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ORIGINAL ARTICLE

Clopidogrel Improves Microvascular Endothelial Function in Subjects with Stable Coronary Artery Disease

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Background Clopidogrel therapy has recently been shown to reduce cardiovascular events in patients with stable vascular disease. This benefit may be due to effects not exclusively related to platelet aggregation. The aim of this study was to evaluate the effect of clopidogrel therapy on microvascular endothelial function in subjects with stable coronary artery disease (CAD).

Methods and Results Forty subjects with stable CAD were randomised to clopidogrel therapy (75 mg/day) or control. Blood and endothelial function testing occurred at baseline, one week and three months following randomisation. Microvascular endothelial function was assessed via reactive hyperaemic index (RHI). Platelet function was assessed by adenosine diphosphate (ADP)-induced whole blood aggregation and the VerifyNow^{TM} system. Plasma markers of endothelial function (asymmetric dimethylarginine, ADMA) and oxidative stress (myeloperoxidase, MPO) were also tested. The primary endpoint was endothelial function assessment (RHI) at three months.
At one week RHI increased by 20±10% in the clopidogrel group; this effect was maintained at three months (21±9% increase from baseline; P<0.01). A significant decrease in ADP-induced platelet aggregation and P2Y12 reaction units was observed in the clopidogrel therapy group (P<0.01). There was no correlation between endothelial function and platelet function testing in the clopidogrel therapy group.

Conclusion Clopidogrel therapy is associated with improved microvascular endothelial function in patients with stable CAD. This effect is independent of its effects on ADP-induced platelet reactivity.

Keywords Clopidogrel • Endothelial function • Coronary artery disease • Platelet aggregation • Nitric oxide

Introduction

Clopidogrel is an anti-platelet agent that works by inhibiting adenosine diphosphate-induced platelet aggregation through the P2Y12 receptor. While newer anti-platelet agents such as Prasugrel [1] and Ticagrelor [2] are increasingly replacing clopidogrel in patients presenting with 'high-risk' acute coronary syndromes, clopidogrel remains widely used and recommended in such patients [3]. Furthermore clopidogrel remains the treatment of choice in patients with stable

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disease undergoing a percutaneous coronary intervention (PCI).

The published Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events (CHARISMA) trial [4] evaluated the effect of clopidogrel in combination with aspirin in reducing cardiovascular events in high-risk subjects including those with known vascular disease. Overall the CHARISMA trial found no benefit in the entire study cohort. However, in sub-group analysis significant benefit was seen in patients with established vascular disease. While it is hypothesised that the benefit of clopidogrel in the context of this sub-group analysis is in reducing intra-arterial thrombosis associated with rupture/vulnerable plaque, this may be a simplistic view of the potential mechanistic benefits of this medication. Previous studies have shown that short-term clopidogrel has pleiotrophic effects relating to vascular protection such as beneficial effects on endothelial function [5,6] and apoptosis [7], however little information is known about any longer-term pleiotrophic effects of clopidogrel. The primary aim of this study was to evaluate the effect of three months of clopidogrel therapy in stable coronary artery disease (CAD) on microvascular endothelial function.

Method

Patient Population

Forty patients (35 men and five women) with known CAD (defined by at least one of the following, known acute myocardial infarction, previous PCI or coronary artery bypass grafting) were recruited into this study. Patients were excluded if they: (i) had a history of atrial fibrillation, (ii) had an acute coronary syndrome or myocardial infarction within the last three months, (iii) had documented cerebrovascular disease, (iv) had severe renal impairment (creatinine clearance <25 ml/min), (v) were considered to have a significant bleeding risk (for example, previous haemorrhagic stroke or peptic ulcer disease), (vi) taking a proton pump inhibitor, due to the potential interaction between these drugs and clopidogrel [8] or (vii) taking clopidogrel at time of randomisation. All patients were required to be taking low-dose aspirin therapy (100-300 mg/day).

This protocol was approved by the Ethics of Human Research Committee of the Royal Adelaide Hospital and written informed consent was obtained from all patients prior to study entry.

Study Protocol

This study was a blinded, randomised, control trial to evaluate the effect of three months clopidogrel treatment on endothelial function in patients with stable coronary disease. Patients were randomised in a ratio of 1:1 to either treatment or control groups. Those in the treatment group received clopidogrel (oral, 75 mg daily) in addition to their regular medications. The control group had no changes to their medical management throughout the course of this three-month study. Patient's demographics were taken at baseline. Patients had blood taken and endothelial function testing evaluated at baseline, one month and three months after the first dose (Fig. 1). Vasoactive medications were withheld for 24 hours prior to each visit. At the baseline visit, patient demographics were recorded. All testing was conducted between 0730 and 1100 hours to avoid potential circadian effects on endothelial or platelet function. All women studied were post-menopausal.

Endothelial Function Testing

Peripheral (microvascular) endothelial function was assessed by reactive hyperaemia peripheral arterial tonometry (RH-PAT) using the Endo-Pat2000™ system (Itamar Medical, Caesarea, Israel) as previously described [9–11]. This non-invasive assessment of endothelial function uses a validated methodology [12,13] which evaluates changes in pulse-wave amplitude (PWA) recorded with a PAT device placed over the tip of the index finger in response to a flow-mediated stimulus. The peripheral arterial tonometry hyperaemia index (RHI) is defined as the ratio of the average pulse wave amplitude during the 1-minute period of reactive hyperaemia compared with the average pulse wave amplitude during a 210-second preocclusion baseline period normalised to the signal from the non-hyperaemic control arm to compensate for spontaneous systemic changes. To assess endothelial-independent vasodilation, 400 µg glyceryl trinitrate (GTN) (Nitrolingual Pumpspray, Sanofi-Aventis, Bridgewater, NJ) was administered as a sublingual spray and PWA recorded for 10 mins. Endothelial-independent function was expressed as the GTN-PAT ratio, which is calculated as the ratio of the average peak PWA during a 1-min period post-GTN divided by the average baseline PWA during a 1-min period pre-GTN. As GTN produces systemic dilation, GTN-PAT ratios from both hands were averaged, as previously described [12]. Studies were performed in the supine position in a comfortable, quiet and temperature-controlled room following a 10-min equilibration period.

Blood sampling

Blood samples were obtained via venipuncture of an antecubital vein and collected into tubes containing 3.8% sodium citrate at 9:1 ratio. Laboratory personnel who conducted endothelial function testing and platelet function testing were blinded to patient characteristics and randomisation. Platelet function testing was performed using the point-of care VerifyNow™ device and via whole blood impedance aggregometry. A systemic marker of nitric oxide synthase inhibition (asymmetric dimethylarginine, (ADMA)) and a measure of oxidative stress (myeloperoxidase, (MPO)) were measured from plasma samples utilising an enzyme linked immunosorbent assay (ELISA). Lipid profile was also assessed.

VerifyNow™

The VerifyNow™ system (Accumetrics, San Diego, USA) is a rapid cartridge-based point-of-care device used to measure