

Bydgoszcz, June 28, 2022

Re: Evaluation of the doctoral thesis by Mr. Piotr Wójcik

The evaluation has been prepared as a reply to the letter of Prof. Wojciech Milyk, the Dean of the College of Pharmaceutical Sciences, which followed the decision of the Senate of the Medical University of Białystok (161/2022).

General information

The basis for the application of Mr. Piotr Wójcik for the doctoral degree is the dissertation entitled “Redox balance and changes in lipid and protein metabolism in patients with psoriasis”. The supervisors of the thesis are: Prof. Elżbieta Skrzydlewska from Department of Analytical Chemistry, Medical University of Białystok and Prof. Neven Žarković from Laboratory of Oxidative Stress, Rudjer Boskovic Institute in Zagreb, and the auxiliary supervisor is Dr. Agnieszka Gęgotek from Department of Analytical Chemistry, Medical University of Białystok. The doctoral dissertation is based on a series of six papers. It is provided with a few pages of introduction followed by the statement of the aim of the presented studies. The subsequent chapters contain articles included in the achievements submitted as a doctoral dissertation; all are published between the year 2019 and 2021 in the journals from JCR list. Four of them are original research papers and two are reviews. The total Impact Factor of the presented articles is 26.518 and the total MNiSW (MEiN) scores is 760. In all publications the candidate is the first author and his leading contribution is stated in the separate documents confirmed by the Supervisor. Also, the statements of contribution from all co-authors are provided and they are in line with the first author’s statements. Following the publications, the thesis includes chapters with short discussion, conclusions, the list of references, the summary of the work in English in Polish, the copy of the approval from the designated bioethics committee along with the application for the approval of the planned experiments. At the end of the dissertation the Author included again the list of the publications which are basis of the thesis as well as other papers of his co-authorship (two with total IF 8.548 and MNiSW 240) and the list of the conference abstracts. The last chapter is the Curriculum vitae of the Candidate. The dissertation is written in clear and logistic way, with good English.

Assessment of the doctoral dissertation

The biological problem undertaken by Mr. Wójcik and the co-authors of his papers concerns the mechanisms related to imbalance in redox reactions and modifications of lipid and protein metabolism in patients with psoriasis. Although, the disease has been well studied, there are still gaps in the knowledge how particular biochemical pathways are activated in different clinical forms of the disease and what factors play major role in those modulations. In the Introduction, the Candidate presents the current knowledge about the disease and molecular mechanisms of psoriasis pathophysiology emphasizing the role of reactive oxygen and nitrogen species (ROS and RNS, respectively) and explaining implications of their overproduction. Mr. Wójcik clearly explains the consequences of interaction between the ROS/RNS and lipids and proteins, particularly in lymphocytes and granulocytes, which are directly involved in development of psoriasis. In the next section, he describes the link between the aforementioned processes and abnormalities occurring in keratinocytes, which leads to observable symptoms of the disease. The author explains the role of particular cytokines, their origin and how they modulate the biochemical processes in psoriasis. At the end of the introductory section there is a short description of cannabidiol, the phytocannabinoid, which seems to have a serious potential in treatment of psoriasis and which became one of the research objectives in the presented thesis. The aim of the studies is well defined; the main goal was to reveal the exact mechanisms of ROS action in lymphocytes of psoriasis patients. Subsequently, the influence of ROS onto development and manifestation of different forms of psoriasis was determined. Finally, the authors examined how cannabidiol affects regulatory pathways in psoriasis and investigated if it can be useful in combine therapy with UVB radiation.

The first article was published in 2020 in Journal of Biochemistry (IF 2.476 and MNiSW 100). The work considered investigation of redox imbalance and its influence on protein modification in lymphocytes in patients with psoriasis. According Scopus the article had 9 citation on the day of preparation of this evaluation. The authors performed wide range of analytical assays including but not limited to: luminescence, electron spin resonance, Western Blot, capillary electrophoresis, liquid chromatography coupled to mass spectrometry. The comprehensive insight into the biochemical changes of the lymphocytes permitted them to recognise enhancement of pro-oxidative conditions in psoriasis arthritis (PsA) when

compared to psoriasis vulgaris (PsV). Monitoring of the activators of the Nrf2 transcription factor showed their higher level in PsV. Additionally, they have noted the increased expression of proapoptotic caspases, which resulted from the modifications of proteins by lipid peroxidation products identified as 4-oxononenal and malondialdehyde. Interestingly, the authors found significant differences between both studied groups in terms of the expression of caspase 3 – it was enhanced in PsV and decreased in PsV. However, they emphasized that the final apoptosis is a result of coexistence of several pathways involving Bcl2, cytochrome c or p53. The expression level of the aforementioned proteins as well as levels of NF κ B and TNF α were determined and compared for PsV and PsV. Based on the obtained results it was concluded that stronger Nrf2 pathway activation might be a result of the lower oxidative stress occurring in lymphocytes of patients with PsV. In the same time, the profiles of the proteins modified by the lipid peroxidation products suggest that this factor is the key player in activation of pro-inflammatory and pro-apoptotic pathways in PsV. These findings may become the basis for further research aimed at searching for targeted therapies in psoriasis.

The second paper in the series concerns the study of differences between PsV and PsA with regards to lipid alteration in blood mononuclear cells. The article was published in 2019 in International Journal of Molecular Sciences (IF4.556, MNI_{SW} 140) and received already 26 citations, which is an impressive number, particularly for the original research paper. Usually the Impact Factor of the journals is the metrics we take into account when evaluating the achievements of PhD candidates because the timeframe of the individual articles is too narrow to evaluate the value of the papers, but in this case the number of citations directly reflects the value of the work. Similarly to the previous work, the authors used multidimensional methodology to investigate different aspects of the topic. Majority of the analysis was performed on triple quadrupole mass spectrometer coupled to ultrahigh performance liquid chromatography. Among compounds determined with UHPLC-MS/MS were unstable species like endocannabinoids or lipid mediators. As the publication was focused on the biological investigation the authors did not provide the results of method validation, but referred to the original protocols. Because of my personal experience with oxylipins and endocannabinoids I would like to ask the Candidate about his experience in this regards and the results of the stability of the reported metabolites – what was the stability of the investigated metabolites and how the stability was monitored over the time of sample

preparation and instrumental analysis. The authors obtained very interesting results indicating strong influence of the oxidative stress on the alteration of phospholipids, endocannabinoids and eicosanoids in mononuclear cells, which consequently leads to development of psoriasis. However, the most interesting and important findings were the differences in the profiles of phospholipids, levels of lipid metabolites and endocannabinoids as well as activities of COX-1 (but not COX-2) between PsV and PsV. This study confirms that investigation of lipid species and the metabolic pathways they are involved in might be the key to understand distinct pathologies of related diseases thus to help in finding the effective therapy.

Next paper is the review article summarizing how oxidative stress and lipid mediators modulate functions of immune cells in autoimmune diseases. The work was also published in International Journal of Molecular Sciences (IF 5.924 and MNI SW 140) in 2021 and received 9 citations as of the day of preparation of this evaluation. The second review included in the thesis was published in 2020 in Biomolecules (IF 4.082, MNI SW 100) and it was focused on the involvement of metabolic lipid mediators in the regulation of apoptosis. This work was cited 13 times. Although reviews are often not treated as equal as original papers in assessing scientific achievements, in my opinion they are an extremely important factor in the process of preparation for scientific work in a given field, particularly for the early stage career researcher. A well-prepared review of world literature allows to broaden the horizons and look at the research conducted by the author through the prism of the research conducted in other institutes. The dynamics and interdisciplinarity of research in the field of biomedical sciences, including methodological diversity, especially at the molecular level, forces scientists to update their knowledge. On the other hand, the ability to critically look at research results, select the most key reports and summarize their impact on the development of a given field in a way that is valuable to the reader, is a very appreciated attribute of a good scientist. In my opinion, the quality of the discussion presented in the Candidate's original papers at least partially results from the in-depth review of the literature in the field during preparation of the review papers. It is also worth noting that the Figures and schemes in these papers which illustrate various mechanisms described in the text seem to be prepared by the authors as they do not have references to any source materials.

Two last publications presented as a part of the doctorate thesis are original papers, both published in International Journal of Molecular Sciences in 2020 (IF 5.556, MNI SW 140) and 2021 (IF 5.924, MNI SW 140). The first work, which deals with the involvement of

cannabidiol in neutrophils extracellular trap (NET) formation in neutrophils of psoriasis patients, has already 6 citations, while the second one, which focuses on the investigation of correlation between cannabidiol (CBD) and antiapoptotic effect in keratinocytes during UV phototherapy was cited one time up to date. The cannabinoids, and cannabidiol in particular, are recently widely studied in various diseases including autoimmune diseases and idiopathic diseases. Mr. Wójcik and his co-workers made an attempt to evaluate the influence of CBD on neutrophils isolated from the blood of PsV patients by monitoring the percentage of neutrophils undergoing NETosis and the level of several NETosis markers. To be able to reliably indicate what changes are associated with what compounds added to the studied system, the authors designed “an internal controls” i.e., neutrophils+LPS (NETosis activated neutrophils) and neutrophils+CBD, which were compared with neutrophils+LPS+CBD. At first, the authors observed that the percentage of neutrophils undergoing NETosis with no stimuli is significantly higher in the PsV group vs. control. Then, for the activated neutrophils they were able to proof that CBD decreases the NETosis markers, thus suggesting its potential in treatment of psoriasis and possibly other autoimmune disease. It should be emphasized that the methodology required in this study was different from the previous papers. This shows the high skills of the researchers, particularly Mr. Wójcik, who was the main analyst as stated in the contribution section. The follow-up CBD study concerns interesting aspect of using the compound as the agent potentially reducing apoptosis in healthy keratinocytes after exposure to UVB radiation. The UVB treatment is frequently used therapy in patients with psoriasis, but it is not selective towards diseased cells and also activates proapoptotic pathways in healthy keratinocytes, which in turn is related to the occurrence of side effects and forces introduction of anti-inflammatory treatment. As previously, the experimental design of the study was carefully planned and all necessary “external” (healthy cells) and “internal” controls (cell cultured with individual stimuli) were considered. Comprehensive protein and prostaglandin analysis were performed using Western Blot, ELISA and LC-MS/MS. The obtained results confirmed the hypothesis about the inhibitory effect of CBD on the apoptotic pathways in keratinocytes with stronger effect observed in healthy cells. However, interesting findings were reported with respect to discrepancies in the effect of UVB irradiation on healthy and psoriatic cells pointing out additional increased expression of caspase 2, decreased expression of p-AKT along with elevated 15-d-PGJ2 level and p-53 expression in the diseased keratinocytes. The mentioned increase of caspase 2 defines UVB irradiation as a

stressor to the endoplasmic reticulum and this action seems to be abolished in the presence of CBD. Therefore, the potential adjuvant UVB-CBD therapy needs to be further studied and carefully planned in order not to weaken the UVB treatment effects but to take the advantage of protective effect of CBD on the healthy skin cells.

Conclusions

In my opinion, the accomplishment presented by Mr. Piotr Wójcik as his doctoral thesis constitutes a well-planned, coherent whole of high scientific value. The presented works show that the Candidate was well prepared for the conducted research, both in terms of the content and the experience. The dissertation is devoid of factual, spelling and stylistic errors. Out of the reviewer's duty, I draw attention to editorial errors and shortcomings e.g., errors in numerations of publications in Chapter 1 and numbers assigned to particular chapters in Chapter 3 (The aim of the studies). Also, the list of abbreviations placed at the beginning of the dissertation would be helpful. However, these small neglects do not affect the overall assessment of the dissertation.

As a reviewer, I allow myself to extend the assessment of the presented dissertation to the general achievements of the Candidate, because undoubtedly his overall activity impacted the quality of articles constituting his doctoral dissertation. It is worth mentioning that the current Hirsch index of Mr. Piotr Wójcik is 6, and the total number of citations of the articles he co-authors is 127. The Candidate also actively attended conferences; his works and the works of his co-authorship were presented eight times at national and international conferences. He also completed six internships lasting between 1 week and 2 months, four in Poland and two in Croatia in the laboratory of his Supervisor, prof. Žarković. The Candidate also reported one research project but no detail information if he was the leader or the executor was provided.

To sum up, on the basis of the presented doctoral dissertation, I conclude that it contributes significantly to the development of the Discipline of Medical Sciences and fully meets the substantive and formal requirements for doctoral thesis. I hereby turn to the High Council of Collegium of Pharmaceutical Sciences of the Medical University of Białystok with a request to admit Mr. Piotr Wójcik to further stages of the procedure in applying for the doctoral degree. At the same time, due to the high level of the dissertation, I am applying for the distinction of the reviewed work.



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