Ruprecht-Karls-Universität Heidelberg
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Dissertations-Kurzfassung

The influence of antihypertensives on ethanol-induced cellular damage in mouse hepatocytes

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Alcohol-associated liver damage is continuously increasing in developed countries and is a leading cause of death worldwide. Chronic alcohol uptake induces synthesis and activation of TGF-β.

Many patients with alcohol abuse also suffer from high blood pressure and rely on antihypertensive treatment. Aim of this work was to investigate the influence of widely used antihypertensives Amlodipine, Captopril, Furosemide, Metoprolol, Propranolol and Spironolactone on damage stress of cultured mouse hepatocytes as a cellular representative of chronic liver disease progression.

We determined hepatocyte damage by LDH release and measured oxidative stress by reactive oxygen species (ROS) production and glutathione (GSH) reduction. Neutral lipids were visualized by Oil Red O staining. TGF-β signaling was analyzed by PCR, immunoblot and an adenovirus infection based Smad3/4 responsive luciferase reporter assay.
To investigate the role of heme oxygenase-1 (HO-1), we compared LDH release in cells treated with hemin as an inducer of HO-1 or zinc protoporphyrine (ZnPP9) as a blocker of HO-1.

Ethanol, as well as TGF-β, rapidly induces oxidative stress in primary hepatocytes cultures. Along with an increase in ROS and a reduction of cellular GSH, about 35% of the total LDH is released into the culture supernatant after 72 h. The antihypertensives Amlodipine, Captopril, Furosemide, Metoprolol, Propranolol and Spironolactone reduced oxidative stress and LDH release in a dose dependent manner.
Furthermore, ethanol-induced neutral lipid accumulation in hepatocytes was strongly decreased by the pharmaceuticals investigated.
These antihypertensives also seem to induce expression of heme HO-1, which buffers oxidative stress. Consequently, blocking HO-1 activity by ZnPP9 reduced the protective effect of the antihypertensives on ethanol/TGF-β mediated cellular damage.
Since patients suffering from alcoholic liver disease show increased TGF-β levels and Smad3 is a prominent profibrogenic transcription factor, we performed Smad3/4 responsive luciferase reporter assays and could see both increases and decreases, depending on the substance used. All substances altered TGF-β signaling, as shown by immunoblot and the Smad3/4 reporter assay.
Interestingly, whereas all other substances seemed to have a more ore less beneficial effect (even if non significant), Furosemide sometimes even enhanced profibrotic signaling.

Our results suggest that medication with Amlodipine, Captopril, Furosemide, Metoprolol, Propranolol and Spironolactone may influence progression of fibrotic liver disease.
Further investigations on human hepatocytes and ultimately *in vivo* are needed to strengthen these results and delineate the respective mechanisms, so that in the future patients suffering from alcoholic liver diseases and high blood pressure could benefit from an adjusted antihypertensive therapy, which might also take into account its impact on liver disease.