**Topic 7 – Oxydative stress – A**

**April 24th, Thursday 2014**

**0040**

The EPA: DHA 6:1-evoked endothelium-dependent NO-mediated relaxation in the coronary artery involves a copper-dependent pro-oxidant response triggering the PI3-kinase/Akt-mediated activation of eNOS

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Omega-3 fatty acid products containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to reduce the risk of cardiovascular disease, in part, by stimulating the endothelial formation of nitric oxide (NO), a potent vasoprotective factor. This study determined the mechanism leading to endothelial NO synthase (eNOS) activation in response to the highly active EPA:DHA 6:1 product. Vascular reactivity was assessed using porcine coronary artery rings suspended in organ chambers, the level of oxidative stress in coronary artery sections using the redox-sensitive probe, dihydroethidine, and the phosphorylation level of target proteins in cultured coronary artery endothelial cells by Western blot analysis. EPA:DHA 6:1 caused pronounced endothelium-dependent relaxations in porcine coronary artery rings. Relaxations to EPA:DHA 6:1 were slightly but significantly reduced by an eNOS inhibitor, not affected by inhibition of endothelium-dependent hyperpolarization and abolished by both treatments. Relaxations to EPA:DHA 6:1 were reduced by inhibitors of oxidative stress (MnTMPyP, PEG-catalase), an inhibitor of either Src kinase (PP2) or PI3-kinase (wortmannin), and intracellular copper chelating agents (neocuproine, tetrathiomolybdate) and were insensitive to cyclooxygenase inhibition (indomethacin), chelating agents for iron (desferroxamine), zinc (histidine), extracellular copper (bathocuproine). EPA:DHA 6:1 induced phosphorylation of Src, Akt and eNOS at Ser 1177; these effects were inhibited by MnTMPyP and PEG-catalase. EPA:DHA 6:1 induced the endothelial formation of ROS in coronary artery sections, this effect was inhibited by MnTMPyP, PEG-catalase, and intracellular copper chelating agents. EPA:DHA 6:1 causes endothelium-dependent NO-mediated relaxations in coronary artery rings, and this effect involves an intracellular copper-dependent event triggering the redox-sensitive PI3-kinase/Akt pathway to activate eNOS by phosphorylation at Ser 1177.

**0209**

Monoamine oxidases as novel sources of reactive oxygen species in experimental diabetes

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The classic enzymatic systems that contribute to the generation of reactive oxygen species (ROS) responsible for the development of endothelial dysfunction and neuropathy in diabetes are mitochondrial respiratory chain, NADPH oxidases, xanthine oxidase, and uncoupled endothelial NO synthase (eNOS). Monoamine oxidases (MAOs) are flavo-enzymes with two isoforms, A and B, located at the outer mitochondrial membrane that constantly generate hydrogen peroxide (H₂O₂) as by-product of their catalytic cycle. We have recently shown that MAO-A and B are both expressed in the murine aorta, induced by in vivo angiotensin II and lipopolisacharide treatment and contribute via H₂O₂ production to endothelial dysfunction. Whether MAO-dependent ROS production contributes to the development of endothelial dysfunction in diabetes was determined here in diseased vessels obtained from Zucker obese diabetic rats. To this aim, aortic segments with intact endothelium and denuded endothelium (using CHAPS solution) were studied in organ-bath system. The contractile response was assessed using phenylephrine (10-8-10-6M) in the presence vs. the absence of a MAO A inhibitor, clorgyline (10 microM). H₂O₂ production was measured by a spectrophotometric method (Feric Oxydation Xylenol Orange assay). Our data showed that MAO inhibition reduced the diabetic-induced aortic ROS formation by 30% and partially normalized the contractility of diseased vascular segments (diabetes, 41.34±2.96%, diabetes+clorgyline= 26±3.86% vs. control, 21.5±3.45%). In conclusion, MAO-derived ROS contribute to endothelial dysfunction in the diabetic rat. Further experiments are required to assess the mechanisms underlying MAO-dependent H₂O₂ formation.

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