Methods: Wistar rats were treated with telmisartan (2.7 mg/kg/d or 8 mg/kg/d p.o./10 d) which was combined with GTN (50 mg/kg/d s.c./3 d). Aortic eNOS phosphorylation and S-glutathionylation were assessed using antibodies against phospho Thr495 and Ser1177, or protein-bound glutathione, which regulate eNOS activity and eNOS-dependent superoxide production (uncoupling). Expression of mitochondrial aldehyde dehydrogenase (ALDH-2) was determined by Western blotting. Formation of aortic and cardiac ROS was assessed by fluorescence, chemiluminescence and 3-nitrotyrosine/malondialdehyde-positive protein content.

Results: Telmisartan prevented endothelial dysfunction and partially improved nitrate tolerance. Vascular, cardiac, mitochondrial and white blood cell ROS formation were significantly increased by GTN treatment and inhibited by telmisartan. GTN-induced decrease in Ser1177, increase in Thr495 phosphorylation or S-glutathionylation and decrease in ALDH-2 expression were normalized by telmisartan.

Conclusions: These data identify modification of eNOS phosphorylation as an important component of GTN-induced endothelial dysfunction. Via its pleiotropic "antioxidant" properties, telmisartan prevents at least in part GTN-induced oxidative stress, nitrate tolerance and endothelial dysfunction.

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P.4.5
Comparison of direct and indirect antioxidant effects of linagliptin with other gliptins — Evidence for antioxidant and antiinflammatory properties of linagliptin

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Objective: Gliptins (dipeptidyl peptidase-4 inhibitors) represent a novel class of drugs for the treatment of hyperglycemia. There is preliminary evidence that these compounds may confer antioxidant effects that beneficially influence cardiovascular complications in diabetes. In the present study we compared the antioxidant and antiinflammatory effects of linagliptin with alogliptin, vildagliptin, saxagliptin and sitagliptin.

Materials and methods: Antioxidant and antiinflammatory effects of gliptins were measured in isolated human neutrophils (PMN) by interfering with lipopolysaccharide (LPS) and zymosan A induced oxidative burst (NADPH oxidase activation) as well as adhesion of PMN to endothelial cells (measured by p67phox content from adherent cells). Direct vasodilatory effects of gliptins on isolated aortic vessels were assessed by isometric tension measurements. Antioxidant and antiinflammatory effects of linagliptin were also tested in a rat model of nitroglycerin induced nitrate tolerance and LPS-induced septic shock (isometric tension recording, vascular, cardiac and blood reactive oxygen species (ROS) formation).

Results: Linagliptin was the most potent inhibitor of oxidative burst in isolated human PMN in response to NADPH oxidase activation by LPS and zymosan A. Moreover, linagliptin suppressed PMN adhesion to endothelial cells in the presence of LPS. Linagliptin and alogliptin had the most pronounced direct vasodilatory potency, followed by vildagliptin, whereas saxagliptin and sitagliptin induced no relaxation in the tested concentration range. Finally, linagliptin in vivo treatment ameliorated nitroglycerin- and LPS-induced endothelial dysfunction and improved vascular, cardiac and blood ROS formation in nitrate-tolerant and septic rats.

Conclusions: These observations support pleiotropic antioxidant and antiinflammatory properties of linagliptin, which are not (or to a minor extent) shared by other gliptins. Further studies have to show whether these pleiotropic antioxidant properties of linagliptin translate into superior therapeutic efficacy in diabetic patients with cardiovascular complications.

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P.4.6
N^1-methylnicotinamide improves endothelial dysfunction in human blood vessels
Direct evidence at a single cell level

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N^1-methylnicotinamide (MNA^+) has until recently been thought to be a biologically inactive product of nicotinamide metabolism in the pyridine nucleotide pathway. However, the latest observations suggest that MNA^+ can exert anti-thrombotic and anti-inflammatory effects through direct action on the endothelium. To further investigate the therapeutic endothelial potential of MNA^+, we examined both *in vivo* and *in vitro* whether the compound might induce vasorelaxation in human blood vessels through an improvement of nitric oxide (NO) bioavailability and a reduction of oxidative stress mediated by endothelial nitric oxide synthase (eNOS) function. MNA^+ treatment (100 mg/m2 orally) in healthy normocholesterolemic and hypercholesterolemic subjects remarkably increased the *N*G-monomethyl-l-arginine (L-NMMA)-inhibitable flow-mediated dilation (FMD) of brachial artery responses that also positively correlated with MNA^+ plasma concentrations ($r = 0.73$ for normocholesterolemics and $r = 0.78$ for hypercholesterolemics; $P < 0.0001$). MNA^+ increased FMD at the concentration range at which the compound enhanced NO release from cultured human endothelial cells after stimulation with either the receptor-dependent (acetylcholine), or the receptor-independent eNOS agonists (calcium ionophore A23187). MNA+ normalized the agonist-stimulated NO release after the exposure of the cells to oxidized-LDL. The simultaneous detections of NO with superoxide ($O2^-$) revealed this effect to be also associated with the normalizing [NO]/[$O2^-$] balance in the endothelial cells after the eNOS stimulation by the agonists. Those results demonstrated for the first time that the