Original Article

Aldose Reductase Pathway Inhibition Improved Vascular and C-Fiber Functions, Allowing for Pressure-Induced Vasodilation Restoration During Severe Diabetic Neuropathy

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Pressure-induced vasodilation, a neurovascular mechanism relying on the interaction between mechanosensitive C-fibers and vessels, allows skin blood flow to increase in response to locally nonnociceptive applied pressure that in turn may protect against pressure ulcers. We expected that severe neuropathy would dramatically affect pressure-induced vasodilation in diabetic mice, and we aimed to determine whether pressure-induced vasodilation alteration could be reversed in 8-week diabetic mice. Control and diabetic mice received no treatment or sorbinil, an aldose reductase inhibitor, or alagebrium, an advanced glycation end product breaker, the last 2 weeks of diabetes. Laser Doppler flowmetry was used to evaluate pressure-induced vasodilation and endothelium-dependent vasodilation after iontophoretic delivery of acetylcholine (ACh). We assessed the nervous function with measurements of motor nerve conduction velocity (MNCV) as well as the C-fiber–mediated nociception threshold. Pressure-induced vasodilation, endothelial response, C-fiber threshold, and MNCV were all altered in 8-week diabetic mice. None of the treatments had a significant effect on MNCV. Although sorbinil and alagebrium both restored ACh-dependent vasodilation, sorbinil was the sole treatment to restore the C-fiber threshold as well as pressure-induced vasodilation development. Therefore, the inhibition of aldose reductase pathway by sorbinil improved vascular and C-fiber functions that allow pressure-induced vasodilation restoration that could limit neuropathic diabetic cutaneous pressure ulcers. *Diabetes* 55:1478–1483, 2006

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Received for publication 3 November 2005 and accepted in revised form 7 February 2006.

ACh, acetylcholine; AGE, advanced glycation end product; ARI, aldose reductase inhibitor; MNCV, motor nerve conduction velocity; STZ, streptozotocin.

DOI: 10.2337/db05-1433

During long-term diabetes, progressive microangiopathy contributes to progressive loss of peripheral neurological function, leading to the development of pressure-induced diabetic foot. However, the exact pathway leading to ulceration has not been fully identified, although it is recognized that ulceration may result from microcirculatory failure (1). Up to now assessment of the circulation, the nervous control of sensation, and foot sensitivity to loading are performed to determine the risk of ulceration in the diabetic foot. In contrast, local measurement of the microvascular function is less routinely performed (2).

We reported a novel relationship between nerves and vessels involving neural mechanosensitivity and cutaneous vasodilation, referred to as pressure-induced vasodilation (3). The increase in cutaneous blood flow induced by local pressure application delays the occurrence of tissue ischemia, thus protecting the skin against pressure. The mechanism of pressure-induced vasodilation involves pressure sensing by specialized capsaicin sensory neurons that act at the endothelial level to synthesize and release endothelial factors, such as nitric oxide (NO) (3,4), that induce smooth muscle relaxation. Therefore, neurovascular interaction is crucial for pressure-induced vasodilation development.

More recently, we reported that pressure-induced vasodilation was altered in 1-week diabetic mice with only vascular dysfunction (5), which was correlated with diabetic patients without neuropathy (6). At the point of clinical diabetes complication detection, significant impairments in nerve function may have already appeared and handicapped patients. Therefore, we expected that long-term diabetes in animals with severe neuropathy will dramatically aggravate pressure-induced vasodilation alteration. In this condition, skin blood flow should drop down directly in response to local applied pressure, leading to early ischemia, which could favor diabetic foot occurrence.

Diabetes through hyperglycemia is widely known to be a major factor that leads to microvascular and nervous complications (7). Indeed, hyperglycemia-induced end-organ damage in diabetes is associated with increased flux of glucose through *1*) the polyol metabolic pathway (8–10) and *2*) accumulation of advanced glycation end products (AGEs) (11,12), both participating to increase oxidative

TABLE 1
Effects of pharmacological agents on body weight, glycemia, fructosamine levels, and mean arterial blood pressure in 8-week diabetic and control mice

Groups	Body weight (g)	Glycemia (mg/dl)	Fructosamine (μmol/dl)	MABP (mmHg)
Control	35 ± 1	124 ± 8	188 ± 7	94 ± 5
Diabetic	21 ± 1*	486 ± 24*	300 ± 11*	94 ± 3
Control + sorbinil	35 ± 1	130 ± 11	192 ± 5	100 ± 4
Diabetic + sorbinil	24 ± 1*†	439 ± 27*	299 ± 14*	96 ± 6
Control + alagebrium	36 ± 1	121 ± 7	165 ± 4	106 ± 2
Diabetic + alagebrium	30 ± 1*‡	469 ± 25*	263 ± 9*†	106 ± 4

Control and diabetic mice were untreated or treated during the 2 last weeks of diabetes with sorbinil or alagebrium. $n = 10$ in each group. *$P < 0.001$ vs. respective control; †$P < 0.05$, ‡$P < 0.001$ vs. untreated diabetic. MABP, mean arterial blood pressure.

stress. These biochemical pathways are involved in the development of diabetic neurovascular disturbance. In fact, inhibitors of the polyol pathway (9,13,14) and AGE accumulation were able to prevent or reverse neurovascular dysfunctions in diabetic rats with early or mild neuropathy (11,14,15). However, it recently appeared that a long-term experimental diabetic mice model mimics human diabetes complications, particularly those related to neuropathy (16,17). Thus, the aim of this study was to determine whether pressure-induced vasodilation alteration could be reversed in long-term diabetic mice. For this purpose we used sorbinil, an aldose reductase inhibitor (ARI) that decreases aldose reductase activity and thus decreases sorbitol flux. Sorbinil is known to improve neurovascular dysfunctions (9,14). We also used alagebrium, which is an AGE breaker known to improve arterial elasticity in experimental animals (18,19) as well as in clinical studies (20,21) by its ability to reduce the accumulation of AGEs in diabetes (19,22).

RESEARCH DESIGN AND METHODS

Male Swiss mice (20–30 g) were kept on a 12-h light/dark cycle with food and water available ad libitum. The current investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (publication no. 85-23, revised 1996). Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ; 200 mg/kg; Sigma) in citrate buffer (pH 4.5) during the fasting state. Animals received equivalent doses of citrate buffer solution to be used as controls. Hyperglycemia occurred 2 days after STZ injection and was verified using an Accu-Check Active glucometer (Roche, Lyon, France). We included the mice in the diabetic group when blood glucose was >16 mmol/l 2 days after the injection.

Diabetes duration was 8 weeks, and treatments were given randomly for the last 2 weeks. Mice were randomized into three groups: *1*) untreated control and diabetic mice, *2*) control and diabetic mice treated with sorbinil (70 mg/kg daily by mouth), and *3*) control and diabetic mice treated with alagebrium (1 mg/ kg i.p. injection once daily). The 70-mg/kg sorbinil dose was selected because of its ability to reverse diabetic neurovascular alterations (9). Sorbinil was a kind gift of Pfizer (Groton, CT). The 1-mg/kg alagebrium dose was selected because of its ability to break cross-links of IgG to red blood cells and reduce diabetic arterial stiffness (18). Alagebrium was a kind gift of Alteon Pharma (Parsippany, NJ).

To test cutaneous microcirculation properties, a total of three experiments were conducted in the three groups: *1*) the microvascular response to local pressure increase was examined (pressure-induced vasodilation), *2*) the endothelium-independent vasodilation in response to sodium nitroprusside was evaluated, and *3*) the endothelium-dependent vasodilation in response to acetylcholine (ACh) was evaluated.

To test nervous function, a total of two experiments were conducted in the three groups: *1*) sciatic motor nerve conduction velocity (MNCV) was measured as well as *2*) the nociceptive threshold, which was quantitated using the tail-flick.

Assessment of cutaneous microcirculation. Hair from the top of the skull to the back of the animals was removed with a depilatory lotion to present a hairless area for skin laser Doppler flow measurements, local pressure application, and iontophoretic delivery. This was performed 2 days before the experiments to prevent skin irritation during the experiment from confounding the results.

For the experiments, animals were anesthetized by intraperitoneal injection of thiopental sodium (65 mg/kg). The level of anesthesia was determined by testing eye reflexes and tail pinch. Then, animals were settled in an incubator (MMS, Chelles, France) warmed to maintain a stable cutaneous temperature (35.0 ± 0.5°C). Mice were placed in the prone position followed by a 20-min resting period to stabilize the blood pressure and cutaneous temperature. Noninvasive blood pressure (IITC, Woodland Hills, CA) was recorded before and after the experiments to verify mean arterial blood pressure stability. At the end of each experiment, animals were killed by an overdose of thiopental.

Assessment of pressure-induced vasodilation. Skin blood flow in response to local pressure application was measured by laser Doppler flowmetry. This method was described by Fromy et al. (23), using a weighbridge that was adapted to hold a laser Doppler probe at one end (PF415, Periflux; Perimed, Sweden). The probe was connected to a laser Doppler flowmeter (PF5000 Master, Periflux; Perimed). The weighbridge was carefully equilibrated, with the probe placed in the middle of the hairless skull of the mouse, and the external pressure was increased progressively at 2.2 Pa/s through the laser Doppler probe, using a syringe pump. The laser Doppler flowmetry signal was digitized with a 20-Hz sampling frequency, using a computerized acquisition system (Biopac, Santa Barbara, CA). Data collection started with a 1-min control period before the onset of increasing pressure. The laser Doppler flowmetry signal was averaged every 30 s to reduce the instantaneous variability of the signals as a result of vasomotion.

Assessment of endothelium-independent and -dependent responses. Skin blood flow was recorded, using a laser Doppler multifiber probe (481-1; Perimed) during transcutaneous iontophoresis applied to a 1.2-cm^2 area on the hairless back of the animals. Endothelium-independent response was assessed, using cathodal sodium nitroprusside iontophoretic delivery (67 mmol/l Nitriate; SERB, Paris, France) with a current application of 100 μA for 20 s. Endothelium-dependent response was assessed, using anodal ACh iontophoretic delivery (5.5 mmol/l; Sigma, Saint Quentin Fallavier, France) with a current application of 100 μA for 10 s. Sodium nitroprusside and ACh were dissolved in deionized water. Sodium nitroprusside– and ACh-induced vasodilator responses are reported as the maximal percent increase from baseline in response to the iontophoretic delivery of sodium nitroprusside and ACh, respectively. The iontophoresis technique was chosen to assess the in vivo cutaneous microvascular function to avoid any systemic effects.

Assessment of the nerve function

MNCV in sciatic-tibial fibers. After general anesthesia (65 mg/kg i.p.), MNCV was assessed by stimulating at the exposed sciatic notch and knee while recording the M-wave (compound muscle action potential) from the tibial-innervated dorsal interossei foot muscles. During recording, the temperature of the site surrounding the nerve was kept constant at 37°C.

Thermal nociceptive response. The conscious mouse was maintained in a restrainer and placed on the surface of the tail-flick analgesia meter (Apelex tail-flick analgesymeter, model DS 20; Socrel, Bagneux, France). The mouse was acclimated to the restrainer ~30 min before all measurements. Then, radiant heat was applied from a halogen lamp focused on the dorsal surface of its tail (2–3 cm from the tip of the tail). Movement of the tail activated a photocell that turned off the stimulus light and stopped a reaction timer that recorded the time. The intensity of the radiant heat was adjusted so the baseline tail-flick occurred within 5–6 s. Withdrawal latency was measured in seconds, and a 15-s cutoff time was imposed to prevent tissue damage. For each mouse, four determinations were carried out.

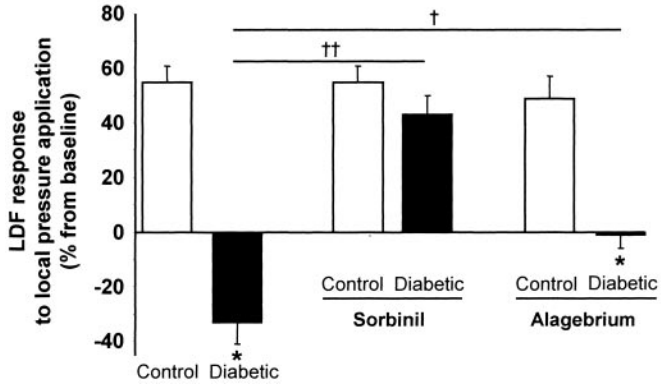

FIG. 1. Effects of pharmacological agents on the percent increase in skin laser Doppler flowmetry from baseline during local pressure application in 8-week diabetic and control mice. Control and diabetic mice were untreated or treated during the last 2 weeks of diabetes with sorbinil or alagebrium. *P < 0.001 vs. respective control; †P < 0.01, ††P < 0.001 vs. untreated diabetic. n = 10 in each group. LDF, laser Doppler flowmetry.

Biochemical assays

Fructosamine. Serum was obtained by centrifugation of blood collected in dry tubes. Serum samples were stored at −20°C. Fructosamine was determined by a nitroblue tetrazolium colorimetric test, based on the ability of the ketoamine group of glycated proteins to reduce tetrazolium salts under alkaline conditions (24).

Data analysis. Data are expressed as the means ± SE. They were first subjected to Bartlett's test for homogeneity of variances. One-way ANOVA was followed by the Student-Newman-Keuls multiple range test to estimate the significance of differences for between-group comparisons. Within a group (untreated, treated with sorbinil, treated with alagebrium) control and diabetic mice were compared, using an unpaired t test. Significance was defined at $P < 0.05$.

RESULTS

At the time of experimentation, all of the diabetic mice lost a significant amount of weight compared with their respective control mice (Table 1). However, treated diabetic mice lost significantly less weight than the untreated diabetic mice. In all of the diabetic mice, blood glucose ($P < 0.001$) and fructosamine ($P < 0.001$) levels were significantly increased compared with their respective control mice (Table 1). Alagebrium treatment significantly decreased the plasma fructosamine levels in diabetic mice compared with untreated diabetic mice ($P < 0.05$). For all the parameters, treated control mice were not different compared with untreated control mice (Table 1).

FIG. 2. Effects of pharmacological agents on the percent increase in skin laser Doppler flowmetry from baseline in response to iontophoretic delivery of ACh in 8-week diabetic and control mice. Control and diabetic mice were untreated or treated during the 2 last weeks with sorbinil or alagebrium. *P < 0.01 vs. respective control; †P < 0.01, ††P < 0.001 vs. untreated diabetic. n = 10 in each group. LDF, laser Doppler flowmetry.

TABLE 2
MNCV and tail-flick responses in 8-week diabetic mice and control mice

Groups	MNCV (m/s)	Tail-flick latency (s)
Control	59 ± 3	5.7 ± 0.1
Diabetic	43 ± 3*	7.2 ± 0.2*
Control + sorbinil	58 ± 2	5.5 ± 0.3
Diabetic + sorbinil	49 ± 4†	6.1 ± 0.2‡
Control + alagebrium	56 ± 3	5.6 ± 0.3
Diabetic + alagebrium	43 ± 4†	6.8 ± 0.2†

Control and diabetic mice were untreated or treated during the 2 last weeks of diabetes with sorbinil or alagebrium. MNCV: n = 12 in each group. Tail-flick: n = 7 in each group. *$P < 0.01$ vs. respective control; †$P < 0.05$ vs. treated control; ‡$P < 0.05$ vs. untreated diabetic.

None of the drug had any effect on the neurovascular function in control mice because there was no difference between treated and untreated control mice in pressure-induced vasodilation response (Fig. 1), ACh-induced vasodilation (Fig. 2), MNCV, and thermal nociceptive response (Table 2).

Assessment of the cutaneous microcirculation

Pressure-induced vasodilation assessment. Neither the treatments nor the diabetes induction changed the mean arterial blood pressure (Table 1).

In the untreated control group, we observed an increase in laser Doppler flowmetry in response to the local pressure application that reached a maximal value at 0.4 kPa, corresponding to a pressure-induced vasodilation of 55 ± 6% from baseline. In contrast, in the untreated diabetic group, we did not observe an increase in laser Doppler flowmetry in response to local pressure application, and at 0.4 kPa the percent change of laser Doppler flowmetry from baseline (−33 ± 8%) was significantly reduced compared with the untreated control group ($P < 0.001$) (Fig. 1).

In the sorbinil-treated diabetic group, pressure-induced vasodilation was restored and reached the same level (43 ± 7%) at 0.4 kPa than the treated control group (55 ± 6%, $P > 0.05$) (Fig. 1). In contrast, treating diabetic mice with alagebrium did not restore the pressure-induced vasodilation (−1 ± 6%) compared with treated control mice (49 ± 8%, $P < 0.001$) (Fig. 1). However, the alteration of pressure-induced vasodilation observed in alagebrium-treated diabetic mice (−1 ± 6%) was less dramatic in comparison with untreated diabetic mice (−33 ± 8%, $P < 0.01$).

Assessment of endothelium-independent response. In all groups, laser Doppler flowmetry increased in response to iontophoretic delivery of sodium nitroprusside, and we did not observe any difference in the endothelium-independent vasodilation between control and diabetic mice (control 49 ± 7%, diabetic 45 ± 4%, sorbinil-treated control 48 ± 9%, sorbinil-treated diabetic 44 ± 6%, alagebrium-treated control 45 ± 3%, alagebrium-treated diabetic 46 ± 7%).

Assessment of endothelium-dependent response. In the untreated control group, laser Doppler flowmetry increased in response to iontophoretic delivery of ACh, corresponding to an endothelium-dependent vasodilation of 27 ± 4% that was significantly reduced in the untreated

diabetic group (6 ± 1%, $P < 0.01$) (Fig. 2). The ACh-induced vasodilation observed in the sorbinil-treated (35 ± 7%) and alagebrium-treated (28 ± 4%) diabetic groups was restored at the same level compared with their respective control groups (35 ± 8%, $P > 0.05$, and 30 ± 4%, $P > 0.05$) and was significantly higher than the untreated diabetic group ($P < 0.001$ and $P < 0.01$, respectively).

Assessment of nerve function

MNCV in sciatic-tibial fibers. MNCV was significantly reduced in 8-week untreated diabetic mice compared with untreated control mice ($P < 0.01$) (Table 2). None of the treatments had a significant effect on MNCV.

Thermal nociceptive response. The tail-flick latency was increased in untreated diabetic mice compared with untreated control mice ($P < 0.01$) (Table 2). This diabetes-associated thermal hypoalgesia was only completely corrected by sorbinil treatment because alagebrium failed to reduce the tail-flick latency in diabetic mice.

DISCUSSION

Our study showed that vascular dysfunction associated with severe neuropathy dramatically impaired the pressure-induced vasodilation response in 8-week diabetic mice. Indeed, the decrease of pressure-induced vasodilation response was much more dramatic (−33%) after 8 weeks of diabetes than after 1 week of diabetes (−8%) (5). At this stage of diabetes, pressure-induced vasodilation was only restored when both vascular and C-fiber functions were restored, as demonstrated by sorbinil treatment in diabetic mice. Pressure-induced vasodilation assessment could then provide a direct noninvasive method to assess the cutaneous neurovascular function and should interest both fundamental and clinical research.

ACh-dependent vasodilation was altered in 8-week diabetic mice without any defect in vascular smooth muscle relaxation, as shown by sodium nitroprusside results. Because ACh is the most widely used drug for assessment of the endothelial L-arginine/NO pathway, our results showed a decrease in NO bioavailability that contributes to pressure-induced vasodilation alteration.

A number of findings suggested that increased aldose reductase activity has a key primary role in all signal transduction, biochemical, and metabolic changes that lead to diabetes complications, including oxidative stress and AGE formation (25,26). Hence, aldose reductase inhibition counteracts diabetes-induced peripheral neuropathies as well as endothelial dysfunction (9,13,14).

AGEs that form nonenzymatically with glucose and accumulate on long-lived tissue proteins have been implicated in vascular complications of diabetes (27,28). Exaggerated glycation processes during diabetes and excessive accumulation of AGEs in endoneurial microvasculature and nerve fibers contribute to the development of peripheral neuropathy (28,29). Accordingly, inhibitors of AGE accumulation were able to prevent or reverse neurovascular dysfunctions in diabetic rats with early or mild neuropathy (11,14,15).

In the current study, sorbinil and alagebrium treatments were both able to reverse cutaneous endothelial dysfunction in 8-week diabetic mice. These findings are concordant with previous studies looking at endothelial function in different vascular beds in long-term diabetic rats using ARIs (14,30,31). Indeed, sorbinil, through its ability to inhibit aldose reductase activity, could have protected NO bioavailability by increasing NADPH, an NO synthase cofactor, and by reducing peroxynitrite formation in vessels (14). Alagebrium, through its ability to break AGE cross-linking of matrix proteins, has been shown to exert beneficial cardiovascular effects in animals (18,19,21) as well as in humans (20,21). Furthermore, alagebrium is mainly an AGEs breaker, but it is also an AGE formation inhibitor (19,32) and thus could improve NO diffusion toward vascular smooth muscle (27) and restore endothelium-dependent vasodilation. In the current study, alagebrium's beneficial effect on the reduction in plasma fructosamine level confirmed its ability to reduce AGE formation and showed the efficiency of the given dose. Indeed, fructosamines are stable complexes of carbohydrates and proteins that are produced by an irreversible nonenzymatic glycosylation of serum proteins. Fructosamine level is a good indicator of AGE production over the previous 1–2 weeks, showing its rationale for short-term treatment (24). Because the glucose level in alagebrium-treated diabetic mice did not differ from the other diabetic groups, the decrease in fructosamine level is likely caused by alagebrium-induced inhibition of AGE formation, confirming the efficiency of the given dose. The dramatic pressure-induced vasodilation alteration observed in our study cannot be explained by the sole endothelial alteration because alagebrium restored ACh-dependent vasodilation but failed to restore pressure-induced vasodilation development in long-term diabetic mice.

Because pressure-induced vasodilation development depends on the activation by pressure of sensory nerve C-fibers (3), we tested C-fibers using thermal stimulus. The changes in unmyelinated C-fibers play a significant role for abnormal nociception in diabetes (33,34). In the current study, 8 weeks of diabetes altered the unmyelinated C-fibers response, as seen by an increase of tail withdrawal latency, demonstrating hypoalgesia. Uehara et al. (35) revealed that the pain sensation in diabetic mice was initially hyperalgesic, followed by late hypoesthesia in the presence of severe neuropathy. In diabetic patients, thermal hypoalgesia is associated with degenerative neuropathy, which includes the loss of epidermal C-fiber terminals (33,36). Accordingly, with increasing duration of diabetes (7–9 weeks), there is a loss of C-fibers in diabetic mice (16,37). Therefore, our results showed that 8-week diabetic mice exhibited a reduction of heat-induced C-fiber sensation, suggesting cutaneous C-fiber loss. Added to endothelial dysfunction, this C-fiber loss contributed to worsen the pressure-induced vasodilation observed in long-term diabetic mice compared with short-term diabetic mice exhibiting only vascular alteration.

Sorbinil succeeded to restore both normal heat-pain C-fiber response and endothelial function. Our study showed that glucose metabolism by aldose reductase contributed to the etiology of thermal hypoalgesia, and aldose reductase inhibition also normalized thermal responses. This is concordant with Calcutt et al. (38), who showed the efficiency of ARI to prevent thermal hypoalgesia in 8-week diabetic rats. Thus, we suggest that restoration of normal algesia in sorbinil-treated diabetic mice is caused by recovery of C-fiber terminals, allowing for pressure-induced vasodilation restoration. In contrast, alagebrium restored ACh-induced vasodilation but failed to restore pressure-induced vasodilation, mainly because it had no benefit on heat-induced C-fiber response and then was not able to improve the small unmyelinated C-fiber function that is crucial for pressure-induced vasodilation

development. The fact that pressure-induced vasodilation in the alagebrium-treated diabetic group was less negative than in the untreated diabetic group, although lower than in the control group, suggests a beneficial effect of alagebrium on pressure-induced vasodilation due to restoration of endothelial function.

As expected, after 8 weeks of diabetes, our data showed severe neuropathy because none of the treatments were able to reverse the decrease of MNCV in diabetic mice. This is in accordance with the data from recent studies on long-term diabetic mice (8,10) and the neuropathic process in human diabetes (33), confirming that STZ-induced diabetic mice might be a suitable model to study the neuropathy that arises in human diabetic patients (16,17). In contrast to our study, sorbinil has been shown to completely normalize sciatic-tibial MNCV (9,14) or prevented MNCV alteration (39), but the duration of diabetes was short and performed in rats. The metabolic origin and reversibility of these early conduction deficits distinguish them from true diabetic neuropathy. The latter is associated with clear neuropathy and with a conduction deficit of greater proportion that is hardly reversible (39), as seen in our study where MNCV was not restored but tended to be improved in sorbinil-treated diabetic mice. In long-duration diabetes, large myelinated A-fibers are likely altered, leading to MNCV reduction (33). Our study confirmed that, as we expected, large A-fiber alteration does not affect pressure-induced vasodilation development, strengthening the major need of intact function of small unmyelinated C-fibers in pressure-induced vasodilation development (3).

In the current study, sorbinil efficiently and fully restored the unmyelinated C-fiber function but was less effective in reversing MNCV deficit in long-term diabetic mice. In contrast to experimental studies, clinical trials of various ARIs conducted to date have failed to provide a convincing rationale for their use in the treatment of patients with diabetic neuropathy (25,26,40,41). The failure of ARIs to have had significant benefit could come from the advanced stage of neuropathy that is irreversible, outcomes based on invalid end points, inappropriate study design or analytical methods to correctly evaluate the efficiency of ARIs, low doses used to prevent side effects induced by ARIs, or differences among individuals in the susceptibility to diabetes complications including neuropathies. However, because some ARIs (such as sorbinil) demonstrated beneficial effect in early clinical studies (41), future well-designed clinical studies should strengthen the importance of the aldose reductase pathway in the pathogenesis of diabetes complications, as was already demonstrated by experimental studies.

To conclude, sorbinil was the sole treatment to restore C-fiber function, which was essential in association with endothelial restoration to reverse diabetes-induced pressure-induced vasodilation alteration. This study emphasizes that pressure-induced vasodilation needs intact vascular and C-fiber functions in order to exist. Pressure-induced vasodilation is thus a useful tool, and it is the only one available to explore the neurovascular interaction in diabetic patients. In addition to a decrease of NO bioavailability, the dramatic impairment of pressure-induced vasodilation observed at 8 weeks of diabetes is caused by an alteration of unmyelinated C-fiber response. The inability, with diabetes, of the cutaneous microcirculation to protect the skin against applied pressure, leading to an early occurrence of ischemia, reflects a high risk factor for foot ulcers. When the cross-talk between the nervous and vascular system is deregulated, it contributes to medically important diseases, as suggested by Carmeliet and Tessier-Lavigne (42,43), such as increased diabetic foot ulceration and amputation. The inhibition of the aldose reductase pathway improved the cross-talk between nerves and vessels that could delay diabetes-induced cutaneous pressure ulcers. Pressure-induced vasodilation could be a good end point measurement to evaluate changes in nervous and endothelial functions during diabetes and/or treatment. Pressure-induced vasodilation could thus provide a more direct method for detecting risk of diabetic foot than that which is currently possible. The specific advantage of pressure-induced vasodilation is that its assessment is noninvasive and easy to perform in clinic (6) and should therefore interest both fundamental and clinical research.

ACKNOWLEDGMENTS

Claire Demiot was supported by a grant from the Académie Nationale de Médecine, and Dominique Sigaudo-Roussel was supported by a grant from the Conseil Général du Département de Maine et Loire.

We thank the local animal care unit of the University of Angers for facilities and Jérôme Roux, Pierre Legras, and Dominique Gilbert for their kind help in treating the animals. We thank Jennifer Deschamps for her technical help.

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