Glucose-regulated protein 78 (GRP78) overexpression inhibits doxorubicin cardiotoxicity

H. Tscheschner[1], J. Reinkober[1], P. Most[1], H.A. Katus[1], M. Mueller[2], O.J. Mueller[1], P.W. Raake[1]. *[1]University Hospital of Heidelberg, Internal Medicine III, Dept Cardiology, Angiology & Pneumology, Heidelberg, Germany; [2]German Cancer Research Center, Heidelberg, Germany*

Purpose: Cancer treatment with the anthracycline doxorubicin is accompanied by severe cardiotoxicity. However, any causal treatment is prevented by the underlying mechanisms not being fully understood so far. We focused on the endoplasmic reticulum (ER) stress sensor Glucose-related Protein 78 (GRP78) which binds unfolded proteins and represses adverse ER stress as it was previously discovered to be associated with evasion of tumor cells from anthracycline treatment.

Additionally, membrane bound GRP78 was recently found to be a mediator for pro- and anti-apoptotic pathways. With the investigation of the role of GRP78 in doxorubicin cardiomyopathy we hope to derive a potential therapeutic approach.

Methods: Isolated neonatal rat ventricular cardiac myocytes were treated with 0,5 or 1μM doxorubicin. In vitro GRP78 knockdown was established by 5nM siRNA while GRP78 overexpression was achieved by transfection with a recombinant

adeno-associated virus serotype 6 (AAV6-CMV-MLC-GRP78). Samples were analyzed by western blotting. Unspecific cell death was measured via ToxiLight Assay from the cell culture supernatant and apoptosis was determined by TUNEL assay and Caspase-3 cleavage.

Results: Increasing doxorubicin concentrations in vitro are associated with significant GRP78 downregulation. Unspecific cell death, caspase-3 cleavage and the number of TUNEL positive cells as a marker for apoptotic cell death were significantly increased. While siRNA mediated GRP78 knockdown with doxorubicin treatment led to a significant disproportional increase in cell death, AAV6-mediated GRP78 overexpression in the same setting inhibited cell death and apoptosis. This indicates a contribution of GRP78 downregulation to doxorubicin cardiotoxicity making it an interesting target for gene therapeutic prevention of Doxorubicin cardiotoxicity.

Summary: Doxorubicin treatment leads to downregulation of GRP78 contributing to cardiac myocyte death and cardiac dysfunction. AAV-mediated overexpression of GRP78 could decrease cell death and apoptosis in vitro promising a beneficial outcome for a gentherapeutic approach with GRP78.