PHARMACHOLOGIC INHIBITION OF NOX4 PROVIDES RENOPROTECTION IN CONTRAST INDUCED NEPHROPATHY

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Contrast induced nephropathy may occur, in part, as a result of intrarenal oxidative stress. NADPH oxidases are important sources of reactive oxygen species (ROS). Among various type of NADPH oxidases, Nox4 is expressed predominantly in rodent kidney. The aim of the present study was to assess the effect of Nox4 inhibition on the prevention of contrast induced nephropathy (CIN). Using HK-2 cells, Nox4 mRNA and protein were determined after exposure to iohexol with/without pretreatment of the most specific Nox1/4 inhibitor, GKT137831. Caspase3/7 activity, DHE stain and amplex red activity were also measured. Proinfammatroy and apoptotc markers (pNFkB/NFkB, pp38/p38, pJNK/JNK, pERK/ERK and pcleaved caspase/caspase) were measured for investigation of intracellular pathway associated with Nox4. In addition, the effect of Nox4 inhibition were evaluated in mice model of CIN. The expression of Nox4 in HK-2 cells significantly increased by Iohexol exposure. Pretreatment of GKT137831 resulted in reduced production of ROS, downregulation of proinflammatory marker (p38), that are implicated in contrast induced nephropathy, reduced caspase 3/7 activity and increased cellular survival in Iohexol exposed HK-2 cells. Silencing of the Nox4 gene replicated theses effects by downregulation of proinflammatory markers. In contrast induced nephropathy mice model, pretreatment with GKT137831 resulted in an attenuated vacuolar degeneration, tubular epithelial cell shedding, cellular cast formation and tubular dilatation. Collectively, these results identify Nox4 as a key source of ROS responsible for kidney injury in contrast induced nephropathy and provide proof of principle for an innovative small molecule approach to prevent contrast induced nephropathy.

The role of local IL6/JAK2/STAT3 signaling in high glucose-induced podocyte hypertrophy

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Background: IL6 is an important regulator of cellular hypertrophy through gp130/JAK2/STAT3. We tested the hypothesis that IL6 and its downstream gp130/JAK2/STAT3 signaling pathway were participated in high glucose (HG)-induced podocyte hypertrophy. **Methods:** Levels of IL6 in media and lysates of podocytes were measured by ELISA. WB were performed to determine protein expression levels of gp130/JAK2/STAT3 among podocytes cultured with normal glucose (NG), NG + mannitol (MN), NG + recombinant IL6 (rIL6), HG, and HG + IL6 neutralizing antibody (IL6NAb). Immunoprecipitation assay (IP) were examined to determine whether gp130 interact with JAK2 in response to HG or IL6. Podocyte hypertrophy was verified using protein/cell counts and flow cytometry. Results IL6 levels were significantly increased in the media and lysates of podocytes cultured in HG compared with the NG groups. Nuclear phospho-STAT3/STAT3 ratio was increased by HG and NG + IL6 treatment and attenuated by addition of IL6NAb to HG media, indicating that nuclear STAT3 activated following JAK2 and cytosolic STAT3 in response to IL6 secreted by HG-stimulated podocytes. IP showed increased phospho-JAK2 recruitment to gp130 in the HG and NG + IL6 groups, and IL6NAb in the HG group significantly abrogated these increases. Podocyte hypertrophy was significantly increased in HG and NG + IL6 conditions compared with NG. This effect was diminished by the addition of IL6NAb to the HG group. **Conclusions:** IL6 might play a prominent role in local activation of JAK2/STAT3 in podocyte hypertrophy under HG conditions.