of a particular agent in an individual prior to usage would optimize treatment. The PA score methodology provides a means for testing patients prior to antiplatelet therapy for prevention and treatment of the thrombotic complications of vascular disease. **Key Words:** Platelet aggregation inhibitors—Aspirin—Thrombosis

The effect on aggregation of antiplatelet agents is rarely measured prior to establishment of clinical therapy regimens. Instead, selection of antiplatelet medication for patients is often based on research results that identify a particular agent as a beneficial treatment to prevent a specific vascular event, rather than on the individual patient's platelet aggregation profile. The platelet aggregation (PA) score is a numerical assessment based on quantification of adenosine diphosphate (ADP)-induced aggregation that describes the relationships between platelet behavior, thrombotic conditions, and antiplatelet therapy (1). The long-term patency of polyester femoropopliteal bypass grafts has been found to be associated with a PA score, whether inherent or medicinally achieved (2). The benefits of aspirin (ASA) therapy in reducing the incidence of arterial occlusive thrombotic events have been repeatedly demonstrated in large-scale, randomized, placebo-controlled trials; however, the protective effect of ASA therapy is not seen in all patients (3). ASA unresponsiveness has been found to be prevalent among individuals with PA scores >30; reduction of PA scores to <30 is possible with ASA therapy in only half of these inherently high-aggregation responders (4); thus, alternative antiplatelet treatments are indicated.

In a double-blind study, we demonstrated that in persons previously shown not to respond to ASA, a combination of ASA and citric acid (CTA) was effective in reducing PA scores to a significantly greater degree than either ASA alone, CTA alone, or placebo (4). The purpose of the present study was to extend the comparison of the ASA + CTA combination to include the prescription medication ticlopidine (Ticlid®) (Roche Laboratories, Inc.; Nutley, NJ) and the nonprescription combination of ASA and citrate salts, Alka-Seltzer® (Miles, Inc.; Elkhart, IL). In this open-label crossover study, the effects of ASA, Alka-Seltzer, ASA in combination with CTA, and ticlopidine on platelet aggregation were evaluated in apparently healthy volunteers known to be poor responders to ASA therapy.

SUBJECTS AND METHODS

Subjects

Apparently healthy subjects who had previously demonstrated PA score values >30 were recruited from a voluntary blood testing program approved by the Institutional Review Board of the Providence Seattle Medical Center. Forty-five subjects, 14 men and 31 women, with an age range of 34–81 years (mean 63.6 ± 9.3) were recruited. Only postmenopausal women were included to eliminate possible estrus cycle variations in platelet aggregability. All subjects were instructed to fast after 10:00 p.m. the evening before each blood draw, and to abstain from ASA and/or ASA-containing products other than those included in the study protocol for at least 10

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days before their first appointment and for the duration of their participation in the study. Blood samples were drawn between 8:00 and 10:00 a.m., using the standard venipuncture technique. Samples were collected in 5-mL K_2(EDTA) tubes (Becton Dickinson Co.; Rutherford, NJ) for whole blood cell counts and in 4.5-mL 3.8% buffered sodium citrate tubes (Becton Dickinson Co.) for platelet aggregation studies. PA score assessments were completed within 3 hours of blood collection.

For all study participants, PA score values were determined prior to initiating ASA therapy (325 mg/day) for 2 weeks and within a day of cessation of treatment. If the PA score value dropped below 30 after ASA ingestion, the subject's participation in the study was discontinued. If the PA score value remained ≥30, the individual crossed over into a sequence of three medications: Alka-Seltzer (one tablet in water, daily), a combination of ASA + CTA (162.5 mg each/day), and ticlopidine (250-mg tablets, one twice daily). With the exception of an 11-day course for ticlopidine, all medications were ingested for 2 weeks with a minimum 2-week medication-free interval before initiation of each treatment. As with the ASA therapy, PA score determinations were performed prior to and right after each subsequent medication period. Differential white blood cell counts were performed before and after treatment with ticlopidine to verify the presence or absence of neutropenia (<1200 neutrophils/mm^3). If the pretreatment blood sample showed neutropenia, the subject's participation in the study was discontinued. If no neutropenia was detected, the subjects concluded their participation in the study with the ticlopidine treatment.

Platelet aggregometry studies

Platelet aggregation was evaluated with the sedimented platelet-rich plasma (SPRP) method and the PA score methodology (1), using 2.5 μM ADP reagent (Chrono-Log Corp.; Havertown, PA). Whole blood platelet counts for PA score determinations and white blood cell differentials were obtained on a Technicon H*1 hematology analyzer (Technicon Co.; New York, NY) with the latter test performed only before and after ticlopidine therapy to monitor neutrophil counts. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared by the previously described SPRP method. Platelet aggregation tests were performed on a Chrono-Log 560 VS aggregometer (Chrono-Log Corp.; Havertown, PA) and a Fluke 1752 data acquisition system (John Fluke Manufacturing Co.; Everett, WA). The software and aggregometer/computer interface that automate the PA score test were designed and manufactured by Cybermed Technology Co., Inc. (Edmonds, WA). The PA score was computer generated using the following formula:

$$\text{PA score} = \text{Amp R} \times \text{Sagg} \times \text{Plt R}$$

where Amp R is the ratio of the primary amplitude to the maximum amplitude of the aggregation pattern; Sagg is the area under the aggregation curve; and Plt R is the ratio of the whole blood platelet count to the PRP-adjusted platelet count (250,000 + 25,000/mm^3) in the test sample.

Data analysis

For the statistical analyses, including the effect of each treatment on platelet aggregation response and the comparative evaluation of treatment efficacy, the PA score measurements were used. To confirm comparability of the initial conditions preceding each treatment period, the PA score measurements were taken prior to any therapy (initial baseline) and before inception of each new medication (washout baseline); statistical difference between these baselines was then estimated. The Student's *t* test was used to evaluate null hypothesis of equality for baseline and treatment comparisons. Each treatment's effectiveness was calculated as the percent of mean of post-treatment PA score values to the PA score mean of that therapy baseline.

RESULTS

From 45 subjects recruited for this study, 21 subjects (10 men and 11 women) were discontinued because their PA score value dropped to <30 after the initial 2 weeks of ASA therapy. PA score determinations identified the remaining 24 subjects as ASA-resistant with PA score values >30 after treatment. All 24 of these ASA-resistant subjects (4 men and 20 women) elected to continue in a sequential trial of the other three medications. In the course of the study, nine subjects discontinued participation for the following reasons: low platelet count (one female subject), low neutrophil count (one female subject), ticlopidine side effects (one male subject), and voluntary discontinuance (one male and five female subjects).

Fifteen subjects (2 men and 13 women) completed all four medications. The PA score values pre- and post-treatment for these 15 individuals and the results from statistical evaluation (*t* test) of all nonmedicated (baseline) PA score values are presented in Table 1. No statistical differences between baseline assessments were demonstrated. A comparative evaluation of baseline and medicated PA score values for each of the four medications tested is presented in Table 2. Average percent inhibitions of platelet aggregability were as follows: 20.0% for ASA, 25.6% for Alka-Seltzer, 46.2% for ASA + CTA, and 66.6% for ticlopidine. Relative to PA score baseline levels, statistically significant reductions in PA score values were demonstrated after treatment with each of the medications tested. However, PA score mean values remained >30 after ASA and Alka-Seltzer treatments. A comparative evaluation of the inhibitory effect

TABLE 1. *Statistical evaluation of baseline platelet aggregation scores after washouts*

Group 1 vs group 2	Mean ± SD group 1	Mean ± SD group 2	*t* value	*P* value
Aspirin vs Alka-Seltzer	47.6 ± 9.6	47.7 ± 7.9	−0.027	0.979
Aspirin vs aspirin + citric acid	47.6 ± 9.6	51.3 ± 14.1	−0.844	0.406
Aspirin vs ticlopidine	47.6 ± 9.6	46.5 ± 12.3	0.265	0.793
Alka-Seltzer vs aspirin + citric acid	47.7 ± 7.9	51.3 ± 14.1	−0.870	0.392
Alka-Seltzer vs ticlopidine	47.7 ± 7.9	46.5 ± 12.3	0.306	0.762
Aspirin + citric acid vs ticlopidine	51.3 ± 14.1	46.5 ± 12.3	0.990	0.331

of the medications tested is presented in Table 3. Relative to ASA treatment, no statistical difference was demonstrated with Alka-Seltzer ($P = 0.43$), while significant reductions in PA score values resulted after treatment with the ASA + CTA combination ($P = 0.004$) and ticlopidine ($P < 0.001$).

Ticlopidine was the strongest aggregation inhibitor among all medications evaluated. The inhibitory effect of the ASA + CTA combination on PA score values was greater than that observed for Alka-Seltzer treatment ($P = 0.04$). The combination of ASA + CTA provided better inhibitory action on platelet aggregability than ASA alone in these ASA-resistant individuals.

DISCUSSION

Although antiplatelet drugs have been used extensively for the prevention of stroke and other vascular events, no one treatment has been shown to work in all patients. Limitations in usage and efficacy of antiplatelet agents have been attributed to side effects, as well as inherent differences in platelet aggregability among patients. Measuring the antiaggregation ability of antithrombotic medication to determine effectiveness in individual patients would appear to be useful. Unfortunately, testing to assess the thrombotic potential of the platelet aggregation response and to measure changes after treatment has for the most part remained a research tool. The PA score method of determining and interpreting ex vivo platelet aggregometry is easily performed and has been shown to correlate with independent markers of platelet activation sensitivity (5) to predict the likelihood of femoropopliteal bypass graft failure (2), to predict results of endovascular and open surgical treatment of carotid occlusive disease (6), and to provide a meaningful measure of inherent platelet aggregability and changes after antithrombotic treatment (4).

In a previous study (4) we defined a criterion using the PA score method for identifying a poor aggregation response to ASA and evaluated the combination of ASA + CTA as an alternative treatment for ASA-resistant subjects. The addition of CTA was based on the hypothesis that providing additional free carboxyl groups from this oxyacid would potentiate the acetylation reaction of ASA in blocking cyclooxygenase activity. This combination therapy was found to improve the antiaggregatory effect of ASA in half the subjects in whom ASA alone had a poor effect.

Alka-Seltzer was included in the current study because in its dry form it is a combination of ASA, CTA, and sodium bicarbonate that effervesces when dissolved in water, producing the salts sodium citrate and sodium acetylsalicylate. Since this combination of salts is chemically very close to the ASA + CTA combination therapy, it was possible that it might have a greater inhibitory effect on platelet aggregability in ASA-resistant subjects. Ticlopidine, an antiplatelet agent used mainly for primary and secondary stroke prevention, is thought to mediate platelet activation through three pathways: thromboxane A_2, ADP, and platelet-activating factor (PAF). Unlike ASA, which acts chiefly on thromboxane A_2 and weakly on the other two pathways, ticlopidine has a sig-

TABLE 2. *Comparative evaluation of platelet aggregation scores pre- and postmedication*

Pre- vs post-	Mean ± SD pre-	Mean ± SD post-	*t* value	*P* value
Aspirin	47.6 ± 9.6	38.1 ± 7.6	3.02	0.0054
Alka-Seltzer	47.7 ± 7.9	35.5 ± 9.9	3.73	0.0008
Aspirin + citric acid	51.3 ± 14.1	27.6 ± 10.5	5.21	0.000016
Ticlopidine	46.5 ± 12.3	15.5 ± 9.6	7.68	0.000000

TABLE 3. *Comparative evaluation of the inhibitory effect of medications tested*

Group 1 vs group 2	Mean ± SD group 1	Mean ± SD group 2	*t* value	*P* value
Aspirin vs Alka-Seltzer	38.1 ± 7.6	35.5 ± 9.9	0.805	0.428
Aspirin vs aspirin + citric acid	38.1 ± 7.6	27.6 ± 10.5	3.107	0.004
Aspirin vs ticlopidine	38.1 ± 7.6	15.5 ± 9.6	7.105	0.000
Alka-Seltzer vs aspirin + citric acid	35.5 ± 9.9	27.6 ± 10.5	2.098	0.045

nificant affect on ADP and PAF activity and a weak affect on thromboxane A_2. Ticlopidine inhibits the platelet shape change reaction which prevents further progress of platelet release and aggregation. This difference in mechanism of action might offer an explanation for why, among the medications evaluated in this study, ticlopidine demonstrated the most significant PA inhibition in this cohort of ASA-resistant subjects. The lack of improvement over ASA therapy seen with the Alka-Seltzer treatment correlated with in vitro work that demonstrated the antiaggregatory effect of CTA to be pH dependent, with no effect at the high pH range of the dissolved Alka-Seltzer medication. The results of this study confirmed previous findings that combined ASA + CTA therapy provides greater reduction of the PA score than ASA alone.

As more antiplatelet medications become available, the selection of an agent should be weighed against safety issues, as well as effectiveness for the individual. The inhibitory effect of ticlopidine was the most potent, eliciting a more widespread effect and greater reduction of the PA score than the other medications evaluated; however, the potential for side effects was also greatest. The acute course of treatment in this study was intended to reduce the risk of neutropenia and thrombocytopenic purpura, adverse effects which have limited clinical use of ticlopidine (7), and neither of these side effects was observed. Gastrointestinal side effects have been the most common with ASA therapy, and the incidence is proportional to the dosage. Because CTA is used in many food products, any adverse effects from the combination ASA + CTA medication would tend to mimic ASA therapy but would be lessened by the smaller dose of ASA required. Thus, the combination ASA + CTA therapy would potentially have an even broader range of efficacy and patient toleration than ASA.

Measuring the antiaggregation potential of a particular agent prior to usage would serve to optimize treatment of the individual patient. The PA score methodology has the potential to provide a clinical means for testing patients prior to antiplatelet therapy for prevention and treatment of the thrombotic complications of vascular disease.

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