Evaluation review of dissertation thesis

Charles University, 1st Faculty of Medicine

Workplace: Institute of Pharmacology

Name of student: Mgr. Mahak Arora

Study program: Doctoral study programmes in biomedicine

Field of study: Pharmacology and toxicology

Title of dissertation: Study of pharmacodynamics and pharmacokinetics of potential therapeutic substances in an experimental model of non-alcoholic fatty liver disease and steatohepatitis (NAFLD/NASH)

Supervisor: MUDr. Nikolina Kutinová Canová, Ph.D.

Reviewer: doc. MUDr. Otto Kučera, Ph.D.

Workplace of reviewer: Department of Physiology, Faculty of Medicine in Hradec Králové, Charles University

The dissertation thesis is logically structured and well presented. The work is divided into chapters according to the requirements for this type of scientific work and contains all necessary issues (Abstract, List of Author´s Publications, List of Abbreviations, Introduction, Aims and Hypothesis, Methods, Results, Discussion, Conclusions, and References). The thesis has 141 pages of text with 33 figures, 6 tables and 241 citations (including 91 from the last five years). The thesis contains only a minimum of typos and punctuation errors.

I appreciate a comprehensive introduction of the problem (NAFLD/NASH), where the reader can find a lot of new information about the topic. Due to the high prevalence of NAFLD/NASH and the lack of causal therapy for this disease, the topic of the dissertation is highly relevant and necessary. Hypothesis and aims are well formulated and logical. The methods used are adequately chosen and modern. I also appreciate *in vitro pre*-experiments on primary rat hepatocytes, on which the author first tested selected substances with regard to their toxicity and action against the lipotoxicity of palmitate. The work (*in vivo* experiments) is not only focused on the pharmacodynamics of the tested substances but in the case of Ku-0063794 also on pharmacokinetics. A number of results are new, but the strongest impact can be seen in the beneficial (and safe) effect of celastrol on many parameters of experimental NASH in mice. Discussion is critical and explains the obtained results of the work. The conclusions are clear and include all the essential findings of the work. The scientific content of the thesis is current and relevant in the context of up-to-date research on NAFLD/NASH and may have significant clinical implications in the search for new candidate agents for the treatment of NAFLD/NASH. The thesis fulfills all formal requirements at very high level.

Miss Mahak Arora is the first author or co-author of 5 publications/manuscripts related to the dissertation topic (first author of 1 original paper in Q1, 1 review in Q1, and 1 submitted manuscript of original paper). The works are published in renowned and quality magazines, and there is no need to doubt their quality. Moreover, Mahak Arora is also an author or co-author of 8 other publications.

Remarks/comments:

1. All methods except mitochondrial respiration are described in detail. Also, the methods for measuring mitochondrial respiration (5.4.7.) should be described in more detail so that they can be reproduced.
2. In your work, you report the concentrations/levels of liver enzymes (ALT, AST) in the plasma or culture medium. You should correctly use the term liver enzyme activities.
3. In the Methods, you should exactly specify the densities of hepatocytes when seeded on 24- and 96-well plates (you state only 1 million per 1.5 ml).

Questions:

1. On page 19, you state that "The increased levels of ROS and lipid oxidation products results in decreased levels of antioxidant enzymes such as superoxide dismutase (SOD) and catalase and antioxidant compounds." Do you think that increased oxidative stress always leads to a decrease in the activity of antioxidant enzymes?
2. On page 24, you state that "Resmetirom works by enhancing hepatic fat metabolism, reducing lipotoxicity, and affecting the liver-specific expression of thyroid receptor β (THR-β), thereby lowering cholesterol and TG levels, increasing BA synthesis, and promoting fat oxidation (Kokkorakis, Boutari et al. 2024)". Can you describe in short, the mechanism of action of resmetirom?
3. On page 48, you state that you measured TG content in the hepatic caudate lobe. Why did you choose this lobe to determine the TG content?
4. In figure 15J, fasting glucose levels of STD+vehicle at weeks 12 and 16 are about 15 and 5 mmol/l, respectively. Do you have any explanation why the fasting glucose levels could be so different?

Conclusion:

The dissertation thesis, "Study of pharmacodynamics and pharmacokinetics of potential therapeutic substances in an experimental model of non-alcoholic Fatty liver disease and steatohepatitis (NAFLD/NASH)," brings new knowledge and insight into the pathophysiology of NAFLD.

The author, Mahak Arora, M.Sc., focused on the highly current and important topic of NAFLD, which makes this work highly relevant as she suggests possible new therapeutic approaches to treat NAFLD/NASH.

The author used proper methods to prove the main hypothesis and achieved the goals of her work. She showed a very high level of orientation in approaching and solving the problematics of the work and fulfilled the prerequisites for further independent scientific work.

Based on the above, I can state that the author has fulfilled the set requirements with the submitted thesis, and I recommend the dissertation for defense according to § 47 of the Higher Education Act (Act No. 111/98 Coll.) and after successfully defending the award of the Ph.D.

Hradec Králové, June 17, 2024

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