COMMENTS TO HABILITATION THESIS

RNDr. Milan Číž, Ph.D.: **Modulation of professional phagocyte activity**

Neutrophils and monocytes/macrophages, so called professional phagocytes, as integral constituents of innate immunity serve as an essential first-line of defence against microbial pathogens and foreign substances at sites of inflammation. Upon activation, these cells among other functional manifestations produce reactive oxygen and nitrogen species. While respiratory burst is important for the elimination of invading pathogens, the overproduction of reactive oxygen species or the impairment of endogenous antioxidant defences may lead to a damage of membrane lipids, DNA, proteins, and lipoproteins which results in various autoimmune and inflammatory diseases. Thus, a modulation of professional phagocyte activity is very important task in physiology and medicine.

The major aims of our studies were to evaluate possibilities of professional phagocyte (namely neutrophil) modulation using endogenous (platelet-derived serotonin) and exogenous (polyphenols and polysaccharides of plant origin as food constituents) mediators. Our studies bring the evidence that serotonin released from platelets is a very important modulator of professional phagocyte activity. Its inhibitory activity is manifested by the decrease in the generation of reactive oxygen species due to the inhibition of myeloperoxidase activity and by direct scavenging of reactive oxygen species. The effects of serotonin on professional phagocytes are also partially mediated by 5-HTR2 receptor expressed on monocytes/macrophages but not on neutrophils. We also introduced and optimized several methods to investigate antioxidant properties of plant extracts and individual plant polyphenols. We confirmed that many medicinal plants, vegetables and berries are rich sources of polyphenol compounds and free radical scavengers. Furthermore, we demonstrated that various plant polyphenols and polysaccharides exerted evident immunomodulatory activity. Thus, these compounds represent effective naturally occurring substances with potent pharmacological effects on respiratory burst of professional phagocytes useful for treatment of compromised immune system and control of inflammation.

My thesis is based on 20 selected original research papers. Study 1: I contributed to the study design, experimental procedures, data analysis, preparation of the manuscript and its final revision. Studies 2 – 5: I conceptualized, designed, and directed the studies, participated in the experiments, supervised data collection and analyses, substantially contributed to the manuscripts‘ preparation and their final revisions. Studies 6 – 16: I contributed to the studies’ concept, design and direction and participated in experimental procedures, data analyses and manuscripts’ preparation and finalization. Studies 17 – 20: I participated in the studies’ design preparation, experimental procedures, data analyses and manuscripts’ preparation. My proportional contribution to these articles is summarized in the following tables.

1. Jancinova V, Drabikova K, Nosal R, Petrikova M, Ciz M, Lojek A, Danihelova E (2003) Inhibition of FMLP-stimulated neutrophil chemiluminescence by blood platelets increased in the presence of the serotonin-liberating drug chloroquine. Thrombosis Research 109: 293-298

Experimental work	Supervision of students	Manuscript preparation	Research direction
20 %	0 %	30 %	30 %

2. Okenkova K, Lojek A, Kubala L, Ciz M (2007) Modulation of rat blood phagocyte activity by serotonin. Chemické Listy 101: 245-246

Experimental work	Supervision of students	Manuscript preparation	Research direction
30 %	100 %	40 %	100 %

3. Ciz M, Komrskova D, Pracharova L, Okenkova K, Cizova H, Moravcova A, Jancinova V, Petrikova M, Lojek A, Nosal R (2007) Serotonin modulates the oxidative burst of human phagocytes via various mechanisms. Platelets 18: 583-590

Experimental work	Supervision of students	Manuscript preparation	Research direction
40 %	80 %	30 %	80 %

4. Pracharova L, Okenkova K, Lojek A, Ciz M (2010) Serotonin and its 5-HT2 receptor agonist DOI hydrochloride inhibit the oxidative burst in total leukocytes but not in isolated neutrophils. Life Sciences 86: 518-523

Experimental work	Supervision of students	Manuscript preparation	Research direction
30 %	100 %	40 %	100 %

5. Vasicek O, Lojek A, Ciz M (2019) Serotonin and its metabolites reduce oxidative stress in murine RAW264.7 macrophages and prevent inflammation. Submitted to Journal of Physiol Biochem.

Experimental work	Supervision of students	Manuscript preparation	Research direction
30 %	100 %	30 %	80 %

6. Ciz M, Pavelkova M, Gallova L, Kralova J, Kubala L, Lojek A (2008) The influence of wine polyphenols on reactive oxygen and nitrogen species production by murine macrophages RAW 264.7. Physiological Research 57: 393-402

Experimental work	Supervision of students	Manuscript preparation	Research direction
40 %	100 %	40 %	90 %

7. Kratchanova M, Denev P, Ciz M, Lojek A, Mihailov A (2010) Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds. Comparison of two extraction systems. Acta Biochimica Polonica 57: 229-234

Experimental work	Supervision of students	Manuscript preparation	Research direction
25 %	20 %	30 %	50 %

8. Ciz M, Cizova H, Denev P, Kratchanova M, Slavov A, Lojek A (2010) Different methods for control and comparison of the antioxidant properties of vegetables. Food Control 21: 518-523

Experimental work	Supervision of students	Manuscript preparation	Research direction
30 %	0 %	30 %	50 %

9. Denev P, Ciz M, Ambrozova G, Lojek A, Yanakieva I, Kratchanova M (2010) Solid-phase extraction of berries' anthocyanins and evaluation of their antioxidative properties. Food Chemistry 123: 1055-1061

Experimental work	Supervision of students	Manuscript preparation	Research direction
20 %	50 %	30 %	30 %

10. Jancinova V, Nosal R, Lojek A, Ciz M, Ambrozova G, Mihalova D, Bauerova K, Harmatha J, Perecko T (2010) Formation of reactive oxygen and nitrogen species in the presence of pinosylvin - an analogue of resveratrol. Neuroendocrinology Letters 31: 79-83

Experimental work	Supervision of students	Manuscript preparation	Research direction
20 %	25 %	20 %	25 %

11. Perecko T, Drabikova K, Rackova L, Ciz M, Podborska M, Lojek A, Harmatha J, Smidrkal J, Nosal R, Jancinova V (2010) Molecular targets of the natural antioxidant pterostilbene: effect on protein kinase C, caspase-3 and apoptosis in human neutrophils in vitro. Neuroendocrinology Letters 31: 84-90

Experimental work	Supervision of students	Manuscript preparation	Research direction
10 %	20 %	25 %	25 %

12. Perecko T, Drabikova K, Lojek A, Ciz M, Ponist S, Bauerova K, Nosal R, Harmatha J, Jancinova V (2013) The Effects of Pterostilbene on Neutrophil Activity in Experimental Model of Arthritis. Biomed Research International, Artn 106041

Experimental work	Supervision of students	Manuscript preparation	Research direction
20 %	0 %	20 %	20 %

13. Denev P, Kratchanova M, Ciz M, Lojek A, Vasicek O, Blazheva D, Nedelcheva P, Vojtek L, Hyrsl P (2014) Antioxidant, antimicrobial and neutrophil-modulating activities of herb extracts. Acta Biochimica Polonica 61: 359-367

Experimental work	Supervision of students	Manuscript preparation	Research direction
10 %	40 %	20 %	30 %

14. Denev P, Kratchanova M, Ciz M, Lojek A, Vasicek O, Nedelcheva P, Blazheva D, Toshkova R, Gardeva E, Yossifova L, Hyrsl P, Vojtek L (2014) Biological activities of selected polyphenol-rich fruits related to immunity and gastrointestinal health. Food Chemistry 157: 37-44

Experimental work	Supervision of students	Manuscript preparation	Research direction
10 %	30 %	20 %	25 %

15. Nosal R, Drabikova K, Jancinova V, Perecko T, Ambrozova G, Ciz M, Lojek A, Pekarova M, Smidrkal J, Harmatha J (2014) On the Molecular Pharmacology of Resveratrol on Oxidative Burst Inhibition in Professional Phagocytes. Oxidative Medicine and Cellular Longevity, Artn 706269

Experimental work	Supervision of students	Manuscript preparation	Research direction
10 %	25 %	20 %	30 %

16. Denev P, Ciz M, Kratchanova M, Blazheva D (2019) Black chokeberry (Aronia melanocarpa) polyphenols reveal different antioxidant, antimicrobial and neutrophil-modulating activities. Food Chemistry 284: 108-117

Experimental work	Supervision of students	Manuscript preparation	Research direction
25 %	0 %	25 %	30 %

17. Nikolova M, Ambrozova G, Kratchanova M, Denev P, Kussovski V, Ciz M, Lojek A (2013) Effects of Pectic Polysaccharides Isolated from Leek on the Production of Reactive Oxygen and Nitrogen Species by Phagocytes. Journal of Medicinal Food 16: 711-718

Experimental work	Supervision of students	Manuscript preparation	Research direction
15 %	40 %	20 %	30 %

18. Georgiev YN, Ognyanov MH, Kiyohara H, Batsalova TG, Dzhambazov BM, Ciz M, Denev PN, Yamada H, Paulsen BS, Vasicek O, Lojek A, Barsett H, Antonova D, Kratchanova MG (2017) Acidic

polysaccharide complexes from purslane, silver linden and lavender stimulate Peyer's patch immune cells through innate and adaptive mechanisms. International Journal of Biological Macromolecules 105: 730-740

Experimental work	Supervision of students	Manuscript preparation	Research direction
10 %	0 %	10 %	20 %

19. Georgiev YN, Paulsen BS, Kiyohara H, Ciz M, Ognyanov MH, Vasicek O, Rise F, Denev PN, Yamada H, Lojek A, Kussovski V, Barsett H, Krastanov AI, Yanakieva IZ, Kratchanova MG (2017) The common lavender (Lavandula angustifolia Mill.) pectic polysaccharides modulate phagocytic leukocytes and intestinal Peyer's patch cells. Carbohydrate Polymers 174: 948-959

Experimental work	Supervision of students	Manuscript preparation	Research direction
10 %	0 %	10 %	20 %

20. Georgiev YN, Paulsen BS, Kiyohara H, Ciz M, Ognyanov MH, Vasicek O, Rise F, Denev PN, Lojek A, Batsalova TG, Dzhambazov BM, Yamada H, Lund R, Barsett H, Krastanov AI, Yanakieva IZ, Kratchanova MG (2017) Tilia tomentosa pectins exhibit dual mode of action on phagocytes as beta-glucuronic acid monomers are abundant in their rhamnogalacturonans I. Carbohydrate Polymers 175: 178-191

Experimental work	Supervision of students	Manuscript preparation	Research direction
10 %	0 %	10 %	20 %