556

Effect of Hormone Replacement Therapy on Oxidative Stress in Postmenopausal Women

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Effects of hormone replacement therapy (HRT) on postmenopausal symptoms have been described; however, its effect on OS is controversial. The aim of this study was to determine the effect of HRT on OS in postmenopausal women (POS). A randomized, double-blind and controlled trial was carried out in 68 women from Mexico City: 1) HRT (0.625 mg/d of synthetic conjugated estrogens [Sixdin®] plus 5 mg/10d of medroxiprogesterone [MPA]), 33 POS (52±3 years, E2 20±4 pg/mL, FSH 55±21 pg/mL); 2) placebo, 34 POS (53±3 years, E2 21±3 pg/mL, FSH 53±22 pg/mL). We measured lipoperoxides [LPO] by TBARS assay, erythrocyte superoxide dismutase [SOD], glutathione peroxidase [GPx] and total plasma antioxidant status [TAS] with Randox Laboratories, Ltd kits; pretreatment and after 6 months of HRT. An alternative cut-off value of LPO ≥0.320 µmol/L was defined on the basis of the 90th percentile of young healthy subjects. Results showed that LPO in TRH decreased (0.353±0.05 µmol/L vs. 0.309±0.07 $p<0.05$) and GPx increased (49±15 U/gHb vs. GPx 67±13 $p<0.05$) after 6 month, in placebo did not change. The women with high LPO levels in HRT decreased after 6 month, pre-treatment 24(73%) to 11(32%) post-treatment $p<0.01$, and placebo did not change. Our findings suggest that TRH decreases OS in posmenopausal women.

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557

Comparison of Direct and Indirect Antioxidant Effects of Linagliptin (BI 1356, ONDERO) with other Gliptins – Evidence for Anti-inflammatory Properties of Linagliptin

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Objective: Gliptins (DPP-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 disease, which are secondary diabetic complications. In the present study we compared the direct and indirect antioxidant effects of linagliptin (BI1356, ONDERO) with alogliptin, vildagliptin, saxagliptin and sitagliptin.

Materials and Methods: Direct antioxidant effects of gliptins were assessed by interfering with superoxide formation from xanthine oxidase, peroxynitrite (authentic and Sin-1 derived) or hydrogen peroxide/peroxidase mediated 1-electron-oxidation. These oxidations were detected by fluorescence, chemiluminescence and nitration of phenols (HPLC). Indirect antioxidant effects of gliptins were measured in isolated human leukocytes by interfering with phorbol ester, LPS, zymosan A and fMLP induced oxidative burst (NADPH oxidase activation). Indirect antioxidant effects of linagliptin were also tested in a rat model of nitroglycerin induced nitrate tolerance.

Results: All gliptins, in our assays, only showed marginal direct antioxidant capacity. Minor (but significant) suppression of superoxide formation was observed for vildagliptin and of peroxynitrite formation/-mediated nitration for linagliptin. All gliptins except saxagliptin showed significant interference with 1-ectron-oxidations by the hydrogen peroxide/peroxidase system with linagliptin being the most potent compound. Linagliptin showed the best inhibition of oxidative burst in isolated human leukocytes in response to NADPH oxidase activation by LPS and zymosan A. Moreover, linagliptin suppressed leukocyte adhesion to endothelial cells in the presence of LPS. Finally, linagliptin in vivo treatment ameliorated nitroglycerin-induced endothelial dysfunction and showed minor improvement of ROS formation in isolated cardiac mitochondria and oxidative burst in whole blood from nitrate-tolerant rats.

Conclusions: These observations support pleiotropic antioxidant properties of linagliptin, which is 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 diseases.

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558

Secretion of Antioxidant Peroxiredoxin 4 in Response to Oxidative Stress

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Background: Peroxiredoxins (Prx), ubiquitously expressed thiol-dependent peroxidases, participate in removal of reactive oxygen species (ROS). Conflicting information exists about the intra- and extracellular localization of Prx4. We hypothesize that the N-terminal signal sequence of Prx4 enables secretion via the classical pathway and that oxidative stress enhances this secretion.

Methods: Using either an immunoluminometric assay or Western blot we studied expression and secretion of endogenous or recombinant Prx4 by different epithelial cell lines. Prx4 serum levels were determined in patients with diabetes and myocardial infarction compared to healthy controls. Intracellular Prx4 was indirectly stained by fluorescence-labeled antibodies and visualized with laser scanning microscopy.

Results: Epithelial cell lines (HEK293, HepG2, Huh7, Cos7, Capan1) express and secrete Prx4. In HEK293, HepG2 and Prx4-transfected MDCK1 cells Prx4 protein colocalized with the ER marker protein disulfide isomerase. Prx4 signals appeared in Golgi-like and vesicular structures in agreement with the Prx4 pattern after fractionized centrifugation. Secretion of Prx4 by transiently transfected HEK293 cells increased with time. In principle, lower Prx4 secretion by stably transfected MDCK1 cells was up to 11-fold enhanced by 0.1-0.4 mM hydrogen peroxide (H_2O_2) but not related to H_2O_2 cytotoxicity (MTT assay). No such H_2O_2 effect was seen in HEK293 or HepG2 cells. Up to 3-fold elevated Prx4 levels were observed in sera of patients with diabetes and myocardial infarction, diseases characterized by elevated ROS production, compared to healthy individuals.

Conclusion: The classical secretory pathway of Prx4 can be stimulated by H_2O_2 in a cell-specific manner. Elevated Prx4 in serum may represent a novel indicator of increased local and/or systemic oxidative stress in diabetes and cardiovascular diseases.

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