Dual endothelin receptor antagonism with bosentan reverses established vascular remodeling in diabetic rats: Relevance to glycemic control

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Aim: We have shown that diabetes alters structural and functional properties of the cerebrovasculature in part by the activation of the endothelin (ET) system in a glucose-dependent manner. Here, we tested the hypothesis that established diabetes-induced vascular dysfunction and remodeling could be reversed by glycemic control or dual ET-1 receptor antagonism. Methods: Studies were performed in non-obese type-2 diabetic Goto–Kakizaki (GK) rats. GK rats were treated with vehicle, metformin (300 mg/kg/day) or dual ET-receptor antagonist bosentan (100 mg/kg/day) after onset of remodeling from 18 to 22 weeks by oral gavage. Additional groups included vehicle-treated 10 or 18-week GK rats. Blood glucose and mean arterial blood pressure (MAP) were monitored weekly. At termination, middle cerebral artery (MCA) lumen diameter, media thickness (MT), media: lumen (M:L) ratio, cross-sectional area (CSA) and myogenic-tone were measured using pressurized arteriograph (n = 8–14/group). Results: GK MAP was 102, 105 and 119 for vehicle, metformin and bosentan, respectively. 18 and 22-week diabetic GK rats displayed increased M:L ratio and CSA, but decreased lumen diameter and myogenic tone compared to 10-week animals. Glycemic control with metformin significantly improved blood glucose and partially reversed vascular remodeling by decreasing the MT, M:L ratio and CSA. Myogenic tone was improved only at lower pressures. Bosentan improved the MT and M:L ratio and did not affect CSA. Bosentan showed a significant improvement in MCA myogenic-tone over the pressure range despite elevated MAP. Conclusions: Glycemic control or ET-1 antagonism can partially reverse diabetes-induced cerebrovascular remodeling and dysfunction. These results strongly suggest that either approach offers a therapeutic benefit and combination treatments need to be tested.

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Bosentan restores impaired cerebrovascular relaxation in diabetes

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Aims: Up-regulation of the endothelin (ET) system in type-2 diabetes leads to increased contraction and decreased relaxation in basilar artery. We showed that 1) ET receptor antagonism prevents diabetes-mediated cerebrovascular dysfunction, and 2) glycemic control prevents activation of the ET system in diabetes. The goal of the current study was to determine whether and to what extent glycemic control or ET-receptor antagonism reverses established cerebrovascular dysfunction in diabetes. Methods: Non-obese type-2 diabetic Goto–Kakizaki (GK) rats were administered either vehicle, metformin (300 mg/kg/day) or dual ET-receptor antagonist bosentan (100 mg/kg) for 4 weeks starting at 18-weeks after established cerebrovascular dysfunction (n = 6–8/group). Blood glucose and blood pressure were monitored weekly. At termination, basilar arteries were collected and cumulative dose–response curves to ET-1 (1–500 nM), 5-HT (1–1000 nM) and acetylcholine (Ach, 1 nM–5 μM) were studied by wire myograph. Results: Only metformin decreased blood glucose. MAP was 102, 105 and 119 for vehicle, metformin and bosentan, respectively. The magnitude of basilar artery constriction in response to KCl was similar among groups. Interestingly, constriction to ET-1 and 5-HT (area under curve, AUC) was greater in treated animals as compared to vehicle-treated GK rats; however, there was no difference in Rmax or EC50. Both bosentan and metformin improved sensitivity to Ach and only bosentan increased relaxation (Rmax and AUC) despite elevated blood pressure. Conclusion: These results suggest that augmented contractile response to vasoactive agents is not improved by glycemic control or ET-receptor antagonism but ET-receptor antagonism is effective in improving relaxation response even if started after established cerebrovascular dysfunction and offers therapeutic potential.

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ETA receptor antagonists in the treatment of diabetic ketoacidosis

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In patients with type I diabetes mellitus poor management causes a drastic rise in glucose levels resulting in diabetic ketoacidosis (DKA). About 1% of DKA episodes can be complicated by cerebral edema. ET and its receptors are involved in the regulation of cerebral blood flow. We studied the effect of ETA receptor antagonists in a rat model of DKA. DKA was produced by streptozotocin (150 mg/kg, ip). Group 1: Control (non-diabetic) animals administered citrate. Animals that developed DKA were divided in five additional groups. Group II: DKA animals without treatment; Group III: DKA animals given saline; Group IV: DKA animals given saline and insulin of 1.5 u/kg/h; Group V: DKA animals given saline, insulin of 1.5 u/kg/h and BMS-182874 (9 mg/kg); and Group VI: DKA animals given saline, insulin of 1.5 u/kg/h and BQ123 (1 mg/kg). Blood glucose and ketones markedly increased by day 4 in DKA rats. Saline/insulin treatment in DKA rats increased the plasma and brain ET-1 levels which were not affected by BMS-182874 or BQ123 treatment. There was no change in the expression of ETB receptors in the brain, however, ETA receptor expression increased in DKA rats and was not altered following treatment with insulin, BMS-182874 or BQ123. Animals in insulin/saline group showed a significant increase (160%) in cerebral blood perfusion compared to baseline. This increase in cerebral perfusion was attenuated by BQ123 or BMS-182874. Treatment with BQ123 also improved blood pH and ketones in DKA rats. It can be concluded that ETA receptor antagonists maybe of therapeutic use in the management of DKA and its complications.

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Endothelin antagonism and diabetic erectile dysfunction: Changes in VEGF and NO in type I diabetic penis and effects of endothelin antagonism

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About 50% of male diabetic patients have erectile dysfunction (ED), which is considered as the vascular and neuropathic complications. Vascular endothelial growth factor (VEGF) has been extensively documented for its pathogenic significance in different complications of diabetes and we reported that VEGF signaling is greatly diminished in penis in a rat model of type II diabetes. The present study used 3 weeks duration of streptozotocin (STZ)-induced diabetic (DM) rat model to assess the VEGF expression with NO system in penile tissue and concomitantly the effects of endothelin antagonism has been studied on these changes. Male Sprague–Dawley rats were administered citrate saline vehicle or STZ (65 mg/kg IP). One week after the injection, animals were separated into those receiving endothelin-A/B (ET-A/B) dual receptor antagonist (SB209670, 1 mg/kg/day), endothelin-A (ET-A) receptor antagonist (TA-0201, 1 mg/kg/day) or saline for 2 weeks by osmotic mini pump. The local ET-1 level in DM penis was higher by 20% than that in non-DM rats. A 30% decrease in VEGF expression in penile tissue was seen in DM rats. Penile NO and eNOS levels were decreased in DM rats; greatly restored by ET-A receptor antagonist while unchanged by ET-A/B dual antagonist. iNOS was not significantly changed in penile tissues among non-DM, DM and ET-A antagonist treated groups. Thus, we conclude that (1) VEGF and pAkt were downregulated in type 1 DM penis, and that (2) the ET-A antagonist was potentially effective in reversing the decreased NO and eNOS levels in DM penis than those by ET-A/B dual antagonist.

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An endogenous blocker of oxidized LDL

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Aim: To elucidate the pathophysiological significance of the endogenous oxidized LDL (oxLDL) blocker, which was originally described as an endothelium-derived secreted protein Del-1 (developmental endothelial locus-1). Background: oxLDL can potentiate the induction of foam cell formation and inflammatory responses, the processes which are believed to be integral to atherogenesis. However, no endogenous proteins, which interfere with oxLDL binding to its receptors, have been identified. Methods: Interaction between oxLDL and Del-1 was examined in a cell-free system by ELISA. Inhibition of binding and action of oxLDL was examined with CHO cells expressing LOX-1 and with COS-7 cells transfected with scavenger receptors. Cultured human umbilical vein endothelial cells (HUVEC) and THP-1 were also used to analyze the inhibitory effects of Del-1 on oxLDL action. Results: We found that Del-1 selectively bound to oxLDL, but not to native LDL. Del-1 inhibited the uptake of DiI-labeled oxLDL (DiI-oxLDL) by LOX-1 expressed in COS-7, but did not inhibit DiI-labeled native LDL uptake by LDL receptor. Del-1 inhibited DiI-oxLDL binding to other oxLDL receptors as well, such as SR-A and CD36. In addition, Del-1 inhibited DiI-oxLDL uptake by HUVEC and THP-1-derived macrophages. Site-directed mutagenesis revealed that two arginine residues in Del-1 were crucial for the binding of oxLDL. Furthermore, Del-1 suppressed oxLDL-induced signal transduction in LOX-1-expressing CHO cells. In HUVEC, Del-1 also suppressed oxLDL-induced signaling and endothelin-1 secretion. Thus, we demonstrated that Del-1 is an endogenous protein which protects cells from oxLDL actions. Conclusion: We identified, for the first time, an endogenous oxLDL blocker which may play a regulatory role in interfering progression of atherosclerosis.

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Selective endothelin ETA and dual ETA/ETB receptor blockade improves endothelial function in patients with type 2 diabetes and coronary artery disease

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Purpose: Endothelin-1 contributes to endothelial dysfunction in patients with atherosclerosis and type 2 diabetes. In healthy arteries the ETA receptor mediates the main part of the vasoconstriction induced by endothelin-1 while the ETB receptor mediates vasodilatation. The ETB receptor expression is upregulated in atherosclerosis and may thereby contribute to the vasoconstrictor tone and development of endothelial dysfunction. The aim of the present study was to compare the effects of selective ETA and dual ETA/ETB blockade on endothelial function in patients with type 2 diabetes and coronary artery disease. Methods: Twelve patients were included in this cross-over study with blinded evaluation. Forearm blood endothelium-dependent and endothelium-independent vasodilatation was assessed by venous occlusion plethysmography during intra-arterial infusions of serotonin and nitroprusside, respectively, before and after 60 min of intra-arterial infusion of either the selective ETA antagonist BQ123 or the combination of BQ123 and the ETB antagonist BQ788. Changes between the two treatments were compared using 2-way analysis of variance. Results: Dual ETA/ETB receptor blockade increased baseline forearm blood flow by $30 \pm 14\%$ ($P < 0.01$) whereas selective ETA blockade did not ($14 \pm 8\%$). Both selective ETA blockade and dual ETA/ETB blockade induced a 2-fold increase in endothelium-dependent vasodilatation ($P < 0.001$). The improvement in endothelium-dependent vasodilatation did not differ between the two treatment strategies. Both treatments improved the endothelium-independent vasodilatation. Conclusions: Both selective ETA and dual ETA/ETB improve endothelial function in patients with type 2 diabetes and coronary artery disease. Addition of ETB to ETA receptor blockade increases basal blood flow but does not additionally improve endothelial function.

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2-year follow-up in oxidative stress levels in patients with acute coronary syndrome: Insights from the assessment of lipophilic vs. hydrophilic statin therapy in acute myocardial infarction (ALPS-AMI) study

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Background: Statins reduce the incidence of cardiovascular events in patients with acute myocardial infarction (AMI). Although all statins are equally effective in secondary prevention, there might be certain differences in the effects of lipophilic and hydrophilic statins and its association with oxidative stress levels in AMI patients remains unclear.