In vitro and in vivo analysis of insulin-induced oxidative stress and DNA damage

Hyperinsulinemia is thought to enhance cancer risk in diabetic patients . In the previous studies, we showed for the first time in vitro and in vivo that insulin in high level as in case of diabetes mellitus can induce oxidative stress resulting in genomic damage in different tissues assuming that if the same mechanisms are active in patients, hyperinsulinemia might cause genomic damage through the induction of ROS contributing to the increased cancer risk, against which the use of antioxidants as well as mitochondrial and NADPH oxidase inhibitors might exert protective effects with cancer preventive potential under certain conditions. Furthermore, we investigated the possible signaling mechanism which can lead to insulin genotoxicity and increase cancer risk in diabetic patients especially in kidney and colon. In addition, DNA damage markers in the lymphocytes of diabetic patients were investigated in comparison to healthy individuals.

Recently, the effect of a combination of metformin with insulin was investigated in vitro and in vivo. The rational for this were reported antioxidative properties of metformin and the aim to gain further insights into mechanisms responsible for protecting the genome from insulin mediated oxidative stress and damage. Comet assay, micronucleus frequency test and a mammalian gene mutation assay were used to evaluate the DNA damage produced by insulin alone or in combination with metformin. For analysis of antioxidant activity, oxidative stress and mitochondrial disturbances, the cell-free FRAP assay, the superoxide-sensitive dye dihydroethidium and the mitochondrial membrane potential-sensitive dye JC-1 were applied. Accumulation of p53 and pAKT were analysed. As an in vivo model, hyperinsulinemic Zucker Diabetic Fatty rats, additionally exposed to insulin during a hyperinsulinemic euglycemic clamp, were treated with metformin. In the rat kidney samples, DHE staining, p53 and pAKT analysis, and quantification of the oxidized DNA base 8-oxodG was performed. Metformin did not show intrinsic antioxidant activity in the cell free assay, but protected cultured cells from insulin mediated oxidative stress, DNA damage and mutation. Treatment of the rats with metformin protected their kidneys from oxidative stress and genomic damage induced by hyperinsulinemia. Metformin may protect patients from genomic damage induced by elevated insulin levels. This may support efforts to reduce the elevated cancer risk that is associated with hyperinsulinemia.