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Collegium Medicum w Bydgoszczy

Prof. dr hab. Michał Piotr Marszałł

KATEDRA I ZAKŁAD CHEMII LEKÓW  
ul. Dr Jurasza 2, 85-089 Bydgoszcz,  
tel. +48 52 585 35 32, fax. +48 52 585 35 29  
e- mail: mmars@cm.umk.pl

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### Review

of the doctoral dissertation of Thi Yen Ly Huynh entitled:

*“Metabolomic approach to understand the mechanism of metformin-induced  
PRODH/POX-dependent apoptosis in MCF-7 breast cancer cells”*

written under the supervision of the dissertation supervisor

Prof. Jerzy Pałka, MPharm, PhD

at the Department of Medicinal Chemistry,  
Medical University in Białystok, Poland

Interdisciplinary systems holds great potential to lead to a better understanding of how living organisms work, and subsequently to the development of effective diagnosis and optimal pharmacotherapy of various diseases. Ms Thi Yen Ly Huynh's doctoral dissertation that has been submitted for my review is an example of research using metabolic tools to elucidate and understand the biological and chemical principles of organismal function in response to pharmacotherapy with a selected biguanide derivative. The topic of the doctoral dissertation is very timely, as meta-analyses of randomised trials conducted over the past two decades have shown a significant effect of metformin used in patients with type 2 diabetes mellitus on reducing the risk of cancer.

The research carried out by the doctoral student fits well with the types of research conducted by the team from the Department of Medicinal Chemistry, Faculty of Pharmacy, Medical University of Białystok, Poland. It is the research of Prof. Jerzy Pałka's team conducted so far that addresses, among other things, the effect of the metabolism of selected amino acids on the regulation of apoptosis/autophagy in cancer cells. The dissertation comprises 116 pages and is preceded by a table of contents and a list of abbreviations, which is very useful. The theoretical section represents a very interesting and condensed body of current knowledge regarding the biochemistry and the effect of metformin on inhibiting cancer cell proliferation based on the

literature and on the hypotheses put forward by the doctoral student and the co-authors. This was followed by a presentation of the aim, description of the results, discussion, conclusions, abstract and the list of literature references.

The dissertation is a series of two publications with a total IF of 8.275, in which the doctoral student is the main author. The dissertation is accompanied by the publications described above and statements from the co-authors highlighting the student's considerable contribution. The first publication (*Molecular and Cellular Biochemistry*) includes a description of the state of the art and a discussion of the role of selected amino acids on the regulation of proline dehydrogenase/proline oxidase (PRODH/POX) function. An important element of this publication, as part of the dissertation, is the setting of an interesting hypothesis based on the previous knowledge and experience of both the doctoral student and her supervisor. It assumes the activation of AMP kinase by methionine, which in turn induces the process of apoptosis in cancer cells. The authors hypothesise that apoptosis is influenced by reactive oxygen species generated by proline dehydrogenase/proline oxidase under conditions of proline availability. This hypothesis is presented in detail by the authors in Figure 1 (page 51 of the dissertation) in the publication indicated above.

Consequently, the linking of the above processes and the stimulatory effect of metformin on AMP (AMPK) allows to extend the hypothesis of the aforementioned mechanism of metformin's involvement in apoptosis/autophagy, which would provide evidence of the antineoplastic effect of the drug which has been used for years in the pharmacotherapy of type 2 diabetes mellitus. The above assumptions have become the main objective of Ms Thi Yen Ly Huynh's doctoral thesis, namely to evaluate the mechanism of metformin's antineoplastic effect in MCF-7 breast cancer cells.

The experimental part has been described in the dissertation and published in *Biomolecules*. Verification of the hypothesis required an *in vitro* model, namely wild-type and PRODH/POX-silenced MCF-7 breast cancer cells. The effect of methionine was performed and assessed using metabolic analysis by comparing the metabolic profiles of the above cell lines, assessing the expression of proapoptotic proteins, and by determining proliferation and the cell cycle under different metabolic conditions. The results suggest that methionine has an inhibitory effect on the proliferation of MCF-7 cells and cells with silenced PRODH/POX expression—especially in the absence of methionine. The drug also contributes to the induction of apoptosis by the reprogramming of amino acid metabolism, the tricarboxylic acid cycle, the urea cycle and the pentose-phosphate pathway in the above cells. Metabolic analysis, however,

indicates a dependence of glycolysis on glutamine and proline metabolism. The results described in the paper open up new possibilities in this direction and may be of great importance in the future for a new strategy of cancer pharmacotherapy. Therefore, the conclusions presented by the doctoral student are justified and the results add to the body of knowledge on the mechanism of the antineoplastic action of metformin. One important observation is that the combination of metformin with glutamine synthetase inhibitors may represent a potential strategy in the pharmacotherapy of breast cancer.

When I was reading the dissertation, the following questions came to my mind:

- In the paper published in *Biomolecules*, the doctoral student pointed out in the discussion section that epidemiological studies confirmed the effect of metformin on reducing the risk of bowel, liver and lung cancer and therefore the mortality rate. Can a similar mechanism be attributed to this as is the case with the hypothesis put forward the dissertation?
- Amino acid derivatisation is also recommended for LCMS/MS chromatographic analysis to increase the selectivity of the method, especially of low-molecular compounds with similar structures. Therefore, did the lack of such a procedure cause problems when analysing the selected amino acids?

Of note is the fact that the dissertation is written in English, and Ms Thi Yen Ly Huynh is a doctoral student of the International Doctoral Programme in Medical Biology and Biostatistics in Medical and Pharmaceutical Sciences at the Faculty of Pharmacy with the Laboratory Medicine Branch, conducted within the framework of a project funded by the European Commission: Marie Skłodowska-Curie Action in the Horizon 2020 Framework Programme and with funds from the Ministry of Science and Higher Education. She performed part of her experimental work (the metabolic analysis) during a foreign research fellowship at the Centre for Metabolic Research and Bioanalysis (San Pablo University, Madrid, Spain) under the supervision of Prof. Coral Barbas. The research tasks were part of an ongoing project funded by the OPUS National Science Centre led by the dissertation supervisor. This demonstrates both the relevance of the research topic and its originality.

It also merits mentioning that the doctoral student has also co-authored 15 publications, mainly with scientists of the Medical University of Białystok, which indicates her strong commitment to scientific work at the university and the appreciation of her abilities and skills as a scientist by the collaborating research teams.

In summary, the dissertation is a series of two publications. At each stage of her work, the doctoral student correctly formulates the problems and correctly draws conclusions from the results, while planning the next stages of her research. This skill and the doctoral student's strong commitment to the completion of the research have positively contributed to the achievement of her objectives. In my opinion, her dissertation fulfils the requirements of the applicable regulations and I therefore request the Dean to admit Ms Thi Yen Ly Huynh to the further stages of the doctoral dissertation for the award of the doctoral degree in medical and health sciences in the discipline of pharmaceutical sciences. Considering also the great contribution of the work, the originality of the subject matter and the results obtained, I assess the work presented to me for review very well and request that the student be awarded a distinction.

Kierownik  
Katedry Chemii Leków  
  
prof. dr hab. Michał Marszał