Complement System and MAPK Signaling in Calcineurin-Inhibitor induced Nephrotoxicity

Beatrix Loeschenberger[1], Iris E. Eder[2], Martin Puhr[2], Michael Rudnicki[1], **Hannes Neuwirt**[1]

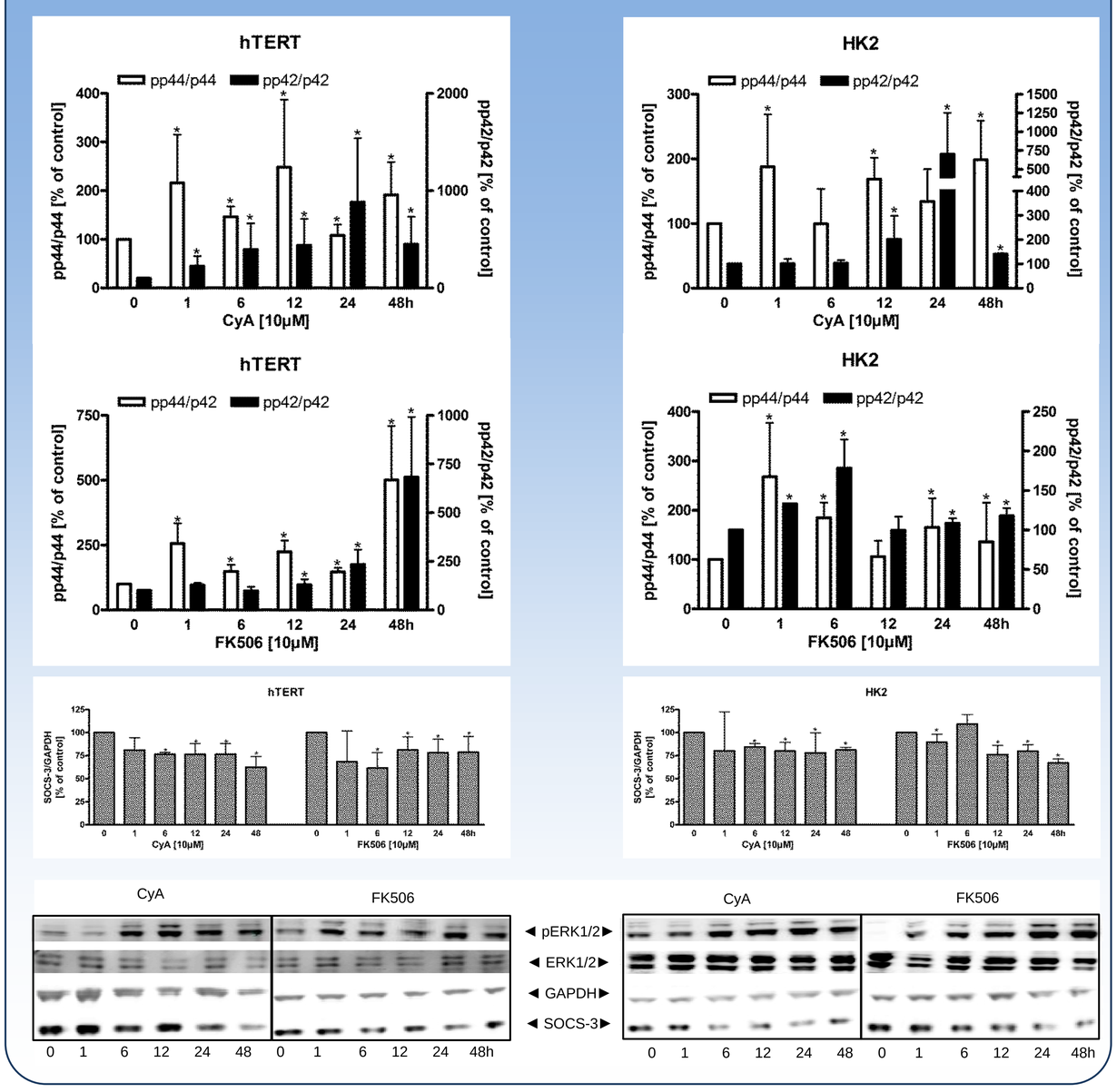
[1]Dept. of Internal Medicine IV - Nephrology and Hypertension, Medical University of Innsbruck, Tyrol, Austria
[2]Dept. of Experimental Urology, Medical University of Innsbruck, Tyrol, Austria.

Background: The gap between organ demand and supply to be used for transplantation is wide and getting wider. One way to improve the situation is to prolong allograft survival. One entity that significantly contributes to renal allograft loss is calcineurin inhibitor (CNI) nephrotoxicity (CIN). Various mechanisms are discussed to play a role in CIN pathogenesis; one of which is complement mediated injury.

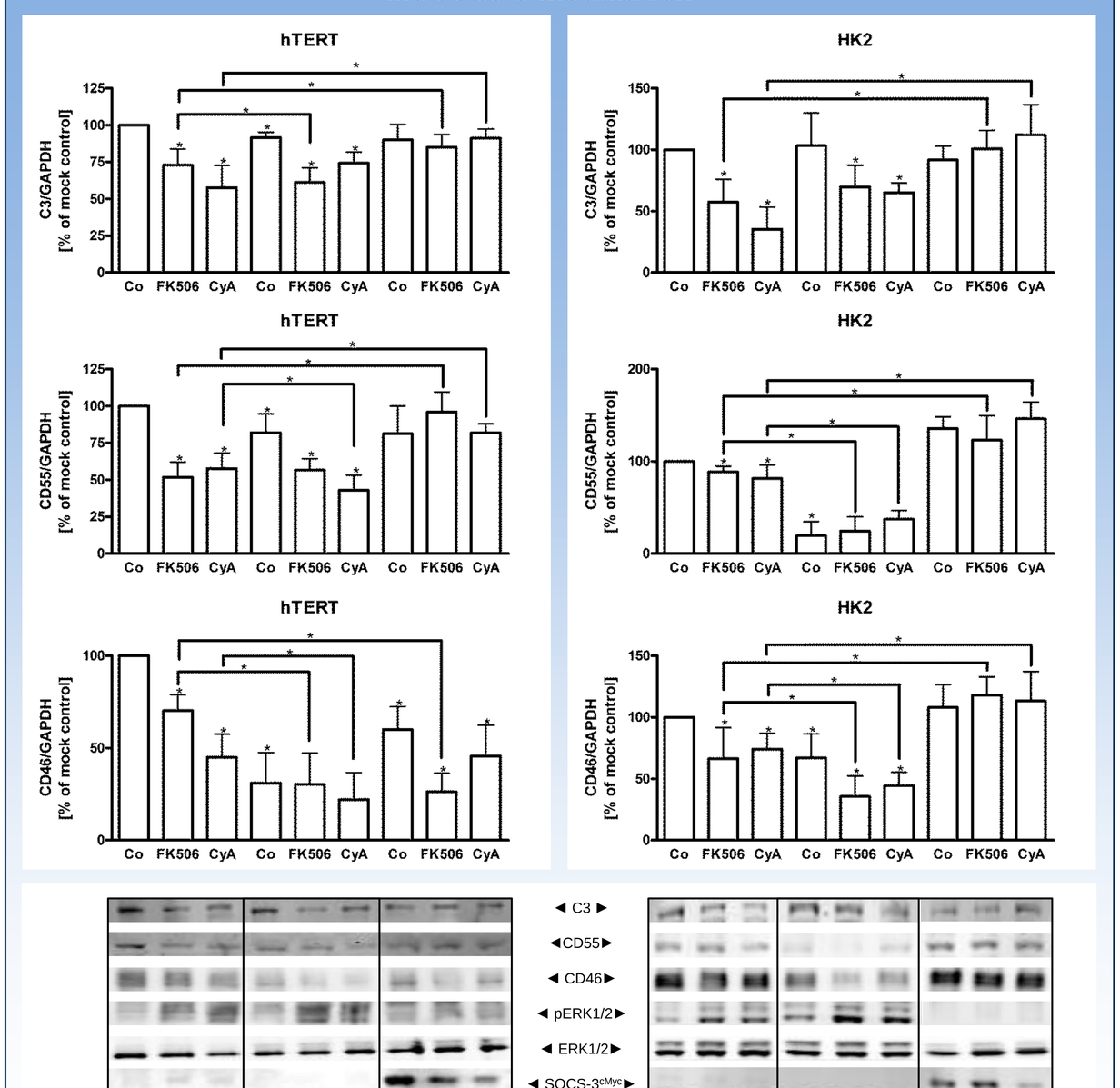
Purpose: To investigate the impact of CNIs on MAPK signaling, complement regulators and complement activation.

Methods / Results: We have performed experiments utilizing two proximal tubule cell lines of human origin, HK2 and hTERT-RPTCs. 10µM cyclosporinA (CyA) and tacrolimus (FK506) treatment induced activation/phosphorylation of MAPK1/-2 in both cell lines.
This was associated with a significant decrease in protein levels of suppressor of cytokine signaling (SOCS)-3, which we have shown recently to act as a negative regulator of MAPK1/-2 signaling. Hence, one might hypothesize that SOCS-3 downregulation enhances MAPK activation.
In order to investigate the regulation of complement regulatory proteins DAF (CD55) and MCP (CD46), which inhibit or accelerate disassembly of C3 convertase and inhibit C3b and C4b, we assessed protein expression levels by Western Blotting and found that CNI treatment caused a down regulation of DAF and MCP. Next we overexpressed and knocked down SOCS-3 via transient transfection with an overexpression plasmid (pBIG2i) and specific siRNA. Under these SOCS-3 modulations we found that MAPK-activation and DAF/MCP regulation is strongly influenced by SOCS-3 expression levels. Thus, one might speculate that a part of CIN may be attributed to dysregulated complement system activation by the SOCS-3-MAPK1/-2 signaling complex, which induces down regulation of complement regulators DAF/MCP.
Finally, we measured cellular proliferation by ^{3}H-thymidine incorporation in cells treated with CsA and FK506 in heat-inactivated normal human serum (HIS) and compared it with the effects in normal human serum (NHS). Interestingly, cellular growth with HIS was approx. 20% better than with NHS. Thus, reduced cell growth could be attributed to enhanced complement system induced cell death and therefore may be of relevance for development of CIN.

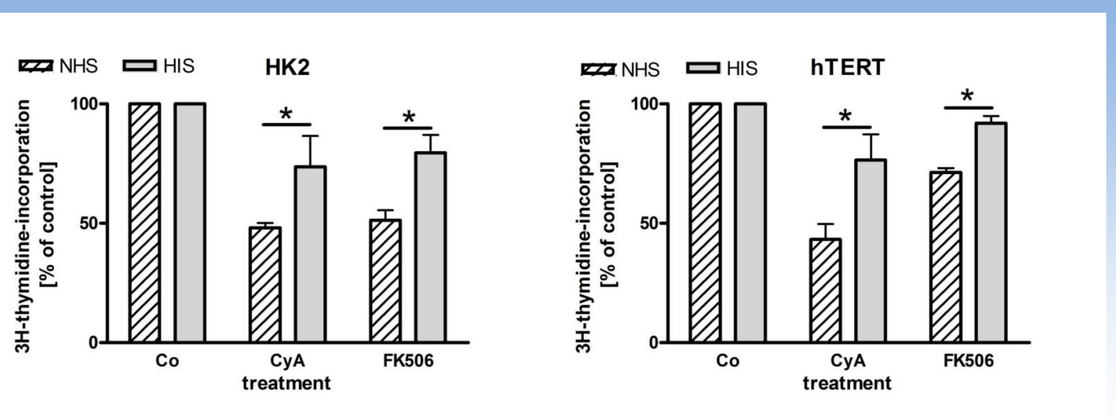
Western blotting of (p)ERK1/2, SOCS3 after CNI treatment



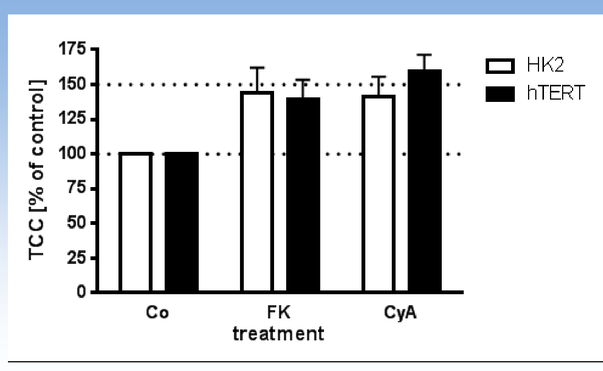
Western blotting of DAF and MCP after CNI treatment and SOCS-3 modulation



Proliferation in normal and heat inactivated human serum after CNI treatment



TCC levels after CNI treatment



Conclusion: Based on our data, we hypothesize that MAPK-activation by CNI, which enhanced by CNI-induced SOCS-3 downregulation, yields downregulation of DAF and MCP and finally complement activation on human proximal tubule cells. This might be one additional pathomechanism of CIN development.

The authors certify that no actual or potential conflict of interest in relation to this article exists.

53 ERA 634--SP

Renal transplantation. Experimental, immune-tolerance of allogenic and xenogenic transplants.

Hannes Neuwirt

DOI: 10.3252/pso.eu.53era.2016

ePosters supported by F. Hoffmann- La Roche Ltd.