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Effect of pirfenidone on endothelium-dependent vasodilatation in type-2 diabetic (db/db) mice

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Introduction: Both endothelial cell dysfunction and vessel stiffness is associated with worsening of the prognosis in patients with cardiovascular disease. In the present study, we investigated the effect of the antifibrotic drug, pirfenidone, on vascular tone. Moreover, we examined whether it restores endothelial function in arteries from diabetic animals.

Methods: The experimental protocol was approved by the Danish Animal Experiments Inspectorate (permission 2014-15-2934-01059). 18-20-Week old wild type mice and normoglycemic (db/+), and type 2 diabetic (db/db) male mice were euthanized by cervical dislocation. Aorta, coronary and mesenteric small arteries were isolated from diabetic db/db and db/+ control mice. The vascular segments were mounted on wires in microvascular myographs for functional studies.

Results: In coronary arteries from wild type mice contracted with U46619, pirfenidone induced concentration-dependent relaxations which at 10^{-4} M pirfenidone were 71±8% (n=10). Relaxations induced by 10^{-4} M pirfenidone were reduced to 18±6% ($P<0.05$, n=7) in the presence of an inhibitor of nitric oxide (NO) synthase, N^G-nitro-L-arginine (L-NOARG, 1 mM) and to 28±12 % ($P<0.05$, n=6) in the presence of high extracellular potassium (30 mM). Relaxations induced by 10^{-4} M pirfenidone were reduced to respectively, 14±7% ($P<0.05$, n=6), 37±9% ($P<0.05$, n=6), and 9±6% ($P<0.05$, n=6) by a blocker of voltage-gated Kv7 channel XE991 (10 µM), and by blockers of large-conductance calcium-activated K channels, tetraethylammonium (1 mM) and iberiotoxin (10 nM). Weight (50±2g, $P<0.05$, n=24) and blood glucose levels (27±2 mM, $P<0.05$, n=24) were higher in diabetic db/db mice compared to normoglycaemic db/+ control mice that weighed 30±1g (n=24) and had blood glucose levels of 8±1 mM (n=24). In aorta, coronary and mesenteric small arteries from diabetic db/db mice and db/+ control animals, relaxations induced by the endothelium-dependent vasodilator, acetylcholine, were markedly reduced. In aorta segments pirfenidone and an opener of K_V7 channels, flurpirtine (10^{-5} M) leftward shifted concentration-response curves for acetylcholine. A blocker of K_V7 channels, XE991 reduced the effect of both pirfenidone and flurpirtine and further reduced acetylcholine relaxations in aorta. In contrast, in mesenteric small arteries the potentiating effect of pirfenidone was absent despite impaired endothelium-dependent vasodilation to acetylcholine in arteries from diabetic db/db mice.

Conclusions: The present findings suggest that pirfenidone is a direct vasodilator and that it improves endothelium-dependent vasodilatation by a mechanism involving K channels in large arteries from diabetic animals. Thus, at relevant therapeutic concentrations the antifibrotic drug pirfenidone may restore endothelial function.