Synergistic antifibrotic effect of Rapamycin and Zoledronic acid in advanced murine biliary fibrosis

Misbah Aslam[1,2], Muhammad Ashfaq-Khan[1], Muhammad Asif Qureshi[1,3], Marcel Senkowski1, Mirko Nick[2], Leonard Kaps[1], Yong Ook Kim[1], Detlef Schuppan*[1,4]

[1]Institute of Translational Immunology, University Medical Center, Mainz, Germany, [2]Shaheed Benazir Bhutto Women University, Peshawar, Pakistan, [3]Dow University Medical Sciences, Karachi, Pakistan, [4]Division of Gastroenterology, Beth Israel Deaconess Medical Centre, Harvard Medical School, Boston, US.

EASL European Association for the Study of the Liver

HARVARD MEDICAL SCHOOL

UNIVERSITÄTSmedizin. MAINZ

1 INTRODUCTION

Hepatocellular carcinoma (HCC) mostly develops in patients with advanced fibrosis; however, the mechanisms of interaction between a genotoxic insult and fibrogenesis are not well understood. Accordingly, this study used a well-known model of liver carcinogenesis induced by a single injection of a low-dose genotoxic agent diethylnitrosamine (DEN) into 6-day-old male Mdr2ko mice. Inflammatory processes are also involved in triggering the molecular and cellular events leading from liver injury to fibrosis and ultimately to HCC. Activation of macrophages and neutrophils, as well as the resultant release of pro-inflammatory cytokines and chemokines (IL-6, IL-8, IL-1β, TNF-α, CCL2), is thought to play an important role in the pathogenesis of human liver cancer.

2 AIM

- There is an urgent need for effective antifibrotic therapies for biliary fibrosis. The *in vivo* microenvironment, especially macrophages, can modulate hepatic stellate cell (HSC) activation
- We therefore assessed the antifibrotic efficacy of a combination of two clinically used drugs, Zoledronic acid (ZA) and Rapamycin (RAPA), that affect macrophage polarization and putative fibrogenic activation

3 METHOD

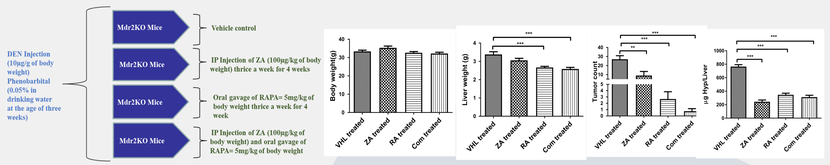
Mdr2-knockout (Mdr2KO) mice that had received one dose of diethylnitrosamine (DEN) at day 5 after birth were treated from month 5-6 (stage of advanced fibrosis with emerging HCC) with 1) ZA alone (100µg/kg body weight i.p., thrice weekly), 2) RA alone (5mg/kg body weight orally), 3) the combination of both drugs. A fourth group of Mdr2KO mice (without DEN and no signs of HCC) was treated with the combination.

The degree of fibrosis and extent of inflammation was quantified histologically using staining with H&E, Sirius Red, for αSMA and CD68. Collagen deposition was confirmed by hydroxyproline determination.

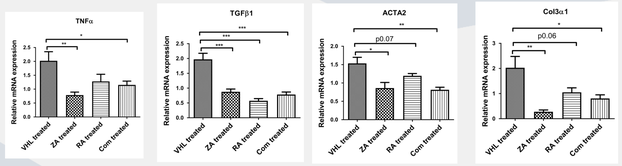
Real time PCR was performed to analyze the hepatic expression of fibrosis-related and macrophage-specific target genes produced by KCs (TNF-α, CCL2, TGFβ1, and several MMPs), liver SECs(VEGF) involved in the activation of HSCs thereby progression of liver fibrosis.

.

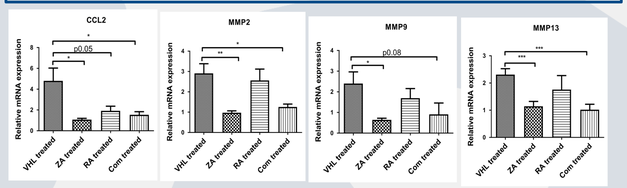
4 RESULTS



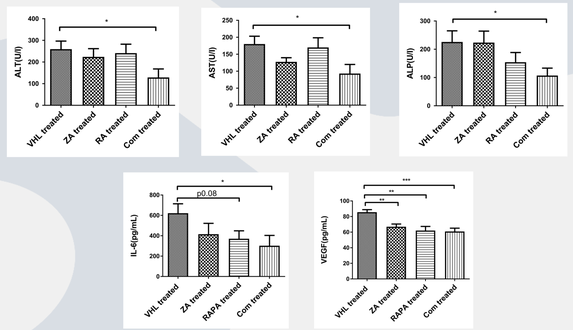
Target factors involved in induction and progression of Liver fibrosis

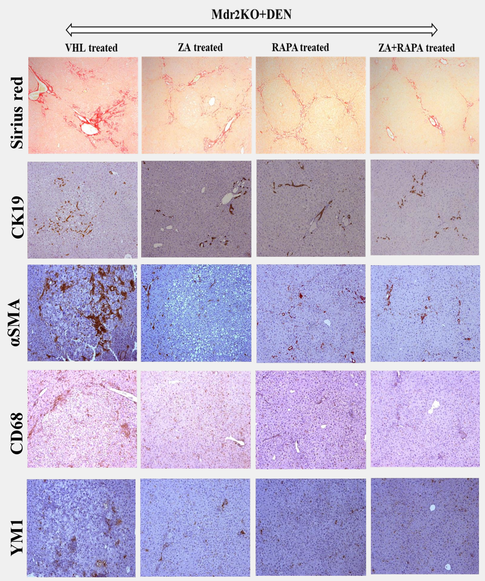


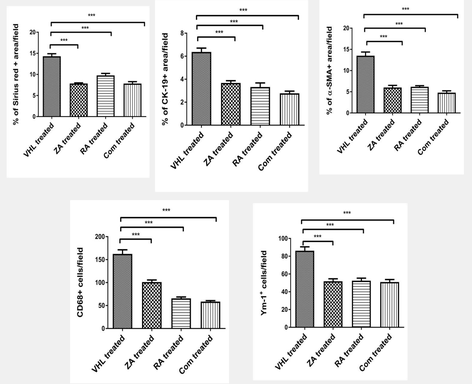
Markers of ECM remodeling and angiogenesis



Serum biochemical indicators of Liver fibrosis







Compared to vehicle treated controls and to mice treated with the single drugs alone, Mdr2KO mice that received the ZA/RA combination at peak fibrosis and for only one month demonstrated a twofold reduced collagen deposition, with elimination of bridging fibrosis ($p<0.0001$).

This was accompanied by a similar reduction of the cholangiocyte-specific marker CK19 and the HSC activation (αSMA) and a significant suppression of profibrogenic transcripts including Col3α1, αSMA, TNFα, and of markers of M2-type macrophages, including CCL2, TGFβ1, and MMP9.

Moreover, the number of YM1+(M2-type) relative to CD68+(total) macrophages was significantly reduced by combination treatment vs all other groups.

5 CONCLUSIONS

- The combination of RA and ZA, two agents with macrophage modulating activity, induces a remarkable regression of even advanced biliary fibrosis.
- This antifibrotic effect goes along with a marked antitumorous activity. Since both drugs are used in the clinic for other indications, with a reasonable safety profile, their clinical testing should be considered in patients with fibrotic and (pre) cancerous PSC and other unaddressed indications.

6 ACKNOWLEDGEMENTS

Supported by a DFG Collaborative Research Center Grant (SFB1066) to DS and a fellowship by Shaheed Benazir Bhutto Women University under Higher Education Commission, Pakistan to Misbah Aslam.

FZI Forschungs-Zentrum Immuntherapie

HEC

Enlightenment Through Knowledge

7 CONTACT

Detlef Schuppan MD, PhD (Detlef.Schuppan@unimedizin-mainz.de)

Misbah Aslam (misaslam@uni-mainz.de)