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Annex No. 11 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

HABILITATION THESIS REVIEWER'S REPORT

Masaryk University Faculty	Faculty of Medicine
Procedure field	Medical Pharmacology
Applicant	PharmDr. Ondřej Zendulka, Ph.D.
Applicant's home unit, institution	Department of Pharmacology – Faculty of Medicine, Masaryk University
Habilitation thesis	The role of cytochrome P450 in drug safety and efficacy
Reviewer	Prof. dr. hab. Władysława Anna Daniel
Reviewer's home unit, institution	Head of Department of Pharmacokinetics and Drug Metabolism, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

The achievements of Dr. Ondřej Zendulka indicate that within the time of his professional activity he has become an experienced scientist. He is a co-author of 121 publications (including 38 articles, 2 chapters, 1 textbook for students, 24 reports in conference proceedings and 56 conference abstracts) resulting from realization of 14 projects. Moreover, he has a lot of teaching achievements, such as lectures in pharmacology and practices for students of the Faculty of Medicine and supervising doctoral theses in medical pharmacology (two theses) at the Masaryk University.

The research of Dr. Zendulka is focused on the pharmacokinetics and metabolism of psychotropic drugs involving cytochrome P450. His habilitation thesis entitled 'The role of cytochrome P450 in drug safety and efficacy' meets the criteria for medical pharmacology. The habilitation thesis consists of 8 original articles containing the results of preclinical and clinical research and 2 review articles, which were published in international peer-reviewed scientific journals, in the years 2008 – 2018. The research was supported by the Masaryk University (five grants), Czech Science Foundation, Internal Grant Agency of Czech Ministry of Health and Ministry of Education, Youth and Sports of the Czech Republic. In three of them, supported by the Masaryk University he was the principal researcher. The thesis is prepared in a very clear and careful way, friendly to go through.

In the 'Introduction' Dr. Zendulka underlines the importance of cytochrome P450 in oxidative drug metabolism, which has an impact on drug concentrations in blood plasma/pharmacological target and, in turn, on the safety and efficacy of the administered drugs. The main part of work is divided into four chapters: 'Drug-drug interactions on the level of P450', 'The role of P450 in drug development and safety', 'Endogenous regulation of P450 activity' and 'P450 phenotyping in clinical practice'. Each main part of the thesis is based on the attached 1-4 original or review articles of Dr. Zendulka's co-authorship, which are briefly reviewed and discussed. The four attractively presented and consistent thematic parts constitute relevant material to fulfill the paramount aim of the thesis. Based on the presented data, the thesis is finalized by concise 'Conclusions" and 'References'.

In the first part of the thesis 'Drug-drug interactions on the level of P450' Dr. Zendulka reviews the P450 system, mechanisms of drug-drug interactions at the P450 level, and possible clinical consequences. A study describing the metabolic interaction at the CYP2D2 level after co-administration of memantine and fluoxetine to male rats is presented as an example of his research in this field. The CYP2D2 activity was inhibited by both drugs in the two model used, i.e. in the model of isolated perfused rat liver and in the *in vivo* rat pharmacokinetic model, though the obtained results were not identical *(Zendulka O., Juřica J., Zahradníková L., Sabová M., Šulcová A. Effects of combined treatment with cognitive enhancer memantine and antidepressant fluoxetine on CYP2D2 metabolic activity in rats. Neuroendocrinology Letters, 32, 727-732, 2011).*

In the second part of the thesis entitled 'The role of P450 in drug development and safety' Dr. Zendulka focuses attention on the studies of interactions with P450 as a standard procedure in drug development. In this instance, the natural substances with promising potential for therapeutic application, such as the neuroactive/psychotropic polyphenolic substances resveratrol, quercetin, and psychotropic substances crocin, safranal, and linalool, were tested for the interactions with rat P450 enzymes after administration *in vivo,* using the model of isolated perfused rat liver or rat liver microsomes. Interestingly, crocin and quercetin decreased, whereas safranal increased the activity of P450 enzymes. On the other hand, the effect of trans-resveratrol on the P450 activity appeared to be enzyme- and sex-dependent. *Trans*-resveratrol inhibited the CYP2D2 activity in both males and females, but with a greater impact in males *(Dovrtělová G., Nosková K., Juřica J., Turjap M., Zendulka O. Can bioactive compounds of Crocus sativus L. influence the metabolic activity of selected CYP enzymes in the rat? Physiological Research, 64 (Supp. 4), S453-S458, 2015; Nosková K, Dovrtělová K., Zendulka O., Řemínek R., Juřica J. The effect of (-)-linalool on the metabolic activity of liver CYP enzymes in rats.*

Physiological Research, 65 (Supp. 4), S427-S440, 2016; Zendulka O., Zahradníková L., Juřica J., Totušek J. The influence of trans-resveratrol and quercetin on the activity of CYP1A2 in rat. Czech Journal of Food Sciences, 26, S60-S64, 2008; Zendulka O., Totušek J., Šulcová A. Intersexual differences in inhibitory influence of trans-resveratrol on activity of cytochrome P450 2D2 in rats. Neuroendocrinology Letters, 30, 88-91, 2009).

The third part of the thesis is devoted to the 'Endogenous regulation of P450 activity'. Dr. Zendulka stresses that the mechanism of endogenous regulation of P450 activity is different from a simple substrate-enzyme interaction, since it usually involves the regulation of gene expression and a change in the amount of enzyme protein. The possible physiological and molecular mechanisms of interaction between cytochrome P450 and bile acids/cannabinoids or the brain endocannabinoid system are described in the two well-written and valuable review articles *(Juřica J., Dovrtělová G., Nosková K., Zendulka O. Bile acids, nuclear receptors and cytochrome P450. Physiological Research, 65, S499-S504, 2016; Zendulka O., Dovrtělová G., Nosková K., Turjap M., Šulcová A., Hanuš L., Juřica J. Cannabinoids and cytochrome P450 interactions, Current Drug Metabolism, 17 (3), 206-226, 2016).* The state of the art of interactions between cannabinoids, a promising target of new therapeutic strategies and P450 is thoroughly analyzed and a hypothesis of how this system may be involved in the enzyme regulation is postulated. Then experimental results with the endocannabinoid substance oleamide are presented based on the rat *in vivo* and *in vitro* models and human *in vitro* models, which were published in the prestigious scientific journal *(Dovrtělová G., Zendulka O., Nosková K., Juřica J., Peš O., Dušek J., Carazo A., Zapletalová I., Hlaváčová N., Pávek P. Effect of endocannabinoid oleamide on rat and human liver cytochrome P450 enzymes in in vitro and in vivo models. Drug Metabolism and Disposition, 46, 913-923, 2018).* Decreases in hormone-dependent P450 enzyme expression and activities in the rat liver, observed after repeated administration of oleamide *in vivo,* might point to a negative enzyme regulation *via* neuroendocrine system, but the problem requires further *in vivo* studies including measurement of plasma growth hormone, which is an important regulator of rat P450 *via* liver membrane growth hormone receptor, GHR (in my opinion).

The fourth part of the thesis 'P450 phenotyping in clinical practice' illustrates the clinical use of research in which Dr. Zendulka has been involved during his scientific career. The author describes methods for clinical phenotyping of P450 polymorphic enzymes and their use for dosage adjusting. He presents the two useful phenotyping methods of CYP1A2 using caffeine metabolism and of CYP2D6 based on dextromethorphan *O*-demethylation, which were developed in cooperation with the Department of Psychiatry of University Hospital in Brno *(Turjap M., Zendulka O., Glatz Z., Brejcha S, Madr A., Juřica J. Determination of caffeine and*

its metabolites in saliva and urine as a measure of CYP1A2 metabolic activity. Current Pharmaceutical analysis, 12 (4), 325-332, 2016; Juřica J., Barteček R., Žourková A., Pindurová E., Šulcová A., Kašpárek T., Zendulka O. Serum dextromethorphan/dextrorphan metabolic ratio for CYP2D6 phenotyping in clinical practice. Journal of Clinical Pharmacy and Therapeutics, 37, 486-490, 2012).

The thesis ends with the 'Conclusions' chapter. I have to admit that I fully agree with the conclusions drawn by Dr. Zendulka stating that "the system of P450 enzymes plays a crucial role in drug safety and efficacy, and this habilitation thesis adds one of the pieces to the final puzzle of P450". Considering the results obtained with memantine and fluoxetine, the natural substances (crocin, quercetin, safranal, trans-resveratrol) and endocannabinoid compound oleamide, the above statement becomes justifiable. I also fully support further statements of Dr. Zendulka: "Most of our experiments are carried out on animal models and enzymes, therefore their clinical relevance should be carefully interpreted with respect to the known interspecies differences between rat and human P450" and "The more knowledge about the function, regulation, and interactions of P450 we gain, the safer and more effective pharmacotherapy with its substrates can be, especially with the drugs with narrow therapeutic ranges".

The articles included in the habilitation thesis of Dr. Zendulka were prepared in cooperation with his colleagues, students, and members of cooperating research units. However, the contribution of Dr. Zendulka, in particular to original articles, was significant and reached up to 75%. Therefore, they can be qualified as constituents of his habilitation thesis.

The presented thesis and scientific opinions expressed therein prove that Dr. Ondřej Zendulka has vast knowledge, experience in experimental work and ability to comprehend and interpret complex phenomena. Therefore, by all means he is entitled to apply for a habilitation degree.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

I would be interested to know the opinion of Dr. Zendulka on the following problem:
Growth hormone is an important regulator of rat cytochrome P450. Do you think that it might be involved in different effects of oleamide on cytochrome P450 in the two models used: 1)

oleamide administered to rats *in vivo* for 7 days (then RLM study) or 2) added to human hepatocytes *in vitro* (48 h- treatment)? (*Dovrtělová et al. Drug Metab.Dispos. 46, 913-923, 2018).*

Conclusion

The habilitation thesis entitled 'The role of cytochrome P450 in drug safety and efficacy'" by Dr. Ondřej Zendulka **fulfils** requirements expected of a habilitation thesis in the field of Medical Pharmacology.

Date: July 31, 2019. Signature: