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To
Prof. Dr. Clara Balsano, PhD, and
Prof. Dr. Wan-Long Chuang, PhD,
Editors in chief
World Journal of Hepatology

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Postanschrift:
Marchioninistr. 15
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Munich, January 7th 2016

Dear Professor Balsano, dear Professor Chuang,

We herewith resubmit our revised manuscript entitled "Role of Interleukin-1 and its Antagonism of Hepatic Stellate Cell-Proliferation and Liver Fibrosis in the Abcb4$^{-/-}$-Model" (Manuscript NO.: 20571). We appreciate the comments and suggestions of the reviewers. They improved the quality of our manuscript.

We reply with a point-by-point response to the reviewers' comments.

Reviewer 00006459:

Comments:

Needed improvements:

1. Fig 2: Please also analyse this data to compare Anakinra treatment versus control: is the Anakinra treatment lowering proliferation compared to control? If so, is there a background level of IL1 signaling?

2. In the methods section please add a statement on whether ethics approval was obtained and the rules adhered to.

3. Please correct the spelling of hydroxyproline and phenotype in abstract and figure labels [fig 1]

Response:

1. According to the reviewer´s comments we analysed the data to compare Anakinra treatment vs. control. We found that Anakinra reduced HSC proliferation at all IL-1β concentrations used, reflecting Anakinra's anti-proliferative potency. The observation that anti-proliferative effects were also observed without

addition of IL-1β might be due to antagonistic effects of serum-derived IL-1β. Nevertheless, the anti-proliferative effect of Anakinra on HSC was confirmed. Figure 2 was revised accordingly.

2. All experiments of this study were approved by the local authorities. Furthermore all institutional and national guidelines for the care and use of laboratory animals were followed. We obtained all required approvals and followed the adhered rules (See Material and methods, Animals, pg. 9). There were no data or material from patients used in this study.

3. We revised the manuscript accordingly (abstract).

Reviewer 00160393:

Comments:

1. The conclusion that "IL-1-antagonsim shows antifibrotic effects in vitro…. is not correct because antifibrotic effects should be tested in vivo. In addition, the author should also observe the effect of Anakinra on HSC activation.

2. Another experimental animal model (such as bile duct ligation) should be used in order to confirm antifibrotic effect of Anakinra. Why the author only chose PSC model?

3. The paper is not well written.

Response:

1. We agree with the reviewer that the efficacy of an antifibrotic agent has to be verified *in vivo*. However discrepant efficacy of antifibrotic compounds has already been described elsewhere in *in vivo* models of liver fibrosis. For example 1,25-(OH) vitamin D3 showed profound antifibrotic effects *in vitro* [1,2] that were translatable to liver fibrosis induced by thioacetamide *in vivo* [1] but lacked beneficial effects on fibrosis in the Abcb4$^{-/-}$-model [2]. Therefore one might conclude that antifibrotic effects could be specific for certain models. We appreciate the reviewer´s recommendation to test the activation of stellate cells *in vitro* since this would give further insights in the antifibrotic efficacy of Anakinra. Unfortunately, these experiments would widely exceed the available time for the revision and are beyond the scope of this study. However, these experiments could be of interest for further projects.

2. In our initial analysis we identified differences in liver-fibrosis between female and male Abcb4$^{-/-}$-mice. Consistent with this finding we observed coherent alterations of interleukin 1β- mRNA-expression-levels. Due to these initial results we hypothesized that the interleukin 1β-pathway could causally be involved in liver-fibrosis in this preclinical model for primary sclerosing cholangitis (PSC). As to date there is no effective therapeutic option to halt disease progression of PSC, identification and translation of potential therapeutic targets is urgently needed and reflected our primary objective. On this background we focused on antifibrotic effects of Anakinra in PSC to halt disease progression to cirrhosis. As Anakinra showed promising effects *in vitro,* we concluded that an antagonism of the interleukin-1 pathway could have therapeutic potency and we investigated the effect of this agent *in vivo* in the Abcb4$^{-/-}$-mouse. However,

due to the missing effects on cholestatic fibrosis in our model *in vivo* we feel it ethically difficult to test this agent in another model of cholestatic fibrosis such as BDL.

3. The manuscript underwent thorough proof-reading and was adapted accordingly.

Reviewer 02861134:

Comment:

The method applied in the study to analyze the research topic is praise-worthy. There is a good consistency of analysis throughout the whole paper. I would like to recommend this paper to publish with no significant modification.

Reviewer 01810523:

Comment:

Activation of HSC is considered a major pathological event in development of liver fibrosis. In this study, Reiter et al. evaluated the role of HSC IL-1 pathway in the pathogenesis of liver fibrosis. The authors provided strong evidence demonstrating the pro-fibrogenic effect of IL-1 pathway, and more importantly the distinct anti-fibrogenic effect of Anakinra in vitro vs. in vivo using the Abcb4 deficient mouse model. These results suggest that multiple pathways need to be considered for therapeutic intervention of different types of liver fibrosis.

Response:

We appreciate the reviewer´s comment and have included this interpretation in our manuscript (See Discussion, pg. 17).

According to the reviewers' comments we revised the manuscript. Furthermore we modified the manuscript according to the ***Guidelines and Requirements for Manuscript Revision: Basic Study***. We revised the statistical analysis according to the recommendations of an external biostatistician. The changes that were made in the revised manuscript are cited in this response letter and/or are highlighted in the updated version of the manuscript (marked in red). We hope that our revised manuscript meets the high standards of the World Journal of Hepatology and warrants publication.

Sincerely,

PD Dr. Gerald Denk

Dr. Florian Reiter

References:

[1] Abramovitch S, et al. Vitamin D inhibits proliferation and profibrotic marker expression in hepatic stellate cells and decreases thioacetamide-induced liver fibrosis in rats. Gut 2011; 60(12):1728-37.

[2] Reiter FP, et al. 1,25-(OH)2-vitamin D3 prevents activation of hepatic stellate cells in vitro and ameliorates inflammatory liver damage but not fibrosis in the Abcb4-/- model. Biochem Biophys Res Commun 2015; 459(2):227-33.