Chronic low-dose aspirin therapy attenuates reflex cutaneous vasodilation in middle-aged humans

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Holowatz LA, Kenney WL. Chronic low-dose aspirin therapy attenuates reflex cutaneous vasodilation in middle-aged humans. J Appl Physiol 106: 500–505, 2009. First published November 26, 2008; doi:10.1152/japplphysiol.91215.2008.—Full expression of reflex cutaneous vasodilation is dependent on cyclooxygenase- (COX) and nitric oxide synthase- (NOS) dependent mechanisms. Low-dose aspirin therapy is widely prescribed to inhibit COX-1 in platelets for atherothrombotic prevention. We hypothesized that chronic COX inhibition with daily low-dose aspirin therapy (81 mg) would attenuate reflex vasodilation in healthy human skin. Two microdialysis fibers were placed in forearm skin of seven middle-aged (57 ± 3 yr), normotensive, healthy humans with no preexisting cardiovascular disease, taking daily low-dose aspirin therapy (aspirin: 81 mg), and seven unmedicated, healthy, age-matched control (no aspirin, 55 ± 3 yr) subjects, with one site serving as a control (Ringer) and the other NOS inhibited (NOS inhibited: 10 mM Ni2+–nitro-L-arginine methyl ester). Red cell flux was measured over each site by laser-Doppler flowmetry, as reflex vasodilation was induced by increasing core temperature (oral temperature) 1.0°C using a water-perfused suit. Cutaneous vascular conductance (CVC) was calculated (CVC = flux/mean arterial pressure) and normalized to maximal CVC (CVCmax, 28 mM sodium nitroprusside). CVCmax was not affected by either aspirin or NOS inhibition. The plateau in cutaneous vasodilation during heating (change in oral temperature = 1.0°C) was significantly attenuated in the aspirin group (aspirin: 25 ± 3% CVCmax vs. no aspirin: 50 ± 7% CVCmax, P < 0.001 between groups). NOS inhibition significantly attenuated %CVCmax in both groups (aspirin: 17 ± 2% CVCmax, no aspirin: 23 ± 3% CVCmax, P < 0.001 vs. control), but this attenuation was less in the no-aspirin treatment group (P < 0.001). This is the first observation that chronic low-dose aspirin therapy attenuates reflex cutaneous vasodilation through both COX- and NOS-dependent mechanisms.

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 were voluntarily obtained from all subjects.

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before participation. Studies were performed on seven control subjects of similar age (55±3 yr, 4 men, 3 women) and seven subjects taking chronic low-dose aspirin therapy (aspirin group; 81 mg/day for >1 yr; 57±3 yr, 3 men, 4 women). Maximal platelet inhibition with enteric-coated oral low-dose aspirin occurs within 7–14 days (27, 28), and recovery of platelet COX activity occurs at a rate of ~10%/day (3, 5). Healthy subjects who were voluntarily taking low-dose aspirin as primary prevention of atherothrombotic disease were recruited for the study. Subjects taking aspirin for >1 mo were eligible to participate in the study; however, all of the subjects had been taking aspirin therapy for >1 yr. Data from a subset of the control group (n = 5) have been previously published (11, 12). Additional control subjects and subjects taking chronic low-dose aspirin therapy were recruited for this study.

Subjects underwent a complete medical screening, including a physician-supervised graded exercise test to evaluate the existence of underlying cardiovascular disease, blood chemistry, 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. Subjects were taking aspirin as recommended by their personal physician, but none had a history or 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. All premenopausal women were studied on days 2–7 (follicular phase) of their menstrual cycle.

Instrumentation and measurements. Protocols were performed in a thermoneutral laboratory with the subject in the semisupine position, with the experimental arm at heart level. Aspirin subjects maintained their normal aspirin regimen and took 81 mg (baby aspirin) the day before reporting to the laboratory for the experiment. To control for acute increases in aspirin plasma concentrations, subjects were instructed not to take aspirin the day of the study. In healthy subjects, low-dose, enteric-coated aspirin reaches peak plasma concentration within 3–4 h after ingestion and has a half-life of 15 min (2, 3, 5). Platelet function returns at ~10%/day with the cessation of daily low-dose aspirin (28). Furthermore, 4 days of low-dose aspirin therapy effectively inhibit current induced vasodilation in human skin (32). On arrival at the laboratory, subjects were instrumented with two intradermal microdialysis fibers (MD 2000, Bioanalytical Systems) (10 mm, 20-kDa cutoff membrane) in the skin on the right ventral forearm. Microdialysis sites were at least 4 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 an anesthetized 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 perfused with lactated Ringer solution during the insertion trauma resolution and baseline measurement periods, thermonutral water (34°C) was perfused through the suit to clamp body temperature. During whole body heating, 50°C water was perfused through the suit to raise subject’s Twa by 1.0°C. Local skin temperature over each microdialysis site was separately maintained at 33°C (Moor Instruments SHO2, Devon, UK).

Experimental protocol. Red cell flux over each microdialysis site was monitored as insertion trauma resolved over a 60- to 90-min period. Microdialysis sites were randomly assigned to receive either 1) 10.0 mM N\textsuperscript{\textdegree} -nitro-L-arginine methyl ester (L-NAME) to inhibit NO production by NOS (15, 18, 25), or 2) lactated Ringer. L-NAME was 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 60 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. At the end of the heating protocol, each microdialysis site was perfused with 28.0 mM sodium nitroprusside (Nitropress, Abbot Laboratories, Chicago, IL) at a rate of 4.0 μl/min to achieve maximal CVC (CVC\textsubscript{max}) in combination with local heating of the skin to 43°C over each microdialysis site to ensure CVC\textsubscript{max} (15, 24, 34).

Data acquisition and analysis. Data were acquired using Labview or Windaq software, and National Instruments or Dataq data-acquisition systems (Austin, TX). 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 every 0.1°C rise in Twa and are presented as a percentage of CVC\textsubscript{max} (%CVC\textsubscript{max}). Absolute CVC\textsubscript{max} in each microdialysis site was calculated as the average of the stable plateau in laser-Doppler flux during 28 mM sodium nitroprusside infusion and local heating to 43°C divided by mean arterial pressure. Student’s unpaired t-tests were used to determine significant differences between the groups for physical characteristics. Two-way repeated-measures ANOVA was conducted to detect differences due to chronic low-dose therapy and pharmacological treatment on CVC\textsubscript{max}; and 2) differences due to chronic aspirin therapy on the change (Δ) in %CVC\textsubscript{max} between the control and the NOS-inhibited sites for every 0.1°C rise in Twa. A mixed-model three-way repeated-measures ANOVA was conducted to detect differences in %CVC\textsubscript{max} between subject groups (chronic low-dose aspirin or age-matched control) at the pharmacological treatment sites within subject groups (control and L-NAME) over the rise in Twa (SAS, version 9.1). Tukey post hoc tests were performed when appropriate to determine where differences between groups and drug treatments occurred. The level of significance was set at α = 0.05. Values are presented as means ± SE.

RESULTS

Subject characteristics are presented in Table 1. Subject groups were well matched for body size, total cholesterol, high-density lipoprotein, low-density lipoprotein cholesterol, and blood pressure (all P > 0.05). Likewise, there was no difference in baseline Twa between the groups (P = 0.73).

Group mean %CVC\textsubscript{max} responses across the rise in body core temperature are presented in Fig. 1. In the nonaspirin group, control and NOS-inhibited sites were first significantly
control subjects, but only minimally decreased \(\%CVC_{max}\) in CVC. \(\%CVC_{max}\) was significantly attenuated in the chronic low-dose aspirin group. NO synthase inhibition significantly reduced \(\%CVC_{max}\) in the age-matched control group. Furthermore, NOS inhibition significantly reduced \(\%CVC_{max}\) at the control sites compared with the age-matched control group (\(P < 0.05\), beginning of significant difference from baseline within site in the aspirin treatment group). NOS inhibition significantly decreased \(\%CVC_{max}\) at the control sites with change in oral temperature (\(\Delta T_{or}\)) of 0.3°C and 0.4°C, respectively (Fig. 1A). There were no significant effects of either chronic low-dose aspirin therapy (no aspirin: 1.7 ± 0.2 vs. aspirin: 1.9 ± 0.4 flux/mmHg; \(P = 0.72\)) or NOS inhibition (no aspirin: 1.8 ± 0.4 vs. aspirin: 1.5 ± 0.2 flux/mmHg; \(P = 0.22\)) on \(CVC_{max}\).

**DISCUSSION**

The principal findings of this study include the important first observation that reflex cutaneous vasodilation is substantially attenuated in healthy humans voluntarily taking chronic low-dose aspirin for the primary prevention of atherothrombotic disease. Although baseline core temperature was similar between the groups, greater increases in core temperature were required to significantly increase skin blood flow from baseline in subjects taking low-dose aspirin. This decreased response is due in part to reduced NO-dependent vasodilation. While the precise mechanism(s) mediating this response remains unresolved, several possibilities exist. Because chronic low-dose aspirin (81 mg) inhibits COX-1 in platelets (28), these data may suggest that platelets may be activated during hyperthermia and release vasodilatory substances that increase skin blood flow. Alternatively, low-dose aspirin may have inhibited COX in platelets and in the cutaneous microvasculature, resulting in reduced cutaneous vasodilation. Finally, decreased blood viscosity resulting from platelet inhibition may decrease the shear stimulus on the cutaneous microvasculature during hyperthermia, resulting in the observed attenuated vasodilation.

In young, healthy humans, COX-dependent vasodilatory pathways contribute to reflex cutaneous vasodilation (22). To date, only non-isofrm-specific COX inhibitors (ketorolac and aspirin) have been used to examine the contribution of COX-dependent mechanisms to reflex vasodilation, using localized administration of these inhibitors (intradermal microdialysis) to examine these mechanisms. Administration of these inhib-
itors at the concentrations used in previous studies is non-selective for COX isoform and location, i.e., platelet vs. vascular, making it difficult to delineate the precise COX isoform and cellular location synthesizing vasodilators during hyperthermia.

While aspirin is also a nonspecific COX inhibitor, the beneficial antithrombotic effects of low-dose aspirin therapy are associated with its ability to alter the ratio of the production of TXA2 and PGI2 (21). Aspirin irreversibly acetylates platelet COX-1 in the presystemic (portal) circulation (30), thereby inhibiting platelet production of TXA2 for the life of the platelet (10 days). Thus it has been suggested that low-dose chronic (5–6 days) aspirin therapy preferentially inhibits platelet COX-1-dependent TXA2 synthesis without affecting endothelial COX-dependent PGI2 synthesis. Much higher doses of aspirin (600 mg) are required to fully but acutely inhibit COX in the microvasculature (9, 27).

In contrast, low-dose enteric coated aspirin reaches peak plasma concentrations within 3–4 h after ingestion and has a half-life of 15 min (3, 27, 28). In the present study, subjects took their last dose of aspirin at least 16 h before the experiment to avoid potential acute inhibition of endothelial COX. Given the pharmacokinetics of enteric-coated, low-dose aspirin, it is unlikely that vascular COX was significantly inhibited. Taken together, these data support a role for platelet involvement in reflex vasodilation.

One interesting finding from the present study was that NO-dependent vasodilation was attenuated in subjects taking chronic low-dose aspirin (Fig. 2). It has been suggested that the NOS and COX vasodilatory pathways are independent of one another because the combined inhibition of both enzymes results in an additive reduction in total cutaneous vasodilation compared with NOS and COX inhibition alone (22). Our data would suggest that there may be cross talk between these two pathways, because chronic COX inhibition with aspirin resulted in a blunted relative NO contribution to reflex heating. There are several possible mechanisms for this finding, including 1) an aspirin-induced reduction cholinergic active vasodilator or vascular sensitivity mechanisms; 2) a decrease in platelet-released vasodilatory substances, which may induce vasodilation through NO-dependent mechanisms; and 3) a reduction in shear stress-mediated vasodilation.

One potential explanation for the severe attenuation in reflex vasodilation that we observed in subjects taking low-dose aspirin is that platelets may release vasodilatory substances that contribute to the increase in skin blood flow during hyperthermia. Platelets are known to release a variety of vasodilator substances, including serotonin and ADP (8, 16, 26). In vitro evidence suggests that vasodilation is attenuated after treatment with platelets from diabetic humans through impairments in the ATP/ADP pathway (26). In contrast to platelet inhibition with low-dose aspirin, specific inhibition with the platelet ADP...
receptor inhibitor clopidogrel does not alter sensory neurogenic vasodilation in human skin (32). Nonetheless, platelet-mediated vasodilation through serotonin and/or the ADP/ATP pathway during reflex heating remains a putative mechanism for the increase in skin that requires further exploration.

For a given rise in body core temperature, skin blood flow was significantly reduced with chronic low-dose aspirin therapy. This reduction in skin blood flow may be through lower endothelial shear stress. Although NO has not been found to cause cutaneous vasodilation after exposure to shear stress (37, 39), there is some indication that shear stress induces vasodilation through an interaction between NO and COX pathway (23). Also, platelet inhibition with aspirin therapy decreases blood viscosity, which further reduces the shear stimulus on the endothelium. These data suggest that platelet and vascular COX inhibition with aspirin may attenuate any shear-mediated cutaneous vasodilation during whole body heating.

Finally, COX-derived products can alter central hypothalamic thermoregulatory mechanisms, and we cannot discount the possibility that chronic systemic administration of low-dose aspirin may have altered central mechanisms. However, in the nonfebrile state, the administration of aspirin (50 mg/kg) (4) or other nonsteroidal anti-inflammatory drugs (800 mg ibuprofen) (6) has not been shown to alter the reflex vasodilatory responses as assessed by the thresholds for reflex vasodilation. In the present study, vasodilation was significantly attenuated in the aspirin therapy group, but baseline body temperature was similar between groups. Additional research assessing sweat output in conjunction with skin blood flow measurements is necessary to determine whether there is a decreased sensitivity of the sweating response, which would suggest that chronic low-dose aspirin may be altering central thermoregulatory mechanisms.

Limitations. Subjects tested in this study were voluntarily taking 81 mg of aspirin daily for the primary prevention of atherothrombotic events. The subjects were otherwise healthy, did not have a significant family history of cardiovascular disease (first-degree relative), and did not have any underlying cardiovascular risk/disease detectable with a maximal graded exercise test or blood chemistry analysis (Table 1). While we cannot exclude the possibility that these subjects may have had other unreported risk factors for vascular disease that lead them to engage in a low-dose aspirin regimen, it seems unlikely that underlying disease could explain the significantly attenuated skin blood flow responses observed in this study.

The 81–mg over-the-counter dose has become the standard therapy, proven as an effective antithrombotic agent in the prevention of myocardial infarction and stroke (28). In this study, we did not titrate the dose of aspirin based on body weight or blood volume: subjects were simply taking the over-the-counter dose based on availability and recommendation from their physician. It has been suggested that doses as low as 30 mg/day may be as effective as the 75-mg dose (29). It is possible that, by using the standard, commercially available 81-mg/dose, we observed significant COX inhibition in the vasculature and in the platelets not observed with lower doses. However, we did observe a significant attenuation in the skin blood flow response in otherwise healthy middle-aged subjects taking this commercially available dose of aspirin. Because primary human aging is also associated with attenuated reflex vasodilation, the combined effects of aging and chronic aspirin therapy may have significant thermoregulatory consequences.

In summary, we found that, in middle-aged men and women taking chronic low-dose aspirin, reflex cutaneous vasodilation is severely attenuated, due, in part, to reduced NO-dependent vasodilation. These data suggest that 1) platelets and/or platelet vessel wall signaling interactions may be involved in reflex vasodilation; 2) chronic low-dose aspirin may inhibit vascular COX, decreasing the synthesis of key vasodilators involved in reflex cutaneous vasodilation; and/or 3) alteration in blood viscosity due to aspirin therapy may decrease the shear stimulus on the cutaneous blood vessels, resulting in attenuated reflex vasodilation.

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