UNRAVELING THE CAUSATIVE ROLE OF INOS-MEDIATED FREE RADICAL PRODUCTION IN THE PATHOGENESIS OF DIABETES.

Krisztian Stadler, Marcelo G Bonini, Shannon Dallas, JinJie Jiang, Ronald P Mason, Maria B Kadiiska

Free radical production has been implicated as a factor contributing for the pathogenesis of *diabetes mellitus*. However, several questions remain such as the mechanism through which oxidative stress is triggered and what are the oxidants involved. Here, electron paramagnetic resonance (EPR) associated with *in vivo* spin-trapping techniques has been used to investigate free radical formation in streptozotocin-induced diabetic rats. After one month of diabetes, six-line EPR spectra were detected in the diabetic rat bile which were consistent with the trapping of carbon-centered, lipid-derived radicals. Using ^{13}C – labeled DMSO the role of the hydroxyl radical was confirmed in the initiation and progression of lipid peroxidation. To study the possible mechanism of the $^{\bullet}OH$ radical formation several known enzyme inhibitors were used with marginal effects on endogenous free radical production. Interestingly, aminoguanidine (general NOS inhibitor) and 1400W (iNOS inhibitor) largely reduced the formation of free radical adducts in diabetic animals indicating the fundamental role of iNOS in the mediation of radical production and lipid peroxidation. Furthermore this result suggested $^{\bullet}OH$ production independent of transition metal ions by iNOS implicating peroxynitrite production. Through confocal microscopy and Western blots iNOS expression was confirmed in the liver of diabetic animals and increased over the course of the disease in parallel to free radical production. Our work demonstrates the causative role of iNOS in the pathogenesis of diabetes and gives a pioneer example of peroxynitrite production in a chronic metabolic disorder.