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Combined Aspirin and Cilostazol Treatment is Associated with Reduced Platelet Aggregation and Prevention of Exercise-Induced Platelet Activation

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Abstract *Background:* Cilostazol has proven efficacy in increasing walking distance in claudicants, but it has not been demonstrated to be more effective than placebo in secondary cardiovascular prevention. The direct effect of exercise on platelet function remains less well defined. We have investigated the effect of combination treatment with aspirin and cilostazol on platelet activity in claudicants subjected to repeated treadmill exercise.
Methods: Nineteen claudicants completed a double-blind, randomised, controlled, cross-over trial. Each subject received a 2-week course of aspirin (75 mg) and placebo and aspirin and cilostazol (100 mg twice daily). Following each 2-week treatment period, patients participated in a standardised treadmill test (3.2 km h^{-1}, 10° incline) walking to maximal claudication distance. The exercise was repeated thrice in total, and blood was sampled before and after exercise. Platelet activation was measured using free platelet counting aggregation, flow cytometry for surface markers of platelet activation and soluble P-selectin assay.
Results: Compared to aspirin and placebo, combination treatment with aspirin and cilostazol was associated with reduced arachidonic-acid-induced platelet aggregation ($p < 0.01$, Wilcoxon signed-rank test). Aspirin and placebo treatment were associated with elevated P-selectin expression, platelet-monocyte aggregation and reduced CD42b expression ($p < 0.05$, Wilcoxon signed-rank test) post-exercise. No difference was seen in spontaneous platelet aggregation whilst soluble P-selectin was reduced post-exercise with combination treatment with aspirin and cilostazol ($p < 0.05$, Wilcoxon signed-rank test).
Conclusions: Combination treatment with aspirin and cilostazol results in suppression of platelet activation and reduces the effect of exercise on platelets. The benefit seen may be a result of cilostazol enhancing the inhibitory effect of aspirin on the cyclo-oxygenase pathway.

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Cilostazol is a type III phosphodiesterase inhibitor licensed for use in intermittent claudication. A meta-analysis of eight multicentre drug trials involving over 2700 claudicants has indicated that cilostazol is beneficial in improving both initial and absolute claudication distance.[1] Comparable results have also been reported by a Cochrane review.[2]

By inhibiting phosphodiesterases, cilostazol prevents the hydrolysis of cyclic adenosine monophosphate (cAMP). The consequential rise in intracellular cAMP is responsible for its antiplatelet and vasodilatory properties.[3] In addition, cAMP is an inhibitor of the cyclo-oxygenase pathway, and hence reduces production of TxA_2. Within vascular smooth muscle, cAMP inhibits the release of stored intracellular Ca^{2+}, and hence suppresses the activity within contractile proteins. While the direct antiplatelet effects of cilostazol have been confirmed, any possible clinical benefits require further work.[4] In the context of coronary artery disease, a beneficial effect has been seen in the prevention of restenosis after percutaneous coronary intervention.[5,6] Compared to placebo, cilostazol has also been shown to be useful in the prevention of secondary stroke, although there was not a significant benefit over aspirin.[7–9] Although the risk of intra- and post-operative bleeding requires further investigation, the results of a study by Wilhite et al. reassuringly suggested cilostazol does not significantly prolong bleeding time.[10]

Although the effect of a combination therapy with aspirin remains undefined, any synergistic antiplatelet effect may support the use of cilostazol in patients with peripheral arterial disease (PAD). Further studies are needed to ascertain both the antiplatelet effect of combination therapy and the potential clinical benefits. This study was performed as a pilot study to assess whether combination therapy with cilostazol and aspirin had an antiplatelet effect that exceeded that of aspirin alone and attenuated the increased platelet activation seen in PAD patients following exercise.

Materials and Methods

Study design

A double-blind, randomised, placebo-controlled, cross-over trial design was used. A cross-over design was chosen to account for any potential confounding effects of smoking, co-morbidities and medication on platelet function. Using a table of block randomisation, patients were randomised to start either aspirin/placebo or aspirin/cilostazol and then treated with the alternative combination therapy. Both the patient and the principal investigator were blinded. Treatment packs were dispensed by the hospital pharmacy. The treatment regimens revealed only after the completion of the study by all participants and analysis of the assays. Each patient was subjected to a 14-day period of treatment with aspirin (75 mg) and placebo (twice daily) and aspirin and cilostazol (100 mg twice daily). A 10-day 'washout' period was allowed between treatments. On the final day of each treatment, the patients were brought to the hospital and performed a standardised treadmill test with blood sampling as previously described.[11] The local ethical committee and the Research and Development committee approvals were obtained. The study was performed according to the declaration of Helsinki. PAD patients with symptoms of intermittent claudication were recruited from outpatients. All patients were treated with 75 mg aspirin daily, prior to recruitment, as part of their routine antiplatelet therapy.

Inclusion and exclusion criteria

Patients were included if they had PAD with intermittent claudication, ankle–brachial pressure index (ABPI) < 0.9 and a radiologically (either angiographically or with duplex) proven lower limb disease. All patients were aged between 40 and 80 years, were able to complete a treadmill exercise test and were on 75 mg aspirin (prior to commencement of cilostazol or placebo) as part of their routine antiplatelet therapy. Patients were excluded if they had any other co-morbidities known to alter platelet activity. Specifically, chronic renal failure, diabetes mellitus, invasive malignancy, current infectious process (including gangrene), platelet count <150 or >450 and any concurrent platelet disorders were the exclusion criteria. Patients taking steroids, anticoagulants and antiplatelet agents (other than aspirin) were also excluded. Patients with co-morbidities preventing completion of a treadmill test, for example, arthritis, pulmonary disease and heart failure, were also excluded. Cilostazol is metabolised by hepatic cytochrome P-450 enzymes, hence patients taking any medication that induced or inhibited cytochrome P-450 activity were also excluded.

Treadmill testing

Patients were asked to remain on clear fluids only for 12 h prior to attending hospital for the treadmill test, aimed at minimising the effect of dietary agents on platelet activity. Transport to the hospital was provided, and, on arrival, patients were rested supine for 90 min prior to the treadmill test (3.2 km h^{-1} at a 10° incline). All participants were asked to walk to their maximal claudication distance. This was repeated thrice in total with brief rest periods in between to allow resolution of calf pain. Blood samples were taken before commencement of exercise and after completion of all exercises (1 min and 40 min after completion of the third cycle of claudication, respectively).

Assessment of platelet activation

Platelet activation was assessed by a variety of methods. Flow cytometry was used to measure the expression of platelet P-selectin, glycoprotein (GP) Ib-IX-V and platelet–monocyte aggregates (PMAs). Whole blood free platelet aggregation was used to measure spontaneous platelet aggregation and arachidonic acid (AA)-induced aggregation. ELISA was used to quantify changes in soluble P-selectin.

Flow cytometry was performed using a whole-blood two-colour staining technique to quantify platelet P-selectin and GP Ib-IX-V. The method for P-selectin has been previously described.[11,12] GP Ib-IX-V expression was measured with a similar technique but using fluorescein isothiocyanate (FITC)-conjugated CD61 antibodies (Dako, Cambridgeshire,