Oral clopidogrel improves cutaneous microvascular function through EDHF-dependent mechanisms in middle-aged humans

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**Dahmus JD, Bruning RS, Kenney WL, Alexander LM.** Oral clopidogrel improves cutaneous microvascular function through EDHF-dependent mechanisms in middle-aged humans. Am J Physiol Integr Comp Physiol 305: R452–R458, 2013. First published June 26, 2013; doi:10.1152/ajpregu.00366.2012.—Platelet P_{2y12}-ADP and COX-1 receptor inhibition with oral clopidogrel (CLO) and low-dose aspirin (ASA), respectively, attenuates reflex-mediated cutaneous vasodilation, but little is known about how these medications affect local vasodilatory signaling. Reactive hyperemia (RH) results in vasodilation that is mediated by sensory nerves and endothelium-derived hyperpolarization factors (EDHF) through large-conductance calcium-activated potassium channels, whereas slow local heating (LH) elicits vasodilation largely through the production of nitric oxide (NO). We hypothesized that CLO and ASA would attenuate locally mediated cutaneous vasodilation assessed by RH and LH (0.5°C/min).

In a randomized, cross-over, double-blind placebo-controlled study, nine healthy men and women (56 ± 1 yr) took CLO (75 mg), ASA (81 mg), and placebo for 7 days. Skin blood flow was measured (laser-Doppler flowmetry, LDF) and cutaneous vascular conductance (CVC) was calculated (LDF/mean arterial pressure) and normalized to maximal CVC (%CVC_{max} 43°C and 28 mM sodium nitropusside). RH response parameters, including area under the curve (AUC), total hyperemic response (THR), and the decay constant tau (τ) were calculated. NO-dependent vasodilation during LH was assessed by calculating the difference in %CVC_{max} between a control site and a NO synthase-inhibited site (10 mM L-NAME: intradermal microdialysis). CLO augmented the AUC and THR (AUC_{clo} = 3.783 ± 342; THR_{clo} = 2.306 ± 266% CVC_{max/s}) of the RH response compared with ASA (AUC_{ASA} = 3.101 ± 325; THR_{ASA} = 1.695 ± 197% CVC_{max/s}) and placebo (AUC_{placebo} = 3.000 ± 283; THR_{placebo} = 1.675 ± 170% CVC_{max/s}; all P < 0.0001 vs. CLO). There was no difference in the LH response or calculated NO-dependent vasodilation among treatments (all P > 0.05). Oral CLO treatment augments vasodilation during RH but not LH, suggesting that CLO may improve cutaneous microvascular function.

**METHODS**

**Subjects.** Experimental protocols were approved by the Institutional Review Board at the Pennsylvania State University and conformed to the guidelines set forth by the Declaration of Helsinki. Verbal and written consent was voluntarily obtained from all subjects before participation. Experiments were performed on nine healthy middle-aged men and women (See Table 1). All women enrolled in the study were postmenopausal and not taking hormone replacement therapy. The American Academy of Chest Physicians recommends that all men over 65 and all women over 50 with one or more cardiovascular risk factors take a prophylactic ASA (81–350 mg/day) (2). Therefore, we selected a healthy, middle-aged group because it is within the age range recommended to take prophylactic ASA therapy, and it encompasses a large portion of the American population [i.e., the baby boomer generation (49 to 67 years old in 2013)]. All subjects underwent a complete medical screening, including blood chemistry, lipid profile analysis (Quest Diagnostics Nichol Institute, Chantilly, VA), coagulation study (prothrombin time), resting electrocardiogram (ECG), and physical examination. Participants were screened for the presence of cardiovascular, dermatological, and neurological disease, pathways regulating local skin blood flow have not been studied. Considering the number of redundant vasodilator signaling pathways that could be affected by altered platelet receptor function, it is likely that oral clopidogrel and ASA modulate cutaneous vasodilation at the local level, possibly through EDHF and nitric oxide synthase (NOS) signaling pathways (15, 21).

Cutaneous reactive hyperemia and slow local skin heating are two methods used to assess microvascular function and mechanisms of dysfunction in healthy and clinical populations (23). Cutaneous vasodilation during reactive hyperemia is mediated primarily by sensory nerves and endothelium-derived hyperpolarization factors (EDHF) through activation of large-conductance calcium-activated potassium (BKCa) channels (18), with little or no contribution from nitric oxide (NO) (23). With slow local heating, the peak cutaneous vasodilator response is largely mediated by endothelium-derived NO and a combination of adrenergic (7, 13) and sensory nerves (6).

The purpose of this study was to examine effects of oral clopidogrel and low-dose ASA therapy on EDHF and NO-dependent mechanisms using cutaneous reactive hyperemia and slow local heating to induce these vasodilatory pathways, respectively. We conducted a randomized, cross-over, double-blind, placebo-controlled study to examine the effects of 7 days of platelet P_{2y12}-ADP receptor inhibition with clopidogrel (75 mg) and platelet-specific cyclooxygenase-1 (COX-1) receptor inhibition with low-dose ASA (81 mg) on local skin EDHF and NO-dependent cutaneous vasodilator mechanisms. On the basis of evidence from our previous reflex heating studies (3, 9), we hypothesized that clopidogrel and ASA would attenuate the cutaneous vasodilatory responses during reactive hyperemia and slow local heating.

**ORAL PLATELET INHIBITORS, including clopidogrel (Plavix) and low-dose aspirin (ASA), alter cutaneous vasodilator function during physiological stress. Clopidogrel and ASA attenuate reflex-mediated cutaneous vasodilation during passive whole-body heating (9). Moreover, after passive heat stress in warm air (30°C, 40% relative humidity), clopidogrel and ASA shift the onset of reflex vasodilation to higher body temperatures during exercise with ASA, further attenuating the rise in skin blood flow (3). Similarly, others have found that local skin blood flow responses to anodal current are reduced with oral low-dose ASA but not clopidogrel (20, 21). However, the effects of oral platelet inhibitors on cutaneous vasodilator function are two methods used to assess microvascular function and mechanisms of dysfunction in healthy and clinical populations (23). Cutaneous vasodilation during reactive hyperemia is mediated primarily by sensory nerves and endothelium-derived hyperpolarization factors (EDHF) through activation of large-conductance calcium-activated potassium (BKCa) channels (18), with little or no contribution from nitric oxide (NO) (23). With slow local heating, the peak cutaneous vasodilator response is largely mediated by endothelium-derived NO and a combination of adrenergic (7, 13) and sensory nerves (6).
including a graded exercise test on a recumbent bicycle with a 12-lead ECG to screen for the presence of underlying cardiovascular disease. No subjects were previously taking clopidogrel or low-dose ASA or had a family history (first-degree relative) of atherothrombotic disease. Subjects were normally active, nondiabetic, nonsmokers, who were currently not taking medications, including vitamin supplements, oral contraceptives, or hormone replacement therapy.

Systemic drug treatments. A randomized double-blinded placebo control cross-over study was performed with appropriate washout periods between trials. Subjects were instructed to consume nonidentifiable capsules compounded by a registered pharmacist, once per day for 7 days prior to experimentation. Drug treatments consisted of 75 mg clopidogrel (Plavix, Bristol-Myers Squibb), 81 mg of ASA, respectively, occurs within 4 days of initiating treatment (9, 10). Subjects ingested the assigned drug treatments each morning, with their last pill being ingested at 7:00 AM the day of the experiment, dissolved in lactated Ringer solution, and sterilized using an i-STAT.

All protocols were performed in a thermoneutral laboratory (23 ± 0.1°C) with the subject in a semisupine position, with the experimental arms at heart level. A venous blood sample was obtained after the subject had been resting for at least 30 min for a prothrombin time (PT)/international normalized ratio (INR) analysis. The PT is a measure of the time that it takes whole blood to clot when mixed with thromboplastin, and the INR assesses the patients PT time divided by the mean normal PT time assessed with an i-STAT.

Mean skin temperature was clamped at 33°C using a water-perfused suit that covered the entire body, except the hands, feet, head, and experimental arm. The right arm was used for the reactive hyperemia protocol, and the left arm was used for the slow local heating protocol. In our previous studies examining clopidogrel and ASA, we found that skin blood flow was reduced for a given change in oral temperature. To determine whether the oral treatments affected the control of local skin blood flow through sympathetic adrenergic mechanisms bretylium tosylate was used to acutely block the sympathetic adrenergic nerves. Bretylium tosylate iontophoresis (Moor Instruments, Iontophoresis Controller MIC2) was conducted at protocol-specific sites on the ventral side of both forearms to block sympathetic adrenergic neurotransmitter release (14). Iontophoresis of 10 mM bretylium at 200 μA for 20 min was conducted over a 3-cm² area of skin prior to the insertion of the intradermal microdialysis fibers on the left arm for the slow local heating protocol. After the initial iontophoresis-induced hyperemia had subsided (at least 40–65 min), the adrenergic blockade was tested by conducting a 3-min vigorous whole body cold stress using 7°C water perfused through a water-perfused suit at the beginning of the reactive hyperemia and slow local heating protocols. We have previously demonstrated the time course of adrenergic blockade with iontophoresis application of bretylium tosylate (16), confirming that the block remains intact for at least 7 h. During the experimental protocol, blood pressure was measured every 5 min using manual auscultation in the contralateral arm to the one that was being tested.

Intradermal microdialysis. Subjects were instrumented with four intradermal microdialysis fibers (MD2000, Bioanalytical Systems) (10 mm, 20-kDa cutoff membrane) on the left ventral forearm, as previously described (8, 9). Microdialysis sites were at least 4.0 cm apart to ensure no cross-reactivity of pharmacological agents delivered to the skin. After insertion, microdialysis fibers were taped in place and initially perfused with lactated Ringer solution to ensure the integrity of the fiber and during the insertion trauma resolution period. The microdialysis fibers were perfused with lactated Ringer solution for at least 90 min before they were randomly assigned as 1) 10.0 mM N⁵-nitro-L-arginine methyl ester (L-NAME) to inhibit NO production by NOS (8, 10–12), 2) lactated Ringer solution to serve as a control, 3) bretylium pretreatment with continuous perfusion of 10 mM L-NAME to inhibit both adrenergic vasoconstrictor mechanisms and NOS, and 4) bretylium pretreatment with experimental continuous perfusion of lactated Ringer solution to inhibit adrenergic vasoconstrictor mechanisms. All drugs were mixed just before each experiment, dissolved in lactated Ringer solution, and sterilized using syringe microfiltrers (Acrodisc, Pall, Ann Arbor, MI). Throughout the heating protocol, microdialysis drugs were perfused at a rate of 2.0 μl/min. At the end of the experiment sodium nitroprusside (SNP) was perfused at a rate of 4 μl/min to induce maximal endothelium-independent vasodilation (Bee Hive controller and Baby Bee microinfusion pumps, Bioanalytical Systems).

For both reactive hyperemia and slow local heating protocols, an index of skin blood flow was obtained by measuring cutaneous red blood cell flux with an integrated laser-Doppler flowmeter probe (MoorLAB, Temperature Monitor SH02, Moor Instruments). The laser-Doppler probe was held in place by a local heater that was maintained at 33°C during the reactive hyperemia protocol and at baseline of the slow local heating protocol. Each probe was placed on the skin directly above each microdialysis membrane. All laser-Doppler probes were calibrated using Brownian standard solution. Cutaneous vascular conductance (CVC) was calculated as laser-

Table 1. Subject baseline characteristics

<table>
<thead>
<tr>
<th>Subject Baseline Characteristics</th>
<th>Treatment Group</th>
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<tr>
<td>Sex, number of men, women</td>
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<tr>
<td>Age, yr</td>
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<tr>
<td>BMI, kg/m²</td>
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<tr>
<td>Total cholesterol, mg/dl</td>
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<td>HDL, mg/dl</td>
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<td>LDL, mg/dl</td>
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<td>HbA1c, %</td>
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<tr>
<td>MAP, mmHg</td>
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<tr>
<td>PT, s</td>
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<tr>
<td>INR</td>
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<td>Values are expressed as means ± SE; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, glycated hemoglobin; MAP, mean arterial pressure; PT, prothrombin time; INR, international normalized ratio.</td>
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Doppler flux divided by mean arterial pressure and normalized to maximum CVC (%CVC<sub>max</sub>).

Cutaneous reactive hyperemia protocol. Laser-Doppler flux was continuously recorded at both a control site and a site at which sympathetic adrenergic function had been blocked throughout the reactive hyperemia protocol. Two 10-min baseline periods were recorded before and after a rapid whole body cold stress that was performed to test the integrity of the bretylium blockade. Each occlusion was separated by 15 min to allow for flux to return to baseline values. The slow local heating response was elicited from heating the skin at a rate of 0.5°C/min from 33°C to 42°C. The skin blood flow response is represented as cutaneous vascular conductance (CVC) normalized to a percentage of maximum vasodilation (%CVC<sub>max</sub>); solid circles.

Local heating protocol. Microdialysis sites were perfused with pharmacological agents for at least 75 min prior to baseline and heating periods to allow for needle insertion trauma resolution. Baseline data were collected for at least 10 min before the start of slow local heating. Local heater temperature was increased at a rate of 0.5°C/min from 33°C to 42°C. Once a steady plateau was reached for at least 30 min, local heaters were increased to 43°C and 28.0 mM sodium nitroprusside (SNP; Nitropress, Abbott Laboratories) was perfused through all sites.

Data acquisition and analysis. Data were collected using Windaq software and Dataq data acquisition systems. The data were collected at 40 Hz, digitized, recorded, and stored on a personal computer for further analysis. CVC data were averaged over 5-min periods at baseline and during the arterial occlusion. The peak was assessed as the highest point after the rapid release of the occlusion cuff. Maximal CVC was calculated as an average of 10 min during a stable plateau after locally heating the skin to 43°C. The area under the curve (AUC) was calculated by determining the area under the reactive hyperemia response curve (from time of release of occlusion until flux returned to a steady state), as described by Wong et al. (23). Furthermore, a total hyperemic response (THR) was calculated [i.e., THR = AUC - (baseline skin blood flow as %CVC<sub>max</sub>)] (i.e., adrenergic blockade or local microdialysis treatment) on l) the parameters of the reactive hyperemic response [time to peak, peak, AUC, THR, and τ (for reactive hyperemia protocol)] and 2) %CVC<sub>max</sub> across the increase in skin temperature (for the slow local heating protocols). Specific planned comparisons with Bonferroni corrections were performed when appropriate to determine where differences between oral treatment and localized microdialysis drug treatment occurred. The level of significance was set at α = 0.05. Values are presented as means ± SE unless otherwise indicated.

RESULTS

Table 2 illustrates the blood parameters in all trials. There were no differences in the blood parameters in any of the treatment groups (all P > 0.05).

<table>
<thead>
<tr>
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<th>Placebo</th>
<th>Low-Dose Aspirin</th>
<th>Clopidogrel</th>
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<tbody>
<tr>
<td>Hb, g/dL</td>
<td>14.5 ± 0.4</td>
<td>14.5 ± 0.3</td>
<td>14.6 ± 0.5</td>
</tr>
<tr>
<td>Hct, %</td>
<td>42 ± 1</td>
<td>42 ± 1</td>
<td>43 ± 1</td>
</tr>
<tr>
<td>PT, s</td>
<td>12.6 ± 0.3</td>
<td>12.6 ± 0.3</td>
<td>12.7 ± 0.4</td>
</tr>
<tr>
<td>INR</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td>1.1 ± 0.0</td>
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</table>

Values are expressed as means ± SE. Hb, hemoglobin; Hct, hematocrit. Values are given for each trial followed by washout.
Figure 2 shows representative tracings of the skin blood flow responses for the reactive hyperemia and the slow local heating protocols. For the reactive hyperemia protocols, there were no differences between the control sites and adrenergic blockade sites for any of the trials \((P > 0.05)\); therefore, only data for the control sites are illustrated in Fig. 3. Treatment with clopidogrel augmented the AUC and the THR compared with ASA and placebo \((P < 0.0001)\). There were no differences between ASA and placebo trials. Furthermore, there were no differences in the peak skin blood flow response or the \(\%\text{CVCmax}\) among any of the trials \((P > 0.05)\).

The mean \(\%\text{CVCmax}\) responses during slow local heating in the control and the NOS-inhibited sites are illustrated in Fig. 4, A and B. There were no differences in \(\%\text{CVCmax}\) responses among oral treatments at the control site or the NOS-inhibited sites \(\text{(Fig. 4, A and B)}\). The calculated NO-dependent vasodilation was not different among the oral treatments \(\text{(Fig. 4C; } P = 0.87\text{)}\) nor was \(\%\text{CVCmax}\) for the plateau during prolonged heating \(\text{(clopidogrel: } 88 \pm 3\text{, ASA: } 90 \pm 2\text{, placebo: } 92 \pm 2\%\text{ CVCmax; } P = 0.47\text{)}\). Figure 5 shows the \(\%\text{CVCmax}\) responses during slow local heating with adrenergic blockade. There was no difference in the \(\%\text{CVCmax}\) response in the control adrenergic blockade sites among clopidogrel, ASA, or placebo \(\text{(Fig. 5A)}\). However, adrenergic blockade \(\text{(Fig. 5A)}\) reduced \(\%\text{CVCmax}\) compared with the control sites \(\text{(Fig. 4A)}\) in all trials \((P = 0.004)\). There were no differences due to adrenergic blockade in the NOS-inhibited sites between oral treatments \((P = 0.98)\) \(\text{(Fig. 5B)}\). Finally, there were no differences in maximal \(\%\text{CVCmax}\) due to oral treatment \((P = 0.58)\) or local treatment \(\text{(bretylium iontophoresis or microdialysis; } P = 0.32\text{)}\).
The principal new finding of this study was that 7 days of oral platelet P2Y12-ADP receptor inhibition with clopidogrel augmented the local cutaneous reactive hyperemic response. Sympathetic adrenergic blockade did not alter local skin blood flow responses during reactive hyperemia, suggesting that clopidogrel-induced augmentation in reactive hyperemia is not due to inhibition of sympathetic adrenergic vasoconstriction (5). On the basis of what is currently understood about the underlying mechanisms mediating cutaneous vasodilation to reactive hyperemia, these data would suggest that clopidogrel augments sensory nerve and/or BKCa channel EDHF-mediated mechanisms (18). Further, the slow local heating response was not different between treatments, suggesting that oral clopidogrel or low-dose ASA treatment does not attenuate local NO-dependent vasodilation (13).

The mechanisms underlying the cutaneous reactive hyperemic response include 1) a sensory nerve contribution (18), 2) stimulation of BKCa channels (18), and 3) possibly modulation by locally derived COX products (19). However, unlike the conduit circulation, NO contributes little, if any, to the total vasodilatory response to reactive hyperemia in the skin (23). Although we observed an augmentation in the cutaneous reactive hyperemic response with clopidogrel, we did not see an effect of low-dose ASA (platelet COX-1 inhibitor). Several studies have examined the contributions of COX vasoactive products to the reactive hyperemic response with divergent results. Conflicting results are reported when using different agents and doses to inhibit platelets and vascular COX. For example, cutaneous reactive hyperemia is reduced after 1 g of oral acetylsalicylate treatment (which inhibits both platelet and vascular COX) (1), whereas the total magnitude of the response is increased with localized treatment of the skin with the nonspecific COX inhibitor ketorolac in some (19), but not all, studies (18). The findings from the present study suggest that platelet-EDHF microvascular signaling mechanisms are differentially affected by platelet P2Y12-ADP receptor inhibition vs. platelet COX-1 inhibition. Because inhibiting platelet aggregation tendencies with low-dose ASA had no effect on the reactive hyperemic response, these data help to rule out a possible role for platelet COX-1 mechanisms in contributing to the reactive hyperemic response.

To assess possible differences in the local control of skin blood flow through NO-dependent vasodilation (13), we performed a slow local heating protocol with NOS inhibition (6, 13). Slow local heating results in a skin blood flow plateau that is slightly lower than the commonly used faster local heating protocols (i.e., ~80% CVCmax vs. ~90% CVCmax). We originally hypothesized that we may be able to detect greater differences between oral treatments using this slower local heating protocol because the rates of blood flow and the relative magnitude of the shear stimulus would be more similar to what occurs during whole body heating. Although the faster local heating protocol is more commonly used as a measure of NO-dependent microvascular function, both the slow and the fast local heating protocols have a large NO-dependent (~50% CVCmax) plateau. We found that oral clopidogrel and low-dose ASA did not affect the total magnitude of the local heating response or functional NO-dependent vasodilation. Furthermore, there was no difference in the NO-independent portion of the local heating response.

In addition to performing two skin-specific protocols under control conditions, we also assessed these skin blood flow responses in the presence of sympathetic adrenergic vasoconstrictor blockade (bretylium tosylate). In the reactive hyperemia protocols, we did not observe a difference between control sites and those with sympathetic adrenergic blockade in any trial. We wanted to assess oral platelet inhibitors ability to
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affect NO-dependent vasodilation with as little activation of sensory and sympathetic nerves as possible. Therefore, we used a slower local heating protocol, which is known to delay the axon reflex (6, 13) and examine this response with and without adrenergic blockade to assess the sympathetic adrenergic nerve component of this response with oral clopidogrel and low-dose ASA therapy. We did detect a systematic reduction in the skin slow local heating response in the bretylium-treated sites, but not in the bretylium plus NOS-inhibited site across all trials. These findings are similar to what Hodges (6) and Houghton et al. (13) have demonstrated with adrenergic blockade with bretylium delivered through a microdialysis fiber. Together, these data demonstrate that 1) the augmentation in the reactive hyperemic response observed with clopidogrel is not due to a decrease in tonic sympathetic adrenergic vasoconstrictor function, and 2) that neither oral clopidogrel nor low-dose ASA affected the sympathetic adrenergic modulation of the slow local heating response.

Our observation that oral clopidogrel augments the reactive hyperemic response in human skin mirrors what is observed in the conduit vasculature in magnitude, but possibly not in underlying mechanism. In coronary artery disease patients, a single loading dose of clopidogrel improved flow-mediated vasodilation and forearm endothelium-dependent vasodilation to intra-arterial infusions of ACh through NO-dependent mechanisms (21). Similarly, Heitzer et al. (5) found similar enhanced forearm blood flow in coronary artery disease patients during intra-arterial infusions of ACh when oral clopidogrel therapy was added to chronic ASA therapy. However, they found improvement in forearm blood flow with confusion of 1-NAME, suggesting that NO-independent vasodilation was improved or oxidative stress was reduced (5). The reason for the disparate mechanisms of clopidogrel-enhanced vasodilation in our study vs. the latter studies may have occurred for a few reasons. First, the mechanisms mediating the reactive hyperemic response are different in conduit vs. resistance vessels. Second, Warnholtz et al. (21) used a high single loading dose of clopidogrel (300 mg and 600 mg), whereas the studies showing improved NO-independent vasodilation used a lower dose of clopidogrel given once daily. Finally, our study examined healthy middle-aged individuals, whereas the other two studies examined coronary artery disease patients who have impaired NO-dependent vasodilation and heightened oxidative stress and inflammation. Clopidogrel has been shown to reduce production of proinflammatory cytokines in humans and animal models of cardiovascular disease (4, 17), suggesting that with cardiovascular disease clopidogrel improves NO bioavailability. Although we found that short-term oral administration of clopidogrel augmented the reactive hyperemic response (Fig. 3), we did not observe a change in NO-dependent vasodilation to skin local heating with clopidogrel treatment. Considering what is currently known about the mechanisms mediating the cutaneous reactive hyperemic response (i.e., lack of a role of NO) and the evidence from animal models demonstrating a direct endothelium-independent effect of thienopyridines, including clopidogrel (24, 25), our data point to a possible role of oral clopidogrel improving sensory nerve-mediated vasodilation and/or vascular smooth muscle function through EDHF mechanisms.

Limitations. We utilized two skin-specific techniques to assess the effects of oral clopidogrel on the local skin blood flow responses. On the basis of our results and the existing mechanistic work in the literature, we concluded that the augmentation in the reactive hyperemia was due to the effects of oral clopidogrel on sensory nerve and/or EDHF-dependent mechanisms. Although we meticulously analyzed all parameters of the reactive hyperemic response, we focused on NO-dependent mechanisms for the local heating response. We did not specifically scrutinize the axon reflex portion of the local heating response because we utilized a slow heating protocol, in which this initial peak is much less prominent compared with a more rapid heating protocol. However, Rousseau et al. (20) found that oral low-dose ASA, but not clopidogrel, attenuated the axon-reflex contribution to anodal current-induced vasodilation (a model of neurogenic inflammation). In addition, P2Y12 receptors have been isolated on vascular smooth muscle of internal mammary arteries of coronary artery bypass surgery patients (22). Therefore, P2Y12 inhibition with clopidogrel may be able to directly relax cutaneous vascular smooth muscle. Although we cannot rule out an effect of oral clopidogrel on sensory nerve mechanisms, it seems more likely that EDHF mechanisms were augmented with this specific platelet P2Y12 receptor inhibitor.

Perspectives and Significance

In summary, contrary to our original hypothesis, we found that treatment with the oral platelet inhibitor clopidogrel but not low-dose ASA augmented the local cutaneous reactive hyperemic response measured by the area under the curve and the total hyperemic response. The increase in vasodilation during reactive hyperemia was not mediated by a reduction in tonic sympathetic adrenergic function. Further, neither oral ASA nor clopidogrel treatments affected the total magnitude of the cutaneous vasodilator response or NO-dependent vasodilation during slow local heating. These data suggest that clopidogrel may alter sensory nerves and/or EDHFs.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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