Systemic low-dose aspirin and clopidogrel independently attenuate reflex cutaneous vasodilation in middle-aged humans

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Holowatz LA, Jennings JD, Lang JA, Kenney WL. Systemic low-dose aspirin and clopidogrel independently attenuate reflex cutaneous vasodilation in middle-aged humans. J Appl Physiol 108: 1575–1581, 2010. First published April 1, 2010; doi:10.1152/japplphysiol.01362.2009.—Chronic systemic platelet cyclooxygenase (COX) inhibition with low-dose aspirin [acetylsalicylic acid (ASA)] significantly attenuates reflex cutaneous vasodilation in middle-aged humans, whereas acute, localized, nonisoform-specific inhibition of vascular COX with intradermal administration of ketorolac does not alter skin blood flow during hyperthermia. Taken together, these data suggest that platelets may be involved in reflex cutaneous vasodilation, and this response is inhibited with systemic pharmacological platelet inhibition. We hypothesized that, similar to ASA, specific platelet ADP receptor inhibition with clopidogrel would attenuate reflex vasodilation in middle-aged skin. In a double-blind crossover design, 10 subjects (53 ± 2 yr) were instrumented with four microdialysis fibers for localized drug administration and heated to increase body core temperature [oral temperature (Ta)] 1°C during no systemic drug (ND), and after 7 days of systemic ASA (81 mg) and clopidogrel (75 mg) treatment. Skin blood flow (SKBF) was measured using laser-Doppler flowmetry over each site assigned as J control, 2) nitric oxide synthase inhibited (NOS-I; 10 mM Nω-nitro-arginine methyl ester), 3) COX inhibited (COX-I; 10 mM ketorolac), and 4) NOS-I + COX-I. Data were normalized and presented as a percentage of maximal cutaneous vascular conductance (%CVCmax; both P < 0.001). In all trials, localized COX-I did not alter SKBF during significant hyperthermia (ND: 56 ± 7; ASA: 43 ± 5; clopidogrel: 35 ± 5% CVCmax; all P > 0.05). NOS-I attenuated vasodilation in ND and ASA (ND: 28 ± 6; ASA: 25 ± 4% CVCmax; both P < 0.001), but not with clopidogrel (27 ± 4% CVCmax; P > 0.05). NOS-I + COX-I was not different compared with NOS-I alone in either systemic treatment condition. Both systemic ASA and clopidogrel reduced the time required to increase Ta 1°C (ND: 58 ± 3 vs. ASA: 45 ± 2; clopidogrel: 39 ± 2 min; both P < 0.001). ASA-induced COX and specific platelet ADP receptor inhibition attenuate reflex vasodilation, suggesting platelet involvement in reflex vasodilation through the release of vasodilating factors.

aspirin; plavix; thermoregulation

WITH RISING BODY CORE temperature, skin blood flow is first increased by withdrawal of adrenergic vasoconstrictor tone, and then, on reaching a specific core temperature threshold, active cutaneous vasodilation occurs (25). Reflex vasodilation is mediated by cholinergic cotransmission (14), where several putative vasodilator mechanisms are involved, including the cotransmitter vasoactive intestinal peptide (1), histamine receptor activation (31), and neurokinin 1 receptor activation (29). Furthermore, full expression of reflex cutaneous vasodilation in young healthy human skin is dependent on nitric oxide (NO) synthase (NOS) (13, 27) and cyclooxygenase (COX) second-messenger mechanisms (18).

With healthy human aging, there is a significant attenuation in reflex cutaneous vasodilation due to a reduction in both NO- and cotransmitter-dependent vasodilation (8, 15). Our laboratory has recently demonstrated that, in otherwise healthy middle-aged humans, chronic low-dose aspirin [acetylsalicylic acid (ASA)] therapy (81 mg) taken for primary atherothrombotic disease prevention (23), consistently and significantly attenuates reflex cutaneous vasodilation (11). In a subsequent study examining the contribution of vascular COX-derived vasodilators in middle-aged skin, our laboratory demonstrated that acute, localized, nonisoform-specific COX inhibition with ketorolac did not attenuate reflex vasodilator responses during significant hyperthermia (10). Cumulatively, these data suggest that 1) local vascular COX-derived vasodilators do not significantly contribute to reflex vasodilation in this age group; and 2) it is unlikely that chronic low-dose, ASA-induced inhibition of vascular endothelial COX is a potential mechanism underlying attenuated reflex vasodilation observed in humans on chronic low-dose ASA therapy (11).

The primary mechanisms of low-dose ASA for atherothrombotic disease prevention is through the acetylation of platelet COX-1 in the presystemic (portal) circulation (24), thereby inhibiting COX for the life of the platelet (~10 days), while preserving the ability of vascular endothelial cells to produce COX-dependent vasodilators. There are several putative mechanisms involving platelets and their potential role in reflex vasodilation that may be altered in subjects taking low-dose ASA. Importantly, activated platelets release known vasodilators, including NO, ATP, ADP, and 5-HT (5, 12, 20), all of which have the potential to directly stimulate cutaneous vasodilator pathways implicated in reflex vasodilation.

The purpose of this study was to examine the effect of specific platelet inhibition on reflex vasodilator mechanisms in middle-aged skin. We performed a randomized double-blinded crossover design study after 7 days of systemic low-dose ASA (81 mg) to inhibit platelet COX-I and 7 days of clopidogrel (75 mg) to specifically inhibit platelet ADP receptors (purinoreceptor P2Y12). We hypothesized that, similar to low-dose ASA, specific platelet ADP receptor inhibition would attenuate reflex cutaneous vasodilation.

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.

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Verbal and written consent were voluntarily obtained from all subjects before participation. Drug intervention studies were performed on 10 healthy subjects (5 men and 5 women). Three additional subjects served as time controls. Data from the baseline (no drug) experimental period and the first experiment for the time control subjects were pooled and previously reported (10), illustrating the effect of localized COX inhibition on reflex vasodilation.

All 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 (prothrombin time and partial thromoplastin time), lipid profile evaluation (Quest Diagnostics Nichol Institute, Chantilly, VA), resting electrocardiogram, and physical examination. All subjects were screened for the presence of cardiovascular, dermatological, and neurological disease. No subjects were previously taking low-dose ASA, nor did any have a family history (first-degree relative) of atherothrombotic disease. Subjects were normally active, nondiabetic, nonsmokers, who were currently not taking medications, including vitamins, hormone replacement therapy, or oral contraceptives.

Systemic drug treatments. Subjects were tested at enrollment into the study while not taking any systemic drugs. Subjects were instructed not to take anti-inflammatory medications for at least 2 wk before the baseline (no drug) experimental day. After initial testing, nonidentifiable capsules, compounded by a registered pharmacist (Boalsburg Pharmacy), were given to subjects over 7 days. Randomized doubled-blinded drug treatments consisted of a daily oral dose of either 81 mg of ASA (Bayer) or 75 mg of clopidogrel bisulphate (Plavix Bristol-Myers Squibb). Full platelet aggregation inhibition specific to arachidonic acid and ADP has been shown to occur within 4 days of initiating treatment (22, 26). Subjects took the compounded drugs each morning, with their last pill being ingested at 7:00 AM the day of the experiments. Experimental trials in the same subjects were separated by a minimum interval of 3 wk. This washout time period has been shown to be efficacious for full platelet recovery (22).

These subjects underwent the same experimental procedures (whole body heating and intradermal microdialysis for localized drug administration) as the drug intervention groups; however, no systemic drug intervention was used. Experimental trials for the time control subjects were separated by a minimum of 3 wk. The data for the time control subjects were analyzed using a mixed-models repeated-measures analysis of variance (see Statistical analysis section).

Instrumentation and measurements. Protocols were performed in a thermoneutral laboratory with the subject in the semisupine position, with the experimental arm at heart level. On arrival at the laboratory, subjects were instrumented with four intradermal microdialysis fibers (MD2000, Bioanalytical Systems) (10 mm, 20-kDa cutoff membrane) in the skin on the left ventral forearm. Microdialysis sites were at least 4.0 cm apart to ensure no cross-reactivity of pharmacological agents being delivered to the skin. Microdialysis fibers were placed at each site by first inserting a 25-gauge needle through unanesthetized skin, using sterile technique. The entry and exit points were ~2.5 cm apart. The microdialysis fibers were then threaded through the needle, and the needle was withdrawn, leaving the fibers in place. The 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. Following this period, microdialysis sites were perfused with 1) 10.0 mM N\textsuperscript{2}-nitro-L-arginine methyl ester (L-NAME) to inhibit NO production by NOS, 2) 10.0 mM ketorolac to nonspecifically inhibit local vascular COX isomers, 3) a combination of 10.0 mM ketorolac and 10.0 mM L-NAME, and 4) lactated Ringer solution to serve as a control. All microdialysis drugs were perfused at a rate of 2.0 μl/min (Bee Hive controller and Baby Bee microinjection pumps, Bioanalytical Systems) continuously throughout the protocol.

An index of skin blood flow was obtained by measuring cutaneous red blood cell flux with an integrated laser-Doppler flowmeter probe placed in a local heater maintained at 33°C (MoorLAB, Temperature Monitor SH02, Moor Instruments, Devon, UK) 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 flux divided by mean arterial pressure (MAP).

Mean skin temperature was controlled by water-perfused suit that covered the entire body, except head, hands, and experimental arm. Subjects also wore a water-impermeable outer garment over the water-perfused suit to minimize evaporative heat loss. The subject’s electrocardiogram was monitored throughout the protocol, and blood pressure was measured via brachial auscultation with every 0.1°C rise in oral temperature (T\textsubscript{or}). T\textsubscript{or} was continuously monitored during baseline and throughout whole body heating as an index of body core temperature with a thermoster placed in the sublingual sulcus. The thermoster was secured in the same location in the sublingual sulcus, and the subject’s mouth was taped shut. The subjects were instructed to keep the thermoster in the same location in the sublingual sulcus and not to open their mouths or speak during the protocol. Mean skin temperature was calculated as the unweighted average from six copper-constantan thermocouples placed on the chest, middle back, abdomen, upper arm, thigh, and calf. During the period of insertion trauma resolution and baseline measurement periods, thermoneutral water (34°C) was perfused through the suit to clamp mean skin temperature. During whole body heating, 50°C water was perfused through the suit, which increased mean skin temperature to 40°C. 50°C water was continually pumped through the suit to raise subject’s T\textsubscript{or} by 1.0°C. Local skin temperature over each microdialysis site was maintained at 33°C (Moor Instruments SH02, Devon, UK).

Experimental protocol. Red cell flux over each microdialysis site was monitored as insertion trauma resolved over a 75- to 90-min period. Four microdialysis sites were randomly assigned to their specific pharmacological treatment. All drugs were mixed just before each experiment, dissolved in lactated Ringer solution, and sterilized using syringe microfilters (Acrodisc, Pall, Ann Arbor, MI).

Microdialysis sites were perfused continuously for at least 75 min before the start of the baseline and during the baseline and heating periods with assigned pharmacological agents at a rate of 2.0 μl/min. Baseline data were collected for 20 min before the start of whole body heating, after which whole body heating was initiated. After T\textsubscript{or} had increased by 1°C and clamped for 10 min, mean skin temperature was returned to baseline, and 28.0 mM sodium nitroprusside (SNP; Nitropress, Abbot Laboratories, Chicago, IL) was perfused through all sites at a rate of 4 μl/min to achieve maximal CVC (CVC\textsubscript{max}). Additionally, local heating of the skin to 43°C, in combination with 28 μM SNP, was conducted to ensure CVC\textsubscript{max} had been achieved.

Data acquisition and analysis. Data were acquired using Windaq software and Dataq data-acquisition systems (Akron, OH). The data were collected at 40 Hz, digitized, recorded, and stored on a personal computer for further analysis. CVC data were averaged over 3-min periods for baseline and during the baseline and heating period (power α = 0.05) would be sufficient to determine a meaningful physiological difference of 12% CVC\textsubscript{max} between microdialysis treatment sites and systemic drug intervention trials. A three-way mixed-models ANOVA with repeated measures was conducted to determine 1) differences between systemic drug treatments (no drug, ASA, and clopidogrel) across the rise in T\textsubscript{or}; and 2) differences between localized microdialysis drug treatment across...
the rise in $T_a$. A two-way ANOVA with repeated measures was conducted to determine differences in absolute CVC$_{max}$ (flux/MAP) between systemic drug treatment and localized microdialysis drug treatment. A one-way ANOVA with repeated measures was conducted to determine differences in the time required to increase body core temperature 1.0°C. The level of significance was set at $\alpha = 0.05$. Specific planned comparisons with Bonferroni corrections were performed when appropriate. Values are presented as means ± SE.

RESULTS

Subject characteristics are presented in Table 1. There were no differences between the subjects who participated in the systemic drug intervention studies and the time control subjects. Furthermore, no differences were observed between the sexes for localized microdialysis treatment or systemic drug intervention; therefore, the data from both sexes in each group were combined.

%CVC$_{max}$ at thermoneutral baseline and with a 1.0°C rise in body core temperature is illustrated in Fig. 1 for all microdialysis treatment sites. Similar to previously reported studies, localized COX inhibition augmented baseline %CVC$_{max}$ in no systemic drug and systemic low-dose ASA trials ($P < 0.001$) and did not affect %CVC$_{max}$ during significant hyperthermia [change ($\Delta$) in $T_a$ = 1.0°C].

Figure 2 illustrates the control, NOS-inhibited (NOS-I), and COX-inhibited (COX-I) microdialysis sites across the rise in body core temperature. During no systemic drug trials, NOS inhibition attenuated reflex vasodilation with $\Delta T_a \geq 0.5°C$ ($P < 0.001$). Furthermore, there was no difference between control sites and COX-I sites with $\Delta T_a \geq 0.5°C$. Compared with no systemic drug trials with systemic ASA treatment, reflex vasodilation was attenuated with $\Delta T_a \geq 0.5°C$ at the control site. NOS inhibition attenuated reflex vasodilation with $\Delta T_a \geq 0.2°C$, and there was no difference between COX-I sites and the control sites with $\Delta T_a \geq 0.5°C$. Systemic clopidogrel treatment significantly attenuated reflex vasodilation compared with no drug and systemic ASA treatment at $\Delta T_a \geq 0.3°C$ and $\Delta T_a \geq 0.5°C$, respectively (both $P < 0.001$). In contrast to no systemic drug and systemic ASA treatment, there was no difference between the control, NOS-I, or COX-I sites with systemic clopidogrel treatment. In all trials (no drug, ASA, and clopidogrel), there was no difference between the NOS-I sites and the NOS + COX-I sites (omitted for clarity in Fig. 2).

Figure 3 shows the absolute CVC$_{max}$ (flux/MAP) for all localized microdialysis drug treatments and across all trials. There were no differences in absolute CVC due to either local microdialysis treatment ($P = 0.89$) or systemic drug intervention ($P = 0.86$).

The time required to increase body core temperature by 1.0°C is shown in Fig. 4. Both systemic ASA and clopidogrel reduced the time required to increase body core temperature by 1.0°C compared with the no-drug trial (both $P > 0.001$).

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Drug Treatment Group</th>
<th>Time Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M, F)</td>
<td>5, 5</td>
</tr>
<tr>
<td>Age, yr</td>
<td>57 ± 3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>159 ± 8</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>56 ± 3</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>103 ± 7</td>
</tr>
<tr>
<td>Fasting blood glucose, mg/dl</td>
<td>94 ± 3</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>85 ± 3</td>
</tr>
<tr>
<td>Baseline $T_a$, °C</td>
<td>36.2 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. M, male; F, female; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MAP mean arterial pressure; $T_a$, oral temperature.
however, there were no differences between the ASA and clopidogrel trials.

The mean %CVC\textsubscript{max} values for each microdialysis treatment sites (ΔT\textsubscript{or} = 1.0°C) and for the time required to increase body core temperature by 1.0°C for the time control subjects are presented in Table 2. There was no difference in %CVC\textsubscript{max} for each microdialysis site or heating time between the experimental trials for the time control group.

**DISCUSSION**

The principal findings of this study were 1) specific platelet ADP receptor inhibition with clopidogrel significantly attenuated reflex vasodilation to a greater extent than platelet COX-1 inhibition with systemic low-dose ASA; and 2) 1 wk of systemic low-dose ASA therapy in healthy middle-aged humans moderately attenuated reflex cutaneous vasodilation during hyperthermia. Similar to previously reported findings, localized vascular COX inhibition augmented baseline %CVC\textsubscript{max} during thermoneutral conditions, but did not affect %CVC\textsubscript{max} during significant hyperthermia across all systemic drug interventions (9). These data suggest that platelets may be involved in reflex cutaneous vasodilation through either 1) the release of vasodilating factors, and/or 2) by altering blood viscoelastic properties, thus decreasing the shear stimulus on the cutaneous microvascular endothelium during hyperthermia. Finally, both ASA and clopidogrel treatments significantly reduced the time required to increase body core temperature by 1.0°C using the water-perfused suit to induce passive whole body heating, suggesting that systemic platelet inhibition may have functional thermoregulatory consequences through decreased dry heat loss mechanisms.

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**Figure 2.** Group mean ± SE %CVC\textsubscript{max} during passive whole body heating. The control site, cyclooxygenase-inhibited (ketorolac) site, and NOS-I site are shown across the change in core body temperature (ΔT\textsubscript{or}). The combination l-NAME + ketorolac site has been omitted for clarity; however, there was no difference between this site and the l-NAME site. A: no-drug trial: local treatment with ketorolac augmented %CVC\textsubscript{max} compared with control from baseline, but not with substantial hyperthermia. L-NAME significantly attenuated reflex cutaneous vasodilation.

**Figure 3.** Group mean ± SE absolute CVC at maximal vasodilation (sodium nitroprusside + 43.0°C). There was no difference in CVC\textsubscript{max} due to localized microdialysis drug treatment or systemic drug treatment.
causing the release of vasodilating substances during hyperthermia. There is in vitro (3, 17) and in vivo (19, 26) direct evidence for 1) platelet COX-mediated vasodilation in models of neurogenic inflammation; and 2) platelet ADP-receptor-mediated, endothelium-dependent vasodilation. Specifically, platelet ADP-receptor stimulation causes the release of dinucleotides stored in platelet dense granule, which further regulates platelet aggregation and also mediates endothelium-dependent vasodilation (3). The precise mechanism of platelet vessel wall interactions is complex and incompletely understood; however, there is clear evidence for platelets releasing multiple vasodilating factors, some of which are known to contribute to cutaneous reflex vasodilation (2, 13, 18, 29, 31).

Another potential explanation for the attenuation in reflex vasodilation observed with platelet inhibition is through a reduction in shear-mediated vasodilation. Unlike conduit vasculature, there is significant controversy whether shear-mediated vasodilation occurs in the cutaneous vasculature. Part of this controversy is due to 1) a lack of an obligatory role for NO in cutaneous reactive hyperemia (30); and 2) methodological limitations in humans for directly measuring cutaneous microvessel diameter in addition to flow for a calculation of shear rate. Using an elegant model, Green and colleagues (7) have recently demonstrated that repeated heating of the skin can induce improvements in microvascular function, only if it is associated with hyperemia and increased shear stress. Furthermore, these authors suggest that the shear stress stimulus in the cutaneous microvasculature (through either passive heating or exercise training) is obligatory. Along these lines, whole blood viscosity may play an important role that might be capable of modulating the microvascular shear stress stimulus. Whole blood viscoelasticity is determined by plasma volume, total plasma protein concentration, red blood cell number, erythrocyte’s internal viscosity, and erythrocyte and platelet aggregation tendencies (28). Both low-dose ASA and clopidogrel decrease whole blood viscoelastic (4, 21–23) properties. However, it is unclear how changes in whole blood viscoelastic properties induced by platelet inhibition may impact cutaneous vasodilation.

Regardless of the mechanism through which platelet inhibition attenuates reflex cutaneous vasodilation, there may be functional thermoregulatory consequences through decreased dry heat loss mechanisms. Although unexpected, we found that the time required to increase body core temperature by 1.0°C was significantly decreased with both systemic low-dose ASA and clopidogrel treatments. This seems counterintuitive from the perspective of convective heat transfer alone, which should be decreased with a lower skin blood flow. Yet the platele-

![Image](https://example.com/image.png)

Fig. 4. The time required (minutes) to increase body core temperature by 1.0°C using the water-perfused suit in the no-drug, low-dose aspirin, and clopidogrel trials. Both systemic low-dose aspirin and clopidogrel decreased the time required to increase body core temperature by 1.0°C compared with the no-drug trials. *P < 0.05 vs. no-drug trial.

Table 2. Time control data

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control, %CVC&lt;sub&gt;max&lt;/sub&gt;</th>
<th>l-NAME, %CVC&lt;sub&gt;max&lt;/sub&gt;</th>
<th>Ketorolac, %CVC&lt;sub&gt;max&lt;/sub&gt;</th>
<th>l-NAME + ketorolac, %CVC&lt;sub&gt;max&lt;/sub&gt;</th>
<th>Heating time, min</th>
</tr>
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<tbody>
<tr>
<td>Day 1</td>
<td>64 ± 4</td>
<td>35 ± 6</td>
<td>61 ± 7</td>
<td>37 ± 8</td>
<td>46 ± 5</td>
</tr>
<tr>
<td>Day 2</td>
<td>63 ± 5</td>
<td>33 ± 3</td>
<td>61 ± 8</td>
<td>33 ± 8</td>
<td>48 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE. The mean percentage of maximal cutaneous vascular conductance (%CVC<sub>max</sub>) for each microdialysis treatment sites (ΔT<sub>tor</sub> = 1.0°C) and for the time required to increase body core temperature by 1.0°C for the time control subjects are shown. l-NAME, N<sup>ω</sup>-nitro-l-arginine methyl ester.
inhibited conditions consistently led to shorter heating times to reach a 1.0°C increase in $T_{oc}$. We utilized the water-perfused suit passive heating model to induce hyperthermia in our study design, which reversed the core-to-skin gradient and clamped mean skin temperature at a constant 40°C across all systemic treatment conditions (no drug, ASA, and clopidogrel). Further research using more physiologically relevant models of heat stress (i.e., exercise in the heat) is needed to fully address the functional thermoregulatory and cardiovascular consequences of systemic platelet inhibition.

Clinical perspective. Both low-dose ASA and clopidogrel are widely and successfully used for primary and secondary atherothrombotic disease prevention, respectively. Our data demonstrate that, in healthy middle-aged human subjects, these commonly used drugs alter skin blood flow responses during hyperthermia. While these data may provide some insight into the mechanisms mediating reflex vasodilation in healthy subjects, the potential thermoregulatory effects in cardiovascular disease populations are unknown. It may be that, in thrombocytopenic populations, treatment with these drugs helps prevent cardiovascular complications during heat stress and may even help normalize skin blood flow responses. However, given the results of the present study, the potential exists for anticoagulatory therapy to significantly reduce skin blood flow, imposing significant thermoregulatory and cardiovascular risk in these vulnerable populations (16).

Limitations. First, given the multiple and redundant neurovascular pathways that contribute to reflex vasodilation, it is difficult to determine what potential signaling mediators may be underlying the attenuation in skin blood flow with platelet inhibition. In this study, the NOS-I microdialysis sites suggest that the NO pathway may be attenuated. This would be consistent with reduced intraluminal release of either ADP (3) and/or platelet-activating factor (17). Second, we did not specifically test our subjects’ blood after platelet inhibition with either arachidonic acid or ADP, respectively. In healthy subjects, Rousseau et al. (26) found complete platelet inhibition after 4 days of treatment, with either low-dose ASA or clopidogrel (26). We treated our subjects for 7 consecutive days with 100% compliance and found that functional measures of skin blood flow were reduced with these systemic treatments. Third, it may be possible that P2Y₁₂ receptors exist on the vascular endothelium and systemic treatment, with clopidogrel-inhibited important vascular mechanisms contributing to reflex vasodilation through P2Y₁₂ receptors. However, the literature to date only shows improvement in microvascular function with clopidogrel, independent of its effects on vascular P2Y₁₂ receptors in a model of endothelial dysfunction (6). Finally, while 1 wk of systemic low-dose ASA attenuated reflex vasodilation based on our laboratory’s previous cross-sectional study with chronic long-term ASA therapy (>1 yr), we anticipated a more significant attenuation in skin blood flow. These differences between our studies are likely due to the length of systemic ASA therapy (1 wk vs. >1 yr) and potential differences in our human subject populations.

In summary, platelet inhibition with systemic low-dose ASA or clopidogrel independently and differentially attenuated reflex cutaneous vasodilation in healthy middle-aged humans. There are several potential mechanisms that may be mediating this attenuation, including: 1) inhibition of platelet-induced release of vasodilating factors causing endothelium-dependent vasodilation; and 2) a reduction in the shear-stress stimulus on the cutaneous microvasculature, resulting from a decrease in whole blood viscoelastic properties. The consistent and significant reduction in skin blood flow with systemic platelet inhibitors was associated with a reduction in the time required to increase body core temperature by 1.0°C, suggesting greater thermal strain. Further research is necessary to examine the mechanisms of platelet vessel wall interactions underlying this attenuated skin blood flow response, as well as the potential cardiovascular and thermoregulatory consequences.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES