Influence of UPR-pathway on stellate cell activation

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ABSTRACT
Hepatic stellate cells (HSC) are quiescent cells situated in the sub-endothelial space of the liver. HSC are activated due to chronic liver injury mostly caused by alcohol abuse, chronic HCV infection or hereditary disease. Upon activation of HSC, a high production of ECM proteins. Recent studies suggested that one way of activating HSC is over the unfolded protein response (UPR) of the endoplasmatic reticulum (ER). The UPR is the endoplasmatic reticulum (ER) and directs the folding of the newly synthesised proteins and the quality control of their function. The UPR is activated upon ER stress and aims to restore the normal function of the ER. The UPR activates upon ER stress and aims to restore homeostasis in the ER. Two known ER stress inducers, Brefeldin A (BFA) and Tunicamycin (TNC), were used to induce UPR in HERT HSC. The activation of UPR and subsequent HSC activation were analysed with qRT-PCR, TGF-β served as positive control for HSC activation. Both BFA and TNC activated UPR. HSC activation has been shown through αSMA staining upon TNC, BFA and TGF-β treatment.

INTRODUCTION
Liver fibrosis and subsequent liver cirrhosis is the common pathway for many chronic liver diseases and can lead to liver failure and portal hypertension, often requiring liver transplantation. The cause of liver fibrosis is a chronic injury to the liver, conjugated with an excess accumulation of extracellular matrix. The major source of these ECM proteins are activated hepatic stellate cells (HSC). HSC are situated in the sub-endothelial space of the liver and normally in a quiescent state. Upon activation, HSC start a high production of ECM proteins, especially collagen type I as shown in Fig. 1. Recent studies suggested that one way of activating HSC is over the unfolded protein response (UPR) of the endoplasmatic reticulum (ER). The UPR activates upon ER stress and aims to restore homeostasis of the ER. The aim of this study was to analyse HSC activation upon UPR activation. ER stress inducers Brefeldin A (BFA) and Tunicamycin (TNC) were used to induce UPR activation and subsequent HSC activation. TGF-β is a well-known factor to induce HSC activation and was thus used as a positive control. To analyse the influence of TGF-β upon UPR activation and subsequent HSC activation, the experiments were also run with a TGF-β inhibitor SB-525334.

RESULTS
To evaluate the activation of UPR, the expression of UPR markers (spliced and total) XBP1, EDEM1, BiP, CHOP were analysed with qRT-PCR (Fig. 2 and 3). All UPR markers showed a time-dependent up-regulation and hence indicating successful UPR activation. For all tested markers, both TNC and BFA had the highest values after 24h. Furthermore, TNC and BFA showed higher values after 24h than TGF-β for all tested UPR markers. The values of the different markers followed the downstream cascade of the UPR pathway, as the highest values were with the most upstream markers and vice versa. The TGF-β inhibitor generated slightly higher values than samples without the inhibitor, although the differences are only significant for spliced XBP1 with TNC, BFA and TGF-β and for EDEM1 with TNC.

CONCLUSION
The UPR activation with TNC and BFA worked well as that the UPR markers showed a time-dependent up-regulation upon treatment with TNC and BFA. For all tested UPR markers, the values of both TNC and BFA peaked after 24 h. The range of the attained values of the different UPR markers were in accordance to the downstream cascade of the UPR pathway. HSC activation upon TNC and BFA was determined in a significant increase of αSMA protein content upon treatment with TGF-β as well as TNC and BFA, showing that all three substances can lead to HSC activation. The qRT-PCR data indicate, that HSC activation upon UPR and UPR itself are TGF-β independent, but that on contrary, upon treatment with TNC the inhibition of TGF-β leads to an intensified activation.

REFERENCES