Review of PhD thesis

Study of the mechanisms of action of phenolic compounds on vascular smooth muscle

by Patricia Dias

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Reviewer: Milan Stengl, Faculty of Medicine in Pilsen, Charles University

The thesis represents a summary of research performed by the candidate in the field of pharmacology and toxicology of phenolic compounds with focus on cardiovascular system and vascular smooth muscle. Cardiovascular actions of a small phenolic metabolite, 3-hydroxyphenylacetic acid (3-HPAA), and bisphenols, especially the next-generation bisphenols, are analyzed in detail including the possible cellular mechanisms. The analytical depth of the work on several levels of biological complexity should be emphasized; the thesis represents a successful combination of a number of experimental approaches ranging from *in vivo* rat experiments through *ex vivo* vascular rings experiments to *in vitro* cell lines experiments with multiple pharmacologic interventions to unravel cellular mechanisms.

The thesis consists of two major parts, the first part is focused on the small phenolic metabolite 3-HPAA and the second part on bisphenols. The general structure of the thesis is standard, a short Introduction is followed by a detailed chapter of Theoretical background (27 pages), which is divided in 3 subchapters: (1) general description of cardiovascular system including basic concepts of hemodynamics, (2) regulation of vascular tone with mechanisms of vascular smooth muscle contraction and relaxation and endothelium-dependent regulation, (3) phenolic compounds with overview of dietary polyphenols and small phenolic metabolites and bisphenols. After defining the aims of doctoral thesis, the Results section (54 pages), which is based on collection of three peer-reviewed publications and comments, is presented. The candidate is the first author in all articles (in one article shared co-first-authorship), always with a dominant contribution. In the first article (ranked Q2), hemodynamic effects of 3-HPAA (administered as bolus or infusion) were investigated in spontaneously hypertensive rats and both systolic and diastolic pressures were significantly decreased in a doses-dependent way without significant changes in heart rate. Further pharmacologic analysis of the vasorelaxant effect in porcine coronary arteries suggested that the peripheral vasorelaxation probably involved nitric oxide release by the endothelial layer.
The second article (ranked D1) provides a thorough systematic review of currently available literature on (possible) cardiovascular effects of bisphenols. In the review, exposure of humans to bisphenols, pharmacokinetics, animal models, effects of bisphenols on ionic channels, endocrine system, angiogenesis blood pressure, cardiac effects are critically reviewed and discussed in great detail thus providing an excellent overview of the field.
In the last article (ranked Q1), effects of 14 bisphenols (bisphenols A, AF, AP, B, BP, C, E, F, G, M, P, PH, S and Z) were systematically addressed *in vitro* (human and rat cell lines), *ex vivo* (isolated rat aorta) and *in vivo* (Wistar Han rats, acutely or chronically exposed to either low environmental or high toxic doses). Bisphenol AF (BPAF) was found as the most potent vasodilator with the blockade of L-type calcium channels being the dominant mechanism. Although bisphenols could induce relaxation of vascular smooth muscles *ex vivo*, the effective concentrations were found to be too high to produce clear cardiovascular effects *in vivo*, especially with regard to common biological exposure.
The Results/Articles section is followed by detailed Discussion (10 pages) of all findings in both relevant fields of thesis, gut microbiota-derived small phenolic metabolites and

bisphenols. After discussing conclusions and future research directions, the thesis is completed by summary od candidate's contributions and overview of scientific outputs. Beside three publications that form the backbone of the thesis, the candidate co-authored two other IF articles (both in the field of pharmacology). The long list of references (242 references) documents that the candidate is well able to put her research into the context of earlier work.

The findings presented in this thesis are, in my opinion, novel and have important consequences in fields of both pharmacology (small phenolic metabolites) and toxicology (bisphenols). Small phenolic compounds may represent an interesting alternative of antihypertensive therapy and certainly deserve further attention to fully clarify their potential. Bisphenols as ubiquitous pollutants are of general interest and, especially for the next generation bisphenols, our knowledge of their biological effects is scarce. All articles reported in the thesis underwent demanding review process in international scientific journals (1x D1, 1x Q1, 1x Q2), which clearly documents their quality and novelty. The thesis is well written and well organized, with sufficient Introduction and Discussion chapters. Obviously meticulous editing and proofreading are evidenced by the virtual absence of typing errors and the quality of language.

Finally, I have several questions, which are meant to stimulate general discussion but they certainly should not cast any doubts on the validity and quality of thesis findings.

In the first article on the effects of 3-HPAA, (spontaneously hypertensive) rats and porcine coronary arteries were employed. Could you comment on species differences in the vascular physiology of these species and how they could contribute to the discrepancies observed?

Did you test the hypotensive effects of 3-HPAA also in normotensive rats? Is the potency of 3-HPAA similar in normotensive and hypertensive settings?

The hypotensive effect of 3-HPAA infusion is only transient (1-2 min) although the infusion lasts longer (5 min). Any explanation?

Is there any information on consequences of long-term (years) exposure to bisphenols? Any evidence of bisphenol accumulation in some tissues? Could not the levels/effects of bisphenols be modified by specific diseases (e.g. kidney disease)?

Conclusion:
In summary, I gladly and unreservedly recommend acceptance of this Ph.D. thesis toward attainment of a Ph.D. degree in Pharmacology and Toxicology by Patricia Dias.

In Pilsen, November 2, 2023

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