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RAPID COMMUNICATION

Sulodexide Improves Endothelial Dysfunction in Streptozotocin-Induced Diabetes in Rats

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Summary

Diabetes mellitus is associated with many complications including retinopathy, nephropathy, neuropathy and angiopathy. Increased cardiovascular risk is accompanied with diabetes-induced endothelial dysfunction. Pharmacological agents with endothelium-protective effects may decrease cardiovascular complications. In present study sulodexide (glycosaminoglycans composed from heparin-like and dermatan fractions) was chosen to evaluate its protective properties on endothelial dysfunction in diabetes. Effect of sulodexide treatment (SLX, 100 UI/kg/day, i.p.) in 5 and 10 weeks lasting streptozotocin-induced diabetes (30 mg/kg/day, i.p. administered for three consecutive days) was investigated. Animals were divided into four groups: control (injected with saline solution), control-treated with sulodexide (SLX), diabetic (DM) and diabetic-treated with sulodexide (DM+SLX). The pre-prandial and postprandial plasma glucose levels, number of circulating endothelial cells (EC) and acetylcholine-induced relaxation of isolated aorta and mesenteric artery were evaluated. Streptozotocin elicited hyperglycemia irrespective of SLX treatment. Streptozotocin-induced diabetes enhanced the number of circulating endothelial cells compared to controls. SLX treatment decreased the number of EC in 10-week diabetes. Acetylcholine-induced relaxation of mesenteric arteries was significantly impaired in 5 and 10-week diabetes. SLX administration improved relaxation to acetylcholine in 5 and 10-week diabetes. Diabetes impaired acetylcholine-induced relaxation of rat aorta irrespective of SLX treatment. Our results demonstrate that SLX treatment lowers the number of circulating endothelial cells and improves endothelium-dependent relaxation in small arteries. These findings suggest endothelium-protective effect of sulodexide in streptozotocin-induced diabetes.

Key words

Diabetes • Sulodexide • Endothelial Dysfunction • Endothelemia • Acetylcholine-Induced Relaxation

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Diabetes mellitus (DM) is a chronic metabolic disorder associated with long-term complications, including retinopathy, nephropathy, neuropathy and angiopathy. DM is considered to be a major risk factor for cardiovascular disorders as ischemic heart disease, cerebral stroke and peripheral artery disease leading to increased mortality of diabetics. Increased cardiovascular risk is associated with DM-induced endothelial dysfunction. The therapeutic approach to DM should also be focused on the reduction of risk factors for cardiovascular diseases as hypertension, dyslipidemia using antihypertensives, vasodilators, hemorrheologics, antithrombotics, hypolipidemics, etc. (Yki-Järvinen 2000, Kojšová *et al.* 2006, Pecháňová and Šimko 2007). Some of these pharmacological agents (ACE inhibitors, statins, pentoxifylline) may have endothelium-protective effects (Kristová and Kriška 1998), which prevent vascular damage, and thus decrease a risk of cardiovascular complications. In previous studies, we have demonstrated a favorable effect of sulodexide (glycosaminoglycans composed from heparin-like and dermatan fractions) and pentoxifylline, a methylxantine with hemorrheologic properties, in experimental models of endothelial damage (Kristová *et al.* 2000). Sulodexide significantly decreased

Table 1. The effect of sulodexide (SLX) treatment on preprandial and postprandial plasma glucose levels (mmol/l) in control rats (Control), in rats treated with SLX (SLX), diabetic rats (DM) and diabetic rats treated with SLX (DM+SLX) in 5 and 10-week diabetes.

	Control	**SLX**	**DM**	**DM+SLX**
Preprandial glucose 5 weeks	5.62 (4.47; 8.62)	5.35	6.45 (5.43; 18.07)	5.47 (5.16; 6.81)
Postprandial glucose 5 weeks	7.20 (5.34; 9.66)	6.84	24.85 (11.33; 33.58)**	22.00 (15.88; 29.17)$^{+}$
Preprandial glucose 10 weeks	5.62 (4.92; 6.41)	5.93	7.41 (6.83; 9.25)**	9.00 (6.00; 12.44)$^{++}$
Postprandial glucose 10 weeks	7.34 (6.68; 11.68)	7.12	9.89 (8.21; 29.45)*	8.88 (8.13; 31.97)$^{+}$

Data are expressed as medians and their 95 % confidence intervals, *P<0.05, **P<0.01: Control vs. DM, $^{+}$P<0.05, $^{++}$P<0.01: Control vs. DM+SLX, (n = 5-11).

Table 2. The effect of sulodexide (SLX) treatment on number of circulating endothelial cells in plasma [EC/10 µl] in control rats (Control), in rats treated with SLX (SLX), in diabetic rats (DM) and diabetic rats treated with SLX (DM+SLX) in 5 and 10-week diabetes.

DM-duration	**Control**	**SLX**	**DM**	**DM+SLX**
5 weeks	3.00 (1.25; 3.25)	2.00 (1.25; 2.50)	5.15 (3.25; 6.25)**$^{++++}$	4.00 (2.25; 5.75)
10 weeks	1.88 (0.75; 2.25)	2.38 (1.75; 3.00)	3.88 (3.25; 5.75)****$^{++}$	2.25 (1.75; 3.50)$^{\#}$

Data are expressed as medians and their 95 % confidence intervals, **P<0.01, ****P<0.0001: Control vs. DM, $^{++}$P<0.01, $^{++++}$P<0.0001: SLX vs. DM, $^{\#}$P<0.02: DM vs. DM+SLX, (n = 8-14).

the number of desquamated endothelial cells in the model of endothelial damage *in vitro.* Therefore sulodexide was chosen as endothelium-protective agent with possible effects on vessels in the model of experimental diabetes induced by streptozotocin in rats. In this study the effect of sulodexide on endothelial dysfunction associated with diabetes was evaluated on the basis of endothelium-dependent relaxation to acetylcholine in aortas and mesenteric arteries as a marker of endothelial function and of number of circulating endothelial cells in blood as a marker of endothelial injury.

Experiments were carried out in Wistar male rats, which were housed under standard laboratory conditions (temperature 23±1 °C, 12-h light-dark cycle, pelleted ST-1 diet and tap water *ad libitum*). Animals were divided into four groups. The first group was injected with saline solution (Control) and the second group was treated with sulodexide (SLX, 100 UI/kg/day). Diabetes was induced by injection of streptozotocin (30 mg/kg/day, i.p.) administered on three consecutive days in the third and fourth groups. The third group was injected with saline solution (DM) and the fourth group with sulodexide (DM+SLX). Treatment with sulodexide lasted 5 weeks and 10 weeks, respectively. Thereafter, preprandial and postprandial blood glucose levels (Bio-la-test, Lachema, CZ) were determined. The animals were sacrificed in thiopenthal anesthesia. The blood with Na-citrate was used for measurement of circulating endothelial cells in Bürker's chamber after their isolation, cleaning from platelets and an additional treatment with adenosine diphosphate as described previously (Hladovec and Rossmann 1973, Kristová *et al.* 2006). Isolated and cleaned aortas and mesenteric arteries were cut into rings for measurement of isometric contractions. The organ chambers were filled with modified Krebs-Henseleit solution (119 mM NaCl, 4.7 mM KCl, 1.17 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.18 mM KH_2PO_4, 0.03 mM EDTA, 2.5 mM $CaCl_2$, 200 mg/l ascorbic acid, 2 g/l glucose) at 37 °C and bubbled with 95 % O_2 and 5 % CO_2. Vessels were stretch to tension elicited by 1 g and left stabilized for 1 hour. Relaxation responses to acetylcholine (10^{-8}, 3.10^{-8}, 10^{-7}, 3.10^{-7}, 10^{-6}, 3.10^{-6} mol/l) were tested on rings precontracted with phenylephrine (10^{-6} mol/l). Multiple comparisons of independent samples by Kruskal-Wallis test were used. All data are expressed as medians and their 95 % confidence intervals.

Streptozotocin application increased preprandial and postprandial plasma glucose levels in rats (Table 1). Sulodexide did not affect plasma glucose levels in diabetic rats compared to controls. The number of circulating endothelial cells was increased in both 5 and 10-week diabetic groups when compared with control. In 5-week diabetes SLX-treatment decreased the number of endothelial cells compared to DM but without significant

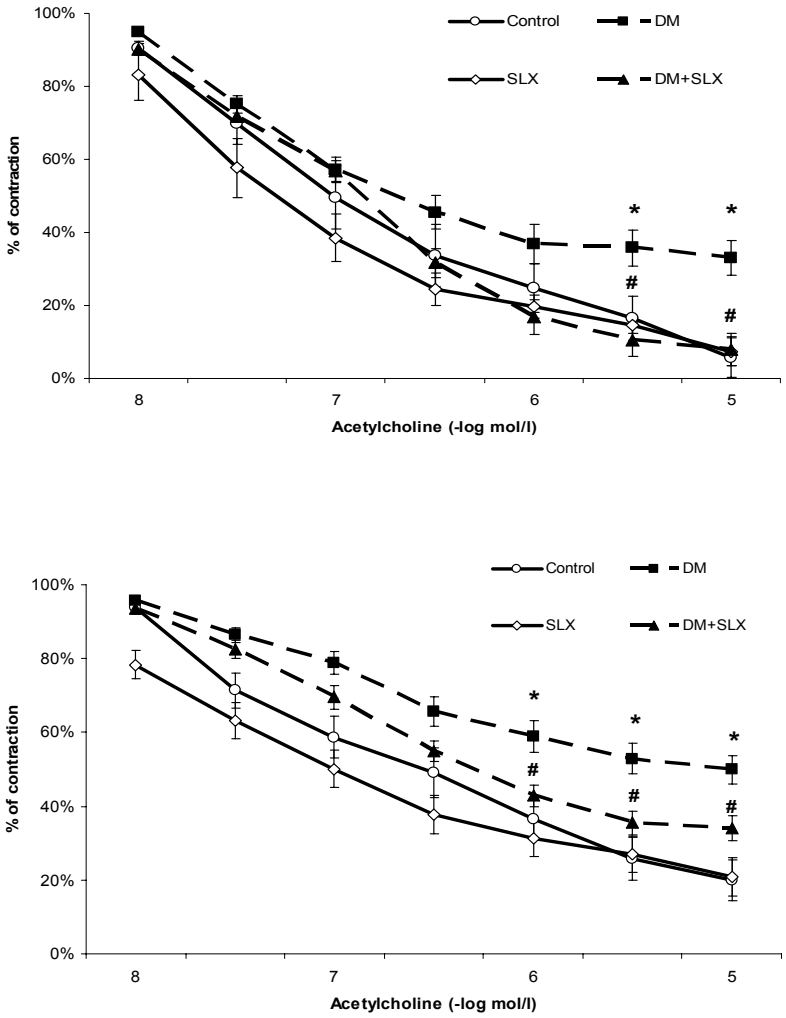

Fig. 1. Upper panel: The effect of sulodexide treat-ment (SLX) on acetylcholine induced-relaxation of me-senteric artery (n = 8) in 5-week diabetic rats (DM) compared to control and diabetic rats treated with sulodexide (DM+SLX). **Lower panel:** The effect of sulodexide treatment (SLX) on acetyl-choline induced-relaxation of mesenteric artery (n = 8) in 10-week diabetic rats (DM) compared to control and diabetic rats treated with sulodexide (DM+SLX). $*P<0.05$: Control vs. DM, $^{\#}P<0.05$: DM vs. DM+SLX.

differences. Contrary, the similar decrease of endothelemia in 10-week diabetes was significant in SLX-treated rats when compared to DM (Table 2).

Acetylcholine-induced relaxation of isolated mesenteric arteries from diabetic rats was significantly impaired compared to controls. The relaxation response was improved to control level after SLX-treatment in 5-week diabetes (Fig. 1, upper panel). Impairment of relaxation response to acetylcholine was found in 10-week diabetes. SLX treatment significantly improved the relaxation, although control level was not reached (Fig. 1, lower panel). Diabetes impaired acetylcholine-induced relaxation of aorta without any effect of SLX-treatment (data not shown).

Streptozotocin-induced diabetes, confirmed by hyperglycemia, reduced endothelium-dependent relaxation to acetylcholine in the rat mesenteric artery and increased the number of circulating endothelial cells. Sulodexide treatment improved endothelium-dependent relaxation of mesenteric arteries in both 5- and 10-week diabetes. Reduced endothelemia after sulodexide was observed only in 10-week diabetes. Sulodexide did not affect preprandial and postprandial plasma glucose levels in 5 and 10-week diabetes. Model of streptozotocin-induced diabetes is often used to study pathological changes of cardiovascular system. These changes involve impairment of endothelial function and further endothelial injury in which hyperglycemia has a crucial role. It was found that hyperglycemia induces reactive oxygen species (ROS) production mediating endothelial dysfunction (Zúrová-Nedelčevová *et al.* 2006). Increased ROS formation has negative effect on nitric oxide (NO) production as the main vasodilator substance in endothelium, with consequent alterations of endothelial function (Maritim *et al.* 2003). The results of present study confirmed the impairment of endothelium-dependent relaxation of aorta and mesenteric artery in diabetic rats. The decreased vascular relaxation may result from disturbances in the balance between vasodilator (NO, PGI_2) and vasoconstrictor substances (endothelin, thromboxane) in endothelium with prevalence of vasoconstrictors.

Endothelial dysfunction and/or injury induced by different stimuli such as stress, hypertension or toxic damage, is manifested by increased number of circulating endothelial cells (Babál *et al.* 2006, Kristová *et al.* 2006). The measurement of endothelemia represents an indirect marker of disturbed endothelium and may have some predictive value in clinics (Dignat-George and Sampol 2000, Rajec *et al.* 2007). Increased endothelemia in our study on streptozotocin diabetes is in agreement with results of previous study on the same model of diabetes (Zúrová-Nedelčevová *et al.* 2006).

Our data have shown that sulodexide decreases endothelemia in 10-week lasting diabetes. More pronounced effect of sulodexide in this model was probably due to longer administration of drug compared to 5 weeks of diabetes. This effect may be explained by ability of sulodexide, a natural glycosaminoglycan with complex effects on the vascular wall (e.g. antithrombotic, fibrinolytic, antiatherogenic), to repair or to prevent endothelial damage. The effectiveness of sulodexide was confirmed by its effects on peripheral occlusive arterial disease (Gaddi *et al.* 1996). Our results demonstrate an improvement of endothelium-dependent relaxation after sulodexide treatment only in the mesenteric artery but not in the aorta. These findings suggest that proposed endothelium-protective effect of sulodexide is exerted preferentially on small arteries. Further studies on resistance arteries are needed to confirm this hypothesis.

Conflict of Interest

There is no conflict of interest.

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