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Evaluation of the PhD-thesis

PhD Student: Thomas Migkos, M.Sc.

Title of the thesis: Effects of isoflavonoids and their metabolites on vascular smooth muscles *in vitro* and *in vivo*

Supervisor: Prof. Přemysl Mladěnka, Pharm.D, Ph.D.

Consultant: Assoc. Prof. Jana Pourová, Pharm.D, Ph.D.

The study is focused on exploring the vasorelaxant properties of isoflavonoids, specifically the impact of individual pure isoflavonoids and their colonic metabolites on the vascular system. Isoflavones are a type of naturally occurring organic compounds belonging to the flavonoid family. They are found predominantly in certain plant-based foods, particularly in legumes, with soybeans being one of the richest sources. Isoflavones have attracted significant attention due to their potential health benefits and biological activities. Isoflavones have a structure similar to the hormone estrogen, and they are referred to as phytoestrogens. Isoflavones are known for their potential health benefits, including antioxidant properties and potential effects on heart health. Other studies have explored their impact on bone health, and certain types of cancer.

The aim of this thesis was to study the impact of sixteen pure isoflavones, many of them were examined for the first time, along with their colonic metabolites, on vascular smooth

muscles. This investigation spanned both in vitro and in vivo levels. The secondary objective was to delineate the underlying mechanism(s) responsible for these observed effects.
The study screened sixteen isoflavonoids, four of their metabolites, and a racemic mixture for their vasorelaxant properties using rat aortas. Biochanin A, glycitein, O-desmethylangolensin (O-DMA), S-equol, and R,S-equol were identified as the most potent vasorelaxants. These compounds induced endothelium-independent relaxation of coronary vasculature, with effective concentrations ranging from 5.5 to 17 μM. Biochanin A, S-equol, and R,S-equol were found to inhibit vasoconstriction induced by various agents in a concentration-dependent manner. Biochanin A specifically inhibited L-type calcium channels in human aortic and coronary smooth muscle cells. O-DMA, S-equol, and R,S-equol were found to dilate smaller resistant mesenteric arteries. O-DMA decreased arterial blood pressure in spontaneously hypertensive rats without affecting heart function. The results suggest that certain isoflavonoids, particularly biochanin A and its metabolites, may have vasodilatory effects in micromolar concentrations. In conclusion, the study provides insights into the vasorelaxant properties of specific isoflavonoids and their metabolites, focussing on potential therapeutic applications for cardiovascular conditions.

The structure of the thesis is well-organized, following a common and logical progression. Thomas Migkos clearly presented the aims of his study. This demonstrates his ability to articulate goals and intentions. To investigate the effects of the isoflavones on arterial blood pressure he used various methods. The use of a variety of methods allowed him to approach the investigation from different angles, increasing the robustness of his findings. The study involved a combination of in vitro, ex vivo, and in vivo approaches to comprehensively explore the effects of isoflavonoids and their metabolites on arterial blood pressure. The results are interesting and the data analyses and presentation is very clear.

The study provides intriguing insights into the potential cardiovascular effects of isoflavonoids and their metabolites, particularly in the context of arterial blood pressure regulation. One notable aspect is the enhancement of vasorelaxant effects by biochanin A, even at lower concentrations, when combined with sodium nitroprusside. This suggests a potential synergistic interaction that could be of clinical importance. Further investigation into the specific signaling pathways and molecular targets of glycitein could provide a more comprehensive understanding of its mode of action. The confirmation of the biological activity of S-equol and O-DMA, compared to their precursors, aligns with previous research but adds a

novel dimension by demonstrating the vasodilator effects of O-DMA in an in vivo rat model. This finding challenges existing assumptions about the primary role of equol in the health benefits associated with isoflavones.
The study's emphasis on the potential clinical relevance of concentrations achievable through dietary intake or supplementation highlights the practical implications of these findings. However, it also raises questions about the variability in individual responses and the long-term effects of sustained exposure to these compounds.

Thomas Migkos published several original articles (Q1 and Q2). He is first author on a scientific publication related to the topic of the thesis. Besides that he presented his work at the Postdoctoral Scientific Conferences in Hradec Králové and had poster presentations at international conferences in Austria, Germany and China. He also did internships at the Université de Strasbourg in France and at the University of Sydney in Australia. Alltogether Thomas Migkos has demonstrated that he is capable to answer scientific questions. The goals of this thesis have been met in a very successful manner. The candidate has proven his expertise in this specific field as well as his ability to contribute original research to the academic community.
For this significant accomplishment I strongly recommend to award the Ph.D. title to Thomas Migkos.

Sincerely

Christian Studenik, M.Sc., Ph.D.
Associate Professor