Linagliptin modulates vascular tone through L-Arginine/eNOS pathway

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Dipetidil-peptidase-4 (DPP4) represents a class of endogenous enzymes involved in the degradation of incretin hormones, such as Glucagon like peptide 1 (GLP-1) and Glucose dependent insulinotropic polypeptide (GIP) (1,2). Incretin system is involved in the regulation of postprandial glycemic control and satiety. In response to food intake, GLP-1 and GIP, released by the gut, induce insulin synthesis and secretion, inhibit glucagon release and reduce glucose production, lowering glycemia. The ipoglycaemic action of GLP-1 is transient, due to its short life-time. DPP4 inhibitors (DPP4i) represents a new approach in the treatment of type 2 diabetes mellitus (T2DM) by virtue of its effects on prolonging the half-life of incretins. At the present stage, five different inhibitors has been approved for the treatment of T2DM *(*sitagliptin, vildagliptin, saxagliptin, linagliptin and alogliptin). Recent studies suggests that DPP4i exert a beneficial pleiotropic action on heart and vessels (3-5). The molecular mechanism through which DPP4i induce their beneficial effects on cardiovascular function is still unclear so the aim of this study is to evaluate the molecular mechanisms involved in beneficial action of DPP4i, in particular of Linagliptin and Sitagliptin. To pursue this goal we perform *in vitro* experiments on isolated aortic and carotid rings harvested from CD-1 mice. Cumulative concentration response curves of Linagliptin and Sitagliptin (100nM-30μM) have been evaluated. In physiological conditions, Linagliptin induces vasodilatation that reaches a maximum effect (Emax) of 89.4 ± 2.58% and 89.7 ± 5.64% in aorta and carotid rings, respectively. Also Sitagliptin induces vasodilatation even though in less extent, indeed sitagliptin achieves an Emax= 49.0 ± 11.3% in aorta and 21 ± 4.48% in carotid. Endothelial removal significantly reduces Linagliptin-induced vasodilation both in aorta and carotid rings. Moreover, incubation with L-NIO, an eNOS inhibitor, or with ODQ, a sGC inhibitor, significantly reduces Linagliptin-induced vasodilation in both tissues. These data are also confirmed by experiments performed on vessels harvested from soluble guanylyl cyclase$_{\hat{i}\pm 1}^{-/-}$ mice in which linagliptin fails to induce vasorelaxation. Recent literature reported that the relaxation induced by DPP4i is partially mediated by the interaction between GLP-1 and its receptor (GLP-1R) (6). To assess if the vasodilating effect of Linagliptin is due to GLP-1R activation, we performed a set of experiments on GLP-1R$^{-/-}$ mice. In aortic rings harvested from these animals, Linagliptin preserves its vasorelaxing property and such vasodilating effect is inhibited by L-NIO or ODQ treatment as well. Cellular and molecular studies performed on Bovine Aortic Endothelial Cells (BAEC) show that Linagliptin: i) increases NOx levels; ii) does not modify calcium flux; iii) rescues eNOS from CAV-1 binding. These results demonstrate that linagliptin-induced vasodilatation is independent from GLP-1 receptor activation and involves eNOS/NO pathway. In particular, linagliptin interferes with the protein-protein interaction i.e. CAV-1/eNOS, leading to an increased eNOS availability with consequent NO increased production. In conclusion we show that the beneficial vascular effect of linagliptin involves a rescue of eNOS activity by disrupting the negative regulation operated by CAV-1.

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