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TARGETING NOX2 NADPH OXIDASE IN INSULIN RESISTANCE RELATED ENDOTHELIAL DYSFUNCTION

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Laboratory based cell culture studies have shown that reactive oxygen species (ROS) induced oxidative stress as an important molecular mechanism in the development and progression of cardiovascular diseases. Despite that, large scale clinical trials have shown that general antioxidants are not only ineffective in treating

cardiovascular diseases but also, in some cases, can have harmful effects. In this context, the next logical step is to specifically targeting critical molecules in ROS generation rather than using non-specific antioxidants. Insulin resistance, the antecedent of type 2 diabetes mellitus, can itself initiate various vascular complications of diabetes before the development of clinically diagnosable diabetes. Insulin resistance is characterised by excessive endothelial cell generation of cytotoxic concentrations of ROS. NADPH oxidases (NOX), a group of ROS producing enzymes, have been recently shown as the major source of excessive ROS in insulin resistant endothelial cells. We hypothesised that specific targeting of NOX could be the way forward in developing antioxidant molecules as therapeutic agents for vascular diseases. In this study, we examined the role of NOX enzymes and specifically Nox2 isoform in superoxide generation in 2 complementary *in vivo* models (endothelial specific and whole body) of human insulin resistance. Using gp91-ds tat peptide, a non-specific NOX inhibitor, we have specifically targeted NOX generated superoxide production. For acute inhibition we have treated cells or tissues with 50 μM gp91ds-tat or control peptide for 30 minutes. For chronic inhibition, mice were implanted with osmotic mini-pumps which delivered 10 mg/kg/day of drug for 28 days.

The excessive level of superoxide found in insulin resistant endothelial cells was significantly reduced by acute or chronic treatment with gp91ds-tat peptide. Also NO dependent vasorelaxation response, which was impaired in insulin resistance, was improved with gp91ds-tat peptide treatment. Since insulin resistant endothelial cells showed higher expression of Nox2 isoform, Nox2 gene was knocked down using siRNA which significantly reduced superoxide levels. Furthermore, to clearly examine the involvement of Nox2 isoform in this context, double transgenic mice with endothelial specific insulin resistance and Nox2 gene knockout were generated. These mice showed reduced superoxide production and improved vascular function. Thus Nox2 is the critical molecule in insulin resistance induced endothelial dysfunction. In conclusion, our study establishes that pharmacological inhibition of NOX as a novel way to treat insulin resistance related vascular disease.