UNIVERZITA KARLOVA
Přírodovědecká fakulta
Katedra biochemie

Examiner's Report on Ph.D. thesis

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

by MSc. Mahak Arora

The submitted Ph.D. thesis is based on 2 articles (1 experimental paper and 1 review) published in respected international journals with cumulative IF 12.7 and 2 papers, which were submitted. In two of these publications, the candidate is the first author. According to WOS, articles by MSc. Mahak Arora are already 18 times cited.

The Ph.D. thesis by MSc. Mahak Arora is focused on the testing of potential therapeutic agents in a mice experimental model of non-alcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH). The aim of the study was to evaluate the prophylactic and/or therapeutic effect of selected modulators in NAFLD and in progression of NAFLD to NASH. Three compounds influencing glucose, lipid and energy metabolism were chosen for the study: i/ mammalian target of rapamycin (mTORC1/C2) inhibitor - Ku-0063794 (KU), ii/ a sterol regulatory element-binding protein (SREBP1/2) inhibitor - fatostatin (FAT), and iii/ a galaninergic system modulator - celastrol (CEL). The research strategy is based on two models: *in vitro* (*ex vivo*) experiments with primary hepatocytes and *in vivo* experiments with mice. Hepatocytes were exposed to palmitate to induce lipotoxicity. To developed NAFLD in animals, they were fed with high fat "western diet" supplemented with fructose/glucose in drinking water. Tested compounds were applied to cell cultures and administered to mice in two regimens to simulate chronic and acute exposure.

In hepatocytes, KU, FAT, and CEL pre-treatments reduced palmitate-induced lipotoxicity. All of these compounds proved to be promising drug candidates for further in vivo studies in mice. To determine the pharmacokinetics of KU, the drug was administered intraperitoneally (i.p.) and orally to experimental mice. Bioavailability after i.p. dosing was approximately 160% of that after oral dosing. Thus, for all pharmacodynamic studies, MSc. Mahak Arora used the i.p. route of drug administration. In NAFLD/NASH mice model KU reduced hepatotoxicity, oxidative stress, liver triglycerides, and TNF-α mRNA but did not fully reverse NASH histopathological progression. FAT decreased glycemia, body, fat tissue and liver weights, serum liver enzymes, cholesterol, liver steatosis, NAFLD activity scores, and some lipogenic and inflammatory genes. However, FAT increased serum TNF-α and showed symptoms of skin toxicity. Animal treatment with CEL resulted in up-regulated *Ppargc1α* and *Galr2* genes and reduced lipid peroxidation in the liver. It is noteworthy that CEL, based on its efficacy and safety, could be a potential therapeutic agent for metabolic disorders such as obesity and NAFLD/NASH in humans.

In summary, I can conclude that the Thesis documents that the author used a wide array of experimental approaches, which allowed providing a complex view into the studied field and revealed unknown effects of tested compounds. The Ph.D. thesis brings original results in the field of NAFLD/NASH, namely in finding new potential human therapeutics.

The Thesis follows a common structure and consists of Introduction (14 pages), Aims & Hypothesis (2 pages), Description of experimental methods (15 pages), Results (46 pages), Discussion (16 pages), Conclusions (3 pages), and References (18 pages).

The Introduction is well written. In the literature overview, the author provides a detailed insight into the field and describes the main theoretical and methodological issues. The dissertation includes clearly formulated Aims & Hypothesis. The section Description of experimental methods contains the list of all experimental approaches used. However, this section of the Thesis suffers from an incomplete and confusing description of some protocols. In Results all outputs obtained are clearly presented and described. The design of the experiments and the research techniques used are appropriate for achieving the objectives of the study. The accompanying text and graphics are mostly sufficient to understand the outcomes of experiments. The Discussion is the most important part of the Thesis. The author compares the current results with those of previous studies and offers possible explanations for her findings. All interpretation is grounded in the data and supported by the results. In addition, she explains how the results contribute to the understanding of the topic and the field as a whole. The Conclusion section recapitulates what has been achieved in the study in view of recent knowledge in the research area. The candidate also points to future work that should be made to identify issues that require further clarification. The section References lists all the cited articles (241 references) in the Thesis. The citation format is inconsistent. Some references are even missing the journal name and/or the names of authors are corrupted (e.g., in reference Cui, A. et al., 2017).

The quality of science has already been verified by the journal referees in the course of the publication of the candidate's articles. Thus, I will focus mainly on wording and adherence to formal requirements for Ph.D. thesis. From my point of view, the text is well written, using good English. It is worth highlighting the author's logic ordering of sections and the precise description of complex issues. Reviewing the whole text, I came across a few typos and errors. From all, I point out some comments as follows:

- I do not agree with the statements: "blood samples were allowed to clot" after being "collected terminally via retro-orbital puncture using heparinized glass capillaries" (page 46) or "... SREBP-1c, which mainly activates genomic transcription of the sterols involved in FA and TG synthesis ..." (page 29).
- The source of Fig. 6 is not cited, abbreviation FXR is not explained (page 24), the legend to Fig. 14 does not correspond to all presented plots in the figure, in the text (page 42) abbreviation FG indicates fructose/saccharose, but fructose/glucose in Figure 9.
- Typos: "Thr severe systemic inflammation ..." (page 114) or "... HSCs by activating the by activating the GalR2 receptor ..." (page 119).

Questions to the candidate:

1. Discuss the mechanism by which dietary changes affect the expression of housekeeping genes such as *β-actin* (page 48).
2. What is the breakpoint event between weeks 12 and 16, which significantly altered measured markers among animal sets and times (page 81)?
3. Tested compounds are also considered "anticancer drugs." Explain the mechanism behind this property.

4. Could the administration of binary or tertiary mixtures of tested compounds be of any benefit in terms of progression of NAFLD to NASH or therapy of NASH?

Finally, I can conclude that MSc. Mahak Arora demonstrated the ability of independent high quality scientific work. The dissertation meets the standards for doctoral dissertations in the medical and life sciences. Therefore, I can fully recommend that the candidate's dissertation proceed to defense and that she be awarded the Ph.D. degree.

In Prague, June 15, 2024

prof. RNDr. Petr Hodek, CSc.