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Reviewer ´s evaluation

of the doctoral thesis of Fatemeh Alaei Faradonbeh MSc „Dynamics of changes in bile acid metabolomics in estrogen-induced cholestasis".

Fatemeh Alaei Faradonbeh MSc. uses a rodent model of estrogen- or iron- induced liver injury in her doctoral thesis „Dynamics of changes in bile acid metabolomics in estrogen-induced cholestasis“ to evaluate the changes in bile acid metabolism under those pathological conditions.

The thesis is written in an English language in the form of annotated set of three original papers published in peer-reviewed international journals with impact factors and the candidate is the first author of two of them.

The theoretical introduction is relevant to the problematics studied, it fully covers the topic of bile acid chemistry, metabolism and transport and is well documented by suitable schemes and figures. However, the sources cited are not always relevant (He et al. 2013 is probably wrong), the author cites often reviews without the effort to find the original source (di Gregorio et al., 2021; Choudhuri and Klaasen, 2022) and sometimes, the citation is missing in place where it should be (for exmple page 1- bottom half is completely without citations). Also, I miss the legend to Table 1 and the source where the data about BA hydrophilic-hydrophobic index came from).

The candidate set three main goals. 1. To study the role of metformin in bile acid homeostasis, 2. to identify Mrp2 transporter role in metabolomics of bile acids, both in the model of ethinylestradiol-induced cholestasis, and finally 3. to characterize bile acid metabolism during iron overload.

The first paper shows that metformin in the intact mouse liver accelerates enterohepatic recycling of bile acids while in cholestatic livers it further impairs the liver transporters of bile acids increasing the serum concentrations of bile acids. This finding has a direct clinical impact as it might help with decision of diabetes treatment in patiens with cholestasis. In the second paper, the authors discovered that Mrp2 transporter has a significant effect on bile acid metabolism and should be considered a risk factor in development of intrahepatic cholestasis of pregnancy. .The third paper describes another novel finding, the role of iron overload on bile acid metabolism, which can have clinical implication in the treatment of iron-induced hepatotoxicity.

The data obtained are thoroughly discussed in the Discussion part of the thesis, compared with the recent relevant literature and reflects the deep insight of the author to the studied topic. The results are clearly and understandably concluded in the Conclusions part of the thesis. Methodologicaly, the study is extensive and

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diverse, the candidate proved the knowledge of number of advanced biochemical, molecular-biological, and analytical methods.

In the substantive part of the work, I miss a discussion of the role of kidney transporters and urine excretion of bile acids as the excretion of conjugated bile acids via kidney is indespensable part of complex bile acid metabolism during cholestasis.

I have following questions and suggestions for discussion:

1. Could metformin treatment and Mrp2 defficiency have an impact on the expression of the renal transporters and bile acids excretion to the urine? Could you speculate on the importance of this excretory pathway in your experimental model?
2. How is ethinylestradiol metabolized in mice and do you think that Mrp2 animals might have increased estrogen concentations in plasma?
3. What was the cause of the increase in plasma bilirubin concentrations (conjugated or unconjugated?) in your iron overload rat model?

In conclusion, I state that the author met all the set goals, mastered number of basic as well as advanced laboratory methods, proved that she was able to present and publish results of her scientific work at an excellent professional level. Doctoral thesis is written clearly and carefully and presents number of original data which contributed significantly to the knowledge of bile acids metabolism under various pathological conditions and enabled to better focus the therapy in estrogen-induced cholestasis and irond overload liver injury. The author demonstrated prerequisities for independent creative scientific work and I recommend the work for defense.

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