Improvement in Dermal Neural Vascular Function with Rosiglitazone in Individuals with Type II diabetes

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ABSTRACT

After baseline data was collected, 5 male and female subjects with a history of Type 2 diabetes were placed on a therapeutic dose of Avandia (rosiglitazone) for 4 weeks. Two tests of endothelial function were performed every 2 weeks. In the first test, the right arm was occluded by a blood pressure cuff inflated to a pressure of 200 mmHg for 4 minutes and the hyperemia following occlusion was assessed by volume plethysmography. In the second test, autonomic reflex function was assessed by first placing subjects in the horizontal posture on a tilt table in a room heated to 32˚C for 30 minutes. After 30 minutes, blood flow was assessed by a laser Doppler flow meter over the left calf. The table was then elevated to 45 degrees (head up), after which blood flow was assessed again. The results of these studies show that both the magnitude and duration of the hyperemia following occlusion increased significantly in subjects treated with Avandia within 2 weeks of administration of a therapeutic dose. Further, the blood flow to the skin in the leg increased significantly in response to heat over the same period. Reactivity of the autonomic nervous system, as assessed from the change in skin flow during change in posture from the horizontal to the vertical position, also increased within 2 weeks of patients taking a therapeutic dose of Avandia. The results of these studies seem to show that Avandia has an effect on increasing circulation and viability of the autonomic nervous system in type 2 diabetics.

INTRODUCTION

The development of the noninvasive

laser Doppler has enabled dermal vascular function to be assessed.[1] Abnormalities in the vasomotor reactivity of the skin microcirculation have been observed in patients with diabetes and are, in part, a result of damage to the peripheral sympathetic nervous system.[1-4] Sympathetic regulation of vascular tone in the dermal microcirculation is complex involving central, short arc, and local reflex control mechanisms.[1,5-7] Defects in both vasoconstriction and vasodilation of dermal vasomotor function have been observed in patients with type 2 diabetes.[1,2,4,8,9]

Patients with type 2 diabetes have shown a profound inability of hairy skin to vasodilate with local heat exposure.[3,4] This lack of local vasodilation most likely results not only from the loss of noradrenergic effectiveness, but also to a greater degree from the loss of the neurogenic action of small nociceptive sensory neurons.[3,10] Neural regulation of skin blood flow is further complicated by the presence of arteriovenous anastomoses involved in thermoregulatory processes.[3] These shunts are maintained in a constricted state by sympathetic tone. Global warming of the body causes centrally mediated cutaneous vasodilation. Recently, we have shown that the blood flow response to global warming is impaired in patients with diabetes.[11]

Dermal blood flow, however, is not only a result of neural regulation, but also a function of local circulatory factors and the microcirculatory endothelium.[4] Endothelial mediated vasodilation through the release of nitric oxide has been shown to be altered by diabetes and the insulin resistance syndrome.[12,13] The thiazolidinediones and metformin have been shown to improve endothelial function.[14-16] In one study, troglitazone was found to enhance dermal vascular blood flow, but its mechanism of action, although attributed in part to improvements in endothelial function, still remains unclear.[3]

The purpose of the present investigation was to study the effect of the thiazolidinedione rosiglitazone on the dermal vascular blood flow on the hairy skin of the legs in 5 subjects with type 2 diabetes. We assessed microcirculatory blood flow using non-invasive laser Doppler flowmetry. Since dermal blood flow is a complex interplay of neural regulation, endothelium, and thermoregulation, we attempted to delineate the mechanism of action by simultaneously measuring the independent contribution of key regulatory factors. In order to assess the contribution of the endothelium, we correlated changes in neural dermal vascular flow to post-ischemic hyperemia. Blood flow was measured in the brachial artery with Whitney plethysmography. Global heat exposure was performed on subjects to observe centrally mediated neurogenic alterations with heat induced vasodilation in the hairy skin. We further examined central sudomotor function with dynamic cardiovascular autonomic testing by assessing orthostatic cardiovascular reactivity during heat exposure.

SUBJECTS

Five patients were studied; average height was 71.6±7.9 cm, average weight was 93.6±27.6 kg, and average age was 65.2±13.8 years. At the onset of the study, the subjects' HbAlc was 8.5±1.5 and the average body mass index was 29.6±6.8. All procedures were approved by the committee on human experimentation at Loma Linda University and all subjects signed a statement of informed consent.

METHODS

Skin Blood Flow

Skin blood flow was measured by a laser Doppler flow meter produced by Moor Instruments, Inc (LDV 304, Oxford, England). The device sat on a stand 35

centimeters above the left lower leg of the subject, while the patient was lying supine. The device scanned the body and produced a picture of blood flow to the skin. The scanned area was 117 x 149 pixels and the scan rate was 4 ms/pixel. This device was completely non-invasive and had no physical contact with the body. The error on repeat measurements was less than 5% from day to day. A 25 cm^2 area was scanned over a 2-minute period and a 10 cm^2 area was chosen in the center of the larger area. Markers were placed on the skin to allow repeat measurements in the same area of interest under different experimental conditions. The laser was warmed for 30 minutes prior to flow measurements to increase stability. After the subject was tilted to a 45° head up position, care was taken to move the laser to the same distance and angle it (90°) relative to the leg of the subject. In preliminary testing on 4 subjects, the laser head was moved ±30 degrees to see if the flows would change due to minor misalignments of the head during tilt; no differences were seen in flows when the head was tilted in this range. To assure that the same area of the leg was scanned, marks were placed on the outside of the scan area and the laser was positioned to scan the same area at each body position. The units of blood flow stated in the results are in "flux" units; the measure of flow was generated by laser Doppler systems.

Forearm Blood Flow

Forearm blood flow was measured by Whitney strain gauge plethysmography. Whitney strain gauge plethysmography is a technique of measuring limb blood flow by volume plethysmography. Briefly, mercury in a rubber strain gauge is placed around the forearm. The gauge is made of silastic and is pre-stretched of 20 g to eliminate hysteresis during the measurements. The gauge is first calibrated by stretching the gauge a set distance and measuring the output on a chart recorder. An arterial occlusion cuff is then placed around the wrist and a venous cuff is placed on the upper arm. Thirty seconds prior to flow measurements, the occlusion cuff is inflated to 200 mmHg on the wrist to remove hand circulation from measurements of forearm flow. During flow measurements, the upper arm cuff placed approximately 4 cm above the elbow is inflated rapidly to a pressure of 55 mmHg. The cuff is inflated for a period of 5 seconds. During this period the change in arm size is transduced by the Whitney string gauge to an electrical output and displayed on a chart recorder. After 5 seconds a vacuum pump rapidly deflates the cuff for a period of 7 seconds. During flow measurements, the arm was placed as close to the level of the heart as possible, while the arm was placed in a controlled temperature water bath at 37°C. The arm was then immersed in the water bath for 15 minutes to allow the entire arm to come to equilibrium at central core temperature. A detailed description of this technique is published elsewhere.[17,18]

Body Temperature

Central core temperature was measured by a thermocouple placed under the back of the tongue (Yellow Springs Instruments, Yellow Springs, Ohio). The mouth was kept closed for 1 minute during temperature measurements and subjects breathed through their noses.

PROCEDURES

Subjects first entered the laboratory and sat in a thermally neutral room for 10 minutes and rested comfortably. Next, the pressure cuffs and Whitney flow gauge were placed on the subjects right arm and the subjects then placed their arms in a 37°C circulating water bath for 15 minutes so that their arms were

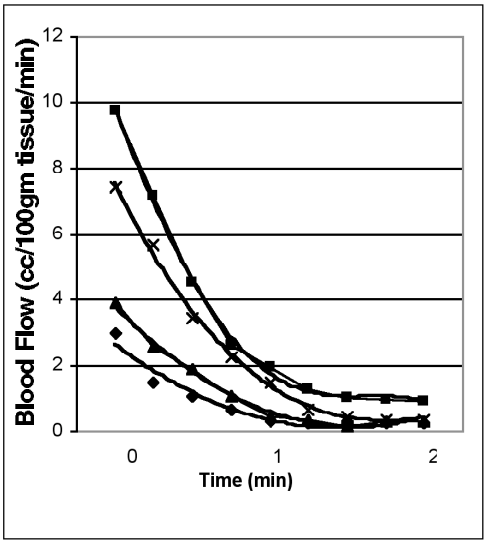

Figure 1. The average forearm blood flows of 5 subjects over a 2 minute period after 4 minutes of vascular occlusion of their forearms in subjects prior to taking Avandia (diamonds) and after 2 weeks (triangles) and 4 weeks on Avandia (crosses) compared to 10 age matched control subjects with no history of Type 2 diabetes (squares).

brought to core temperature. The arterial pressure cuff on the wrist was inflated to remove the hand circulation from the flow measurements. Thirty seconds later, blood flows were recorded for a period of 2 minutes. The circulation to the arm was then occluded at the upper arm for 4 minutes. After the 4-minute period, the wrist cuff was again inflated to arterial pressure and the upper arm cuff was cycled to low venous pressure to measure blood flows for an additional 2 minutes. The subject entered a thermoregulatory room where temperature was controlled at 32.2˚C±2˚C, and humidity was controlled at 35%±5%. The subject laid comfortably in the horizontal posture wearing shorts and a thin T-shirt on a tilt table. At the end of the 30-minute period, an area of 10 square cm just below the knee was scanned with a laser Doppler flow meter, as described under methods. The average flow was then determined in this area. After the flows were measured, the table was tilted to the 45˚ vertical position over a period of 15 seconds. The laser Doppler flow meter was moved such that the flow meter was maintained 35 cm above the table with the laser at 90˚ perpendicular to the leg and flows were measured in exactly the same location with the subject tilted in the 45˚ vertical position. The laser then scanned the body and flows were measured again. This procedure was repeated before subjects took Avandia, and then at 2 and 4 weeks after taking a 4 mg daily dose of Avandia.

Statistical Analysis

Statistical analysis involved the calculation of means, standard deviations and 2 tailed ANOVA. The level of significance was $P < 0.05$. All data in the results section are expressed ± the standard deviation.

RESULTS

The resting flows in the forearm and the flows during the 2-minute period after occlusion, before taking Avandia and at 2 and 4 weeks after taking Avandia are shown in Figure 1. Resting flows for the 5 subjects averaged 0.26 ± 0.13 cc per 100 g tissue/minute. After occlusion, flow increased to an average of 2.97 cc per 100 g tissue/minute and then exponentially decreased to the level of the blood flow with the subject at rest prior to the occlusion. After 2 weeks on Avandia, the average flows as shown in this figure immediately after the release of the occlusion averaged 3.9 ± 1.4 cc/100 g tissue/minute. After 4 weeks dosage on Avandia, the flows increased to 6.1 ± 1.9 cc/100 grams tissue/minute. The increase at 2 and 4 weeks was significant. For a basis of comparison, similar data was collected under the same experimental circumstances on age matched control subjects in other studies.[11,18] As seen in Figure 1, subjects without type 2 diabetes had substantially

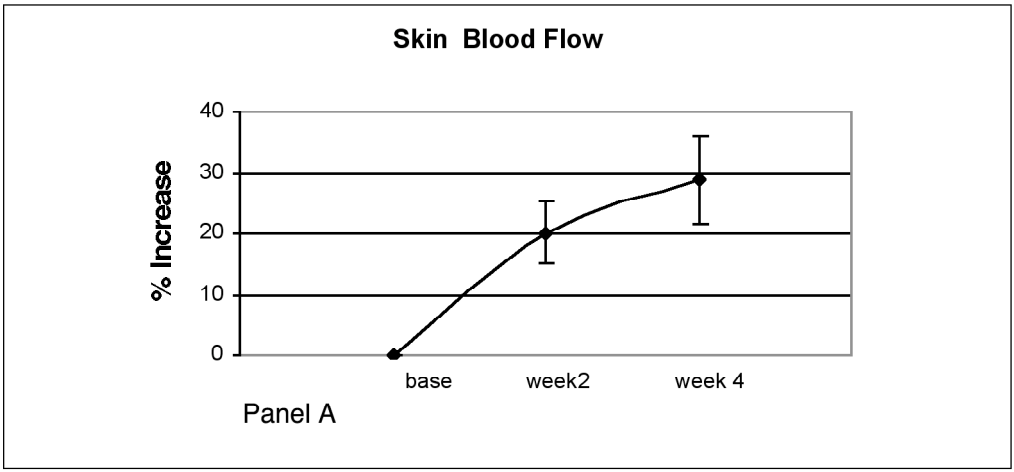

Figure 2a. The average increase in skin blood flow in 5 subjects prior to taking Avandia and after 2 and 4 weeks on Avandia. Flows are expressed as a percent of the control flows at 2 and 4 weeks.

higher resting flows and higher exercising flows than the group with type 2 diabetes.

The average blood flow of the group, when exposed to a warm room for a 30-minute period, is shown in Figure 2a. For ease of presentation the flows had been normalized in terms of the flow during the period prior to taking Avandia. As shown in Figure 2a, after 2 and 4 weeks of taking Avandia, flows increased. For example, the average flow after taking Avandia for 2 weeks, measured in the skin after 30 minutes of exposure to heat, was 120% ± 9% of the flow during the control period. In contrast, after 4 weeks of taking Avandia the flows had increased to 136% ± 13 % of those of the control period. These increases were significant.

Much more dramatic is the data shown in Figure 2b. Normally, when posture is changed from the horizontal to vertical position, in order to maintain blood pressure, the sympathetic nervous system constricts blood flow to the legs by as much as 50% or more.[11] Figure 2b compares the flows measured in the horizontal posture to the flows measured in the vertical posture for subjects, during the control period and after 2 and 4 weeks of taking Avandia. Whereas flows should have been twice as high in the horizontal versus the vertical posture in control subjects, the subjects with type 2 diabetes only showed a small reduction in flows (horizontal versus vertical flow ratio) when changed from the horizontal to the vertical position, as demonstrated previously in another study.[11] Figure 2b shows that the average ratio of horizontal to vertical flows when being tilted was 1 to 58, in the subjects prior to taking Avandia. However, after 2 weeks of taking Avandia, the ratio of horizontal to vertical flows increased to 1 to 66 and finally after 4 weeks of taking Avandia, the flow ratio increased to 1 to 79. During heat exposure, the average core temperature increased by 0.52˚C ±0.23˚C. By week 2, there was still an increase in core temperature of 0.34˚C ±0.31˚C but by week 4, the average core temperature increased by 0.35˚C ±0.31˚C. The reduction in temperature after 2 weeks on Avandia was significant (P<0.05).

DISCUSSION

The results of the present study confirm

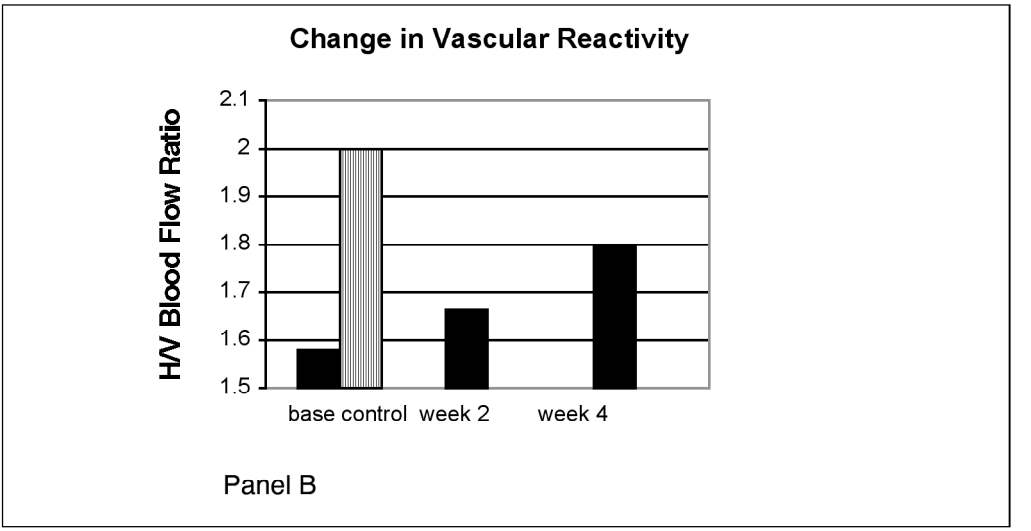

Figure 2b. The average ratio of the skin flows in the horizontal posture to vertical posture of the 5 subjects prior to taking Avandia and after 2 and 4 weeks on the medication.

prior findings with laser Doppler flowmetry which showed significant diminished flow in dermal neurovascular circulation in subjects with type 2 diabetes versus age matched controls. Similar to prior experiments where skin was locally heated, our findings with global heating revealed significant attenuation in cutaneous vasodilation on the hairy skin of our subjects with type 2 diabetes.[3,10,19] The greatest contributing factor to local heating induced vasodilation in hairy skin (75%-90%) is believed to be the small fiber nociceptive sensory neurons.

We verified the affect of global warming in a heat chamber by measuring elevations in core temperature. Core temperature increased during heat exposure, verifying that the central thermoregulatory stimulus needed to increase skin blood flow was present. Although such heat exposure is associated with cutaneous vasodilation, the primary mechanism differs from that of local warming. The action is primarily sympathetically mediated and centrally regulated. Our findings support a defect of centrally mediated sudomotor function, as the reason for attenuated vasodilation in the dermal vascular bed with heat exposure. But curiously, the smallest increase in blood flow during heat exposure occurred in subjects prior to taking Avandia, when core temperature increased to almost double that of core temperature in weeks 2 and 4 post Avandia. There are three possible implications: (1) central sympathetic drive increased even with a smaller change in core temperature and higher gain in central sympathetic drive; (2) sympathetic drive was constant or less in weeks 2 and 4 post Avandia, but vascular endothelial response was much greater; or (3) both. In all likelihood, both 1 and 2 are true. Thus, in patients with type 2 diabetes, it would seem that dermal vascular dysfunction is not only affected at the local and reflex level as shown in prior experiments, but also extends centrally as well.

We further tested central adrenergic function in our diabetic cohort by assessing cardiovascular reactivity to orthostatic stress. Previously, we have

shown that subjects with type 2 diabetes experience diminished orthostatic tolerance during heat exposure.[11] Cardiovascular autonomic neuropathy has been defined as an abnormality associated with heart-rate control and vascular dynamics.[20] Cardiovascular autonomic dysfunction has also been associated with an increased rate of mortality in patients with type 2 diabetes.[21]

In patients with type 2 diabetes, a blunted response of circulating levels of norepinephrine in response to standing as well as diminished responsiveness of the carotid baroreceptors can occur.[11,22-26] A key factor in maintaining adequate compensatory blood pressure response to standing are intact baroreceptor reflexes.[27] Heat exposure reduces the carotid baroreflex responsiveness.[28] Thus, in patients with type 2 diabetes, orthostasis is aggravated further by heat exposure. This central adrenergic impairment was replicated again in our diabetic cohort, in the present investigation, and correlated with diminished cutaneous dermal vascular blood flow and more importantly, showed improvement with Avandia administration.

Endothelial cell function was also assessed simultaneously with dermal vascular function in this group and was also found to be altered. Critical to the health of the vasculature is a properly function endothelium. The endothelium is critical to maintaining a positive balance of opposing states such as coagulation, fibrinolyis, thrombosis, inflammation and oxidation.[29] When the endothelium is healthy, it is resistant to clotting and penetration by monocytes, which form an atheromatous plaque.[16] Healthy endothelium also results in nitric oxide formation, which then results in vasodilation.

Previous attempts to look at the contribution of the vascular endothelium to dermal microcirculation failed to detect any differences in post ischemic hyperemia in the skin.[4] One limitation of prior experiments may have been that this procedure was performed at room temperature. However, in our experiment we used post ischemic blood flow in the brachial artery as the model for endothelial function. Furthermore, we had subjects place their arms in a warm water bath. Attenuation in brachial artery mediated vasorelaxation has demonstrated significant endothelial cell dysfunction in diabetics and those with insulin resistance syndrome.[30] We observed significant reductions in brachial artery flow, which corresponded to dysfunction in dermal vascular function.

While the thiazolidinediones help to improve glycemic control, their beneficial effects on the vascular system have been the focus of recent study.[16] Troglitazone had a positive effect on endothelial function by demonstrating improvement in ischemia induced flow mediated vasodilation in the forearm.[31-33] In vitro human and animal studies with rosiglitazone further support enhancement of endothelial function.[14,15] What was surprising was the fact that improvements in dermal vascular function after initiation of rosiglitazone were not only seen with improvements in endothelial cell function (increased post-ischemic flow) but also accompanied by improvements in central sympathetic function as well (improved vasodilation with heat exposure and improved cardiovascular reactivity to orthostatic stress).

Stansberry and colleagues speculated that the site of impairment for neural vascular dysfunction appeared to be primarily a microvascular abnormality. This was based on the fact that the impairment of responses whether local, reflex or central had the microvasculature as a common endpoint.[1] It is possible that the key to enhancing the

microcirculation of dermal vascular function and perhaps improving circulation throughout the body may be improving the health of the endothelium. Certainly, in our experiment, improvements in endothelial function corresponded with improvements in dermal blood flow. However, these improvements, witnessed in the dermal vascular bed, appeared to be correlated with improvements in cardiovascular reactivity and heat vasodilation. An attractive hypothesis with our limited findings is that enhancements of the microcirculation could result in secondary improvements in autonomic neuropathy, which are centrally mediated and sudomotor in function. This study only examined 5 individuals. In order to draw more definite conclusions, studies conducted with much larger patient populations that cover a greater time period are needed.

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