POSTER SESSION 3

Session held on 6 July 2014
doi:10.1093/cvr/cvu098

P773
Uric acid regulates multiple interacting major cellular pathways in human aortic endothelial cells: a global proteome approach
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Background: Uric acid (UA) is the end product of the purine metabolism and hyperuricemia has been identified as one major risk factor for cardiovascular diseases. Lowering of serum UA levels seems to improve endothelial dysfunction. Though UA is a potent anti-oxidant it can also induce oxidative stress after entering the endothelial cells via urate transporters. There is growing evidence for multiple UA action on various other cellular pathways contributing to the adverse effects of high UA serum concentrations.

Materials and Methods: In this study we sought to elucidate concentration-dependent effects of UA (100 μM, 300 μM, 500 μM) on human aortic endothelial cells. We used NanoLC-MS/MS to analyse protein lysates of HAEC after UA stimulation and ingenuity pathway analysis (IPA) to reveal putative cellular pathways regulated by UA. For validation purposes we measured the abundance or activity of key proteins prototypic for the identified pathway using ELISA, Western blotting, immunohistochemistry and quantified NO production by DAF confocal lasermicroscopy.

Results: Our global proteome shot-gun approach and IPA identified ubiquitin-proteasome system (UPS) and eIF4 signalling as the major pathways regulated by UA. Further k-means clustering analysis revealed 11 additional pathways, of which we further validated: NOS signalling, superoxide signalling and hypoxia as the most interesting pathways in respect to endothelial function. We found a complex regulatory network between those pathways demonstrating that 500 μM UA, which is well above the concentration regarded as pathological in clinical settings, leads to diminishing of NO bioavailability. Abatement of NO was accompanied by an increase of eNOS activity but up-regulation of oxidative stress and increase of nitrotyrosinylated proteins (NTP). These finding speak in favour for a drainage of the NO pool to NTP. In addition we found down-regulation of eIF4 and up-regulation of UPS demonstrating the impact of high UA concentrations on HAEC.

Conclusions: In summary our data indicate that the control of UA levels is of utmost importance for the cellular homeostasis of endothelial cells. Therefore, consequent hyperuricemia therapy is necessary to counteract cardiovascular strain by elevated sUA levels.