

LOW-DOSE ASPIRIN AND CLOPIDOGREL ATTENUATE REFLEX CUTANEOUS VASODILATION IN MIDDLE AGED SKIN

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INTRODUCTION

We recently demonstrated that chronic low-dose aspirin therapy (81mg daily) consistently and significantly attenuates reflex cutaneous vasodilation (VD) in middle-aged (58±3 years) human skin¹; however the precise mechanisms underlying this response are unclear. Full expression of thermoregulatory reflex cutaneous VD is dependent on nitric oxide synthase-(NOS)^{2,3} and cyclooxygenase- (COX) mediated second messenger mechanisms which contribute independently to the rise in skin blood flow during hyperthermia⁴. Low-dose aspirin therapy is increasingly recommended in middle-aged and elderly populations for atherothrombotic disease prevention⁵. Aspirin is an irreversible inhibitor of platelet and vascular cyclooxygenase (COX) I and II. At low doses (81mg daily) aspirin acetylates platelet COX-1 in the presystemic (portal) circulation⁶ inhibiting platelet production of the potent aggregating agent and vasoconstrictor thromboxane A₂ (TXA₂) for the life of the platelet (~10 days), whereas vascular endothelial cells re-synthesize COX in a matter of hours maintaining the ability to produce COX-dependent vasodilators.

There are several putative mechanisms that may underlie reduced reflex cutaneous VD in subjects taking low-dose aspirin including: 1) low-dose aspirin may inhibit COX isoforms in platelets and cutaneous vascular tissue, thus blocking a key enzyme involved in reflex VD⁴, and 2) platelet activation during hyperthermia may release substances that directly stimulate cutaneous VD pathways contributing to reflex cutaneous VD. Therefore, the purpose of this

study was to examine the role of vascular COX and platelet activation in attenuated reflex cutaneous VD. We hypothesized that specific platelet ADP-receptor inhibition (clopidogrel) would not attenuate reflex cutaneous VD but that COX-1 inhibition with low-dose aspirin would attenuate reflex cutaneous VD through COX, rather than NOS-dependent mechanisms.

METHODS

Subjects. Studies were performed on 6 subjects at enrollment and then in a double blinded fashion after 7 days of 81 mg aspirin and/or 75mg clopidogrel with a two week washout period between drugs. Subjects underwent a complete medical screening, including a physician-supervised graded exercise test to evaluate the existence of underlying cardiovascular disease, blood chemistry, coagulation study (PT and PTT), lipid profile evaluation, resting electrocardiogram, and physical examination. No subjects were previously taking low-dose aspirin or clopidogrel.

Instrumentation and Measurements. Subjects were instrumented with four intradermal microdialysis fibers (MD2000, Bioanalytical Systems) in the skin on the left ventral forearm as previously described⁷. MD sites were perfused with 1) 10.0 mM *N*^G-nitro-L-arginine methyl ester (L-NAME) to inhibit NO production by NOS, 2) 10.0 mM ketorolac to inhibit COX isoforms⁸, and 3) lactated Ringer solution to serve as a control.

Skin blood flow (cutaneous red blood cell flux) was measured with an integrated laser-Doppler flowmeter probe placed in a local heater maintained at 33°C on the skin directly above each MD membrane. After baseline thermoneutral measurements whole body heating (water-perfused suit) was conducted as previously described⁹ to increase body core temperature by 1.0°C. Cutaneous vascular conductance (CVC) was calculated as flux divided by mean arterial pressure and

normalized to a percentage of CVC_{max} (% CVC_{max} : 28mM sodium nitroprusside + local heating to 43°C). % CVC_{max} data were averaged for baseline and every 0.1°C rise in T_{or} .

Statistical Analyses. A three-way mixed models analysis of variance (ANOVA) with repeated measures was conducted to determine 1) differences between systemic drug treatments (ASA and Plavix) across the rise in body core temperature, and 2) differences between localized MD drug treatment across the rise in body core temperature. The level of significance was set at $\alpha = 0.05$. Values are presented as means \pm SEM.

RESULTS

Skin blood flow at thermoneutral baseline and at the plateau with a 1.0°C rise in body core temperature is illustrated in Figure 1 for all MD treatment sites. Localized COX-inhibition augmented baseline % CVC_{max} in all trials ($p < 0.001$). During baseline and systemic aspirin treatments NOS- inhibition attenuated reflex VD at the plateau ($p < 0.001$), however there was no difference between control sites and COX-inhibited sites with $\Delta T_{or} \geq 0.4^\circ\text{C}$. With systemic aspirin treatment reflex VD was attenuated with $\Delta T_{or} \geq 0.8^\circ\text{C}$ at the control site. Systemic clopidogrel treatment significantly attenuated reflex VD compared with baseline and systemic aspirin treatment ($p < 0.001$). In contrast to the baseline and systemic aspirin treatment, there was no difference between the control, NOS-I, or COX-I sites with systemic clopidogrel treatment.

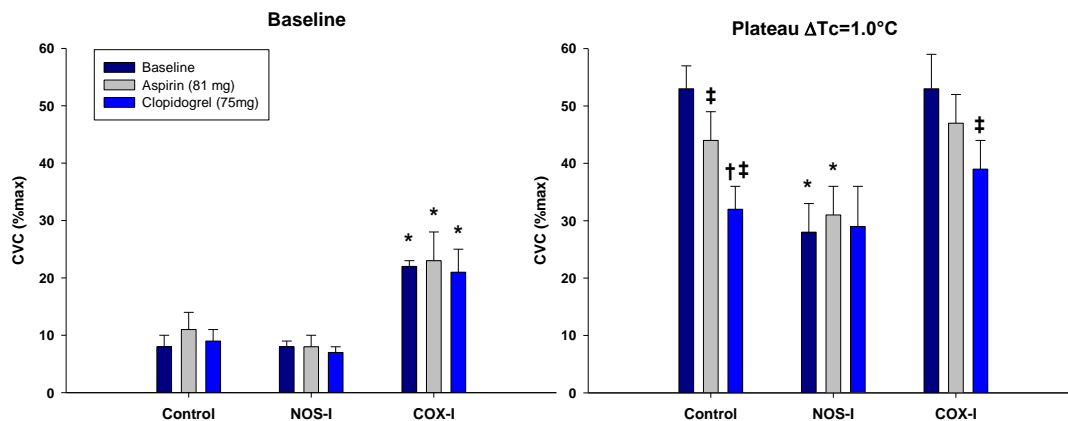


Figure 1: Cutaneous vascular conductance at a percentage of maximum (% CVC_{max}) during thermoneutral baseline (A) and at the plateau with $\Delta T_c = 1.0^\circ\text{C}$ (B) in the control, nitric oxide synthase-inhibited (NOS-I), and cyclooxygenase-inhibited (COX-I) sites, during baseline experiments (no systemic drug) and with systemic low-dose aspirin and clopidogrel treatments. * $p < 0.05$ vs. the control microdialysis site, † $p < 0.05$ vs. the baseline (no drug) experiment, ‡ $p < 0.05$ vs. systemic aspirin treatment

Figure 2 illustrates the absolute maximal CVC (flux/mmHg) for each microdialysis site at baseline and with systemic aspirin and clopidogrel treatment. There was no difference in maximal CVC due to localized microdialysis treatments or across trials (both $p > 0.05$).

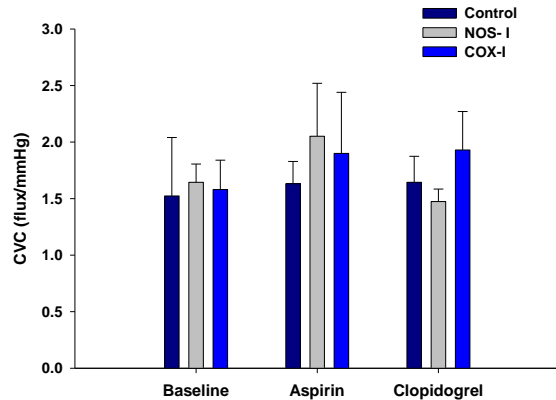


Figure 2: Absolute maximal cutaneous vascular conductance in control, nitric oxide synthase-inhibited (NOS-I) and cyclooxygenase-inhibited (COX-I) sites during baseline experiments (no systemic drug) and during systemic low-dose aspirin and clopidogrel treatments.

Figure 3 shows the time required to increase body core temperature by 1.0°C all of the trial. Systemic aspirin and clopidogrel treatment both decreased the time required to increase body core temperature by 1.0°C ($p < 0.01$).

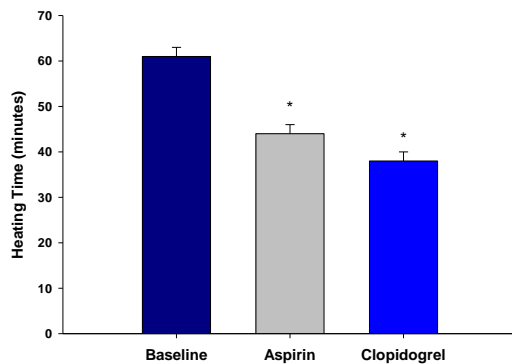


Figure 3: Time (minutes) required to increase body core temperature by 1.0°C using the water-perfused suit during baseline experiments (no systemic drug) and during systemic low-dose aspirin and clopidogrel treatments.
* $p < 0.01$ vs. the baseline experiment

CONCLUSIONS

The principle findings of this study were 1) localized COX-inhibition augments baseline skin blood flow suggesting that vasoconstrictor thromboxanes contribute to basal cutaneous vascular tone, 2) in contrast to young healthy subjects, COX-derived vasodilators do not contribute to reflex cutaneous VD in healthy middle aged subjects (Figure 1), 3) one week of systemic low-

dose aspirin therapy to inhibit platelet COX-1 significantly attenuates reflex cutaneous VD during the plateau phase of the increase in skin blood flow during hyperthermia, and 4) specific platelet ADP receptor inhibition with clopidogrel significantly attenuated reflex VD through NO-dependent mechanisms. These data suggest platelets may be involved in reflex cutaneous VD through either 1) the release of vasodilating factors, and/or 2) by altering blood viscoelastic properties thus decreasing the sheer stimulus on the cutaneous microvessels during hyperthermia. Finally, both aspirin and clopidogrel treatments significantly reduced the time required to increase body core temperature by 1.0°C using the water-perfused suit model of whole body heating, suggesting that systemic platelet inhibition may have functional thermoregulatory consequences through decreased dry heat loss mechanisms.

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