

Kraków, dn. 18.06.2024

THE REVIEW

of the doctoral dissertation entitled:
„Novel approaches for diabetes management by inhibition of DYRK1A kinase”
Author: Agata Barzowska M.Sc.

the work was prepared at Malopolska Centre of Biotechnology:
Jagiellonian University, Krakow, Poland
under the supervision of:
prof. dr hab. Grzegorz Dubin and dr Anna Czarna.

The doctoral dissertation summarizes the effects of four year long work of Agata Barzowska, described in detail in four publications, all published in journals from Journal Citation Reports (JCR) list. In three of them A. Barzowska is the first or the second Author. In the fourth one, she was third, out of ten Authors. Additional activity of PhD. student is shown in five additional publications, which are not directly connected to the subject of the dissertation. The works described here were partially financed from the following sources:

- NCN Grant 2019/34/E/NZ1/00467 (beneficiary: Anna Czarna),
- UJ grant U1U/P03/NO/03.03 (given to B. Pucelik)
- NAWA grant number: PPN/PPO/2018/1/00046/U/00001 (given to A. Czarna).

Summarizing the scientific achievements by the publication activity and based on the data from Web of Science server (checked 18.06.2024) Mrs Barzowska is the



co-author of 12 publications, cited 174 times (without self-citations 164). Her Hirsch index is 5.

The manuscript is prepared in a very aesthetic manner: the dissertation contains of 174 pages in a typical set-up of scientific paper. Main chapters are as follows: abstract, literature review, short summary of research aims and objectives, materials and methods, the longest part: results and discussion, divided into four subchapters. Next: conclusions in the synthetic form, references (348 scientific papers cited) and supplementary materials. It was difficult to find any imperfections in the manuscript. I found only very rare typos, additionally Figure 14 on page 77, has improper description: panel „A” shows levels of C-peptide and glucagon (not „B” as it was described under the figure). There was information at page 84 that: „AC27-treated islets exhibited a 60-fold increase in insulin secretion” while, as it was presented in Figure 18, it should be ca. 60% increase comparing to controls. Also, at page 124 there is an error in the units applied. There should be 50µm (micrometers) instead of 50µM (micromoles) used as the size measurement. Lastly, in Figure 46, page 136, there are no tested compounds concentrations given.

The work is devoted to modification of the activity of DYRK1A kinase using low-MW inhibitors. The major goals were as follows:

- identify the best inhibitors or combination of inhibitors out of known, towards kinase of interest,
- prove efficacy of selected inhibitors in *in vitro* model,
- check the influence of selected molecules on the insulin production and proliferation of β-cells in various models of diabetes,
- test inhibitors delivery methodologies to the cells in an organoid model, using two nanoparticle types.

In the presented investigations, results of the expressed research problems were described in details in four subchapters: the group of harmine and its eight derivatives was tested in two *in vitro* models of INS-1E and MIN6 β-cells cultures. Another group of tests was performed on isolated mouse islets. Then the group of four leucettines was tested in similar experimental conditions. The third, big analytical problem stated in the dissertation, touched encapsulation of DYRK1A inhibitors and their effective delivery to β-cells. Here MIN6 cell line, isolated mouse islets and cell organoids were used. Which was very important, also animal model of diabetes induced by streptozotocin (on Balb/c mice) was used here. The last set of

experiments, concerning influence on the interaction between PDX1 protein and its regulator: SPOP protein was described in details. The interplay between those proteins leading to elevated survival of the β -cells, as well as elevated insulin gene expression, could be achieved by inhibition of PDX1 degradation mediated by SPOP-induced ubiquitination. This goal could be reached by the selected SPOP inhibitors: 200, 245, 259 and 316 tested here. Similarly to previous experiments, those also were done on MIN6 cells, isolated mouse islets and on *in vivo* studies, treated like preliminary ones.

There are two points, which should be clearly stated here: all experiments in cell cultures were preceded by careful toxicity tests, which is very important for the appropriate model application. Toxicity testing is not as common procedure as it should be, in many other dissertations. The second point is a great variety of methods used during experimental procedures. The following methods were used to finalize the investigations: cytotoxicity assays, reverse transcription and RT-PCR, flow cytometry, western blotting, ELISA assays, isolated islets model, 2D and spheroids cell culture models, differentiation of hiPSC into pancreatic cells, transmission electron microscopy, atomic force microscopy, infrared and Raman spectroscopies, DLS, BrdU tests, fluorescent staining and others. Even if the Author of the dissertation is not an expert in every of the listed techniques, the quantity of the data, which must have been processed and understood, especially in the context of the methodological principles, is impressive.

All results, described in detail in the document, were published already in high-quality journals, so every result presented underwent already careful assessment by the Reviewers designated by Journal's Editors. Therefore, I consider it pointless to re-review published material. However, due to my duty as a reviewer of the doctoral dissertation, treated it as a complete work, some questions and minor doubts arose to me while reading the studies So, I would like to express them below.

1. Widely used harmine is a natural psychedelic, being also a strong monoamine oxidases (MAO) inhibitor. The substance can be isolated from *Peganum harmala*, *Banisteriopsis caapi* or other plants and, in its original form it would be rather difficult to use it as a pharmaceutical in the diabetes treatment. On the other hand, some derivatives of harmine are currently used as antidepressant drugs.

Do remaining DYRK1A inhibitors, like AC27 or others, have similar, psychedelic properties?

2. Some DYRK1A inhibitors, like harmine and AC27 look to be strong proliferation stimulants, as proven in Figure 11. Were there any signs of cells overgrowth or mutagenesis observed during the experiments?

3. Which strategy, in the personal opinion of the dissertation Author, would be more prospective for diabetic patient treatment:

a.) direct influence on the β -cells by DYRK1A inhibitors, using eg. targeted drug delivery systems, or

b.) controlled differentiation and stimulation of patient's-derived hiPSC before reimplantation into the patient body as mature β -cells in the form of islets?

Additionally to this question: one of the crucial problems is the constant interaction of the β -cells with activated lymphocytes in autoimmunization being the cause of diabetes type I. Are there any current ideas on how to solve this problem?

4. The phenomenon observed in Figure 45: significantly higher cell viability after application of compound 259 at doses 1.0 and 2.5 μ M looks like a technical error, mainly because it seems to be independent on the dose. Was this observation confirmed in the separate experiment? How to explain this phenomenon if the technical error is excluded?

5. I found the comment in the manuscript (page 145) that after four doses of 40 mg/kg bw of compound 259, all mice taken to the experiment suddenly died. Is it known what was the reason for such an acute reaction? And, which is more important: how far from LD₅₀ was a tenfold lower dose applied in the following experiments?

6. The final comment: I was astonished that, despite a lot of analytical techniques being used, there was no proteomic approach applied. In my opinion, proteomic analysis would significantly help in gaining a broader view of how the cells answer towards pharmacological interventions applied in the experiments.

Regardless of my remarks and comments, I believe that the work has made a valuable contribution to knowledge related to the problem of diabetes therapeutic strategies. The findings are important to scientists working on the problem, as well as for the wide group of patients, hoping to receive more effective therapy than known and routinely used to date. To make up for the formalities, assessed here doctoral dissertation, written by Agata Barzowska, M.Sc. meets all conditions and requirements specified in the Act: „Prawo o Szkolnictwie Wyższym i Nauce (Dz.U.

z 2018 r. poz. 1668 z późniejszymi zmianami)". Based on the scientific achievements of the Candidate, I ask the Members of the Biological Sciences Discipline Council, Jagiellonian University, Krakow for further proceeding, finally leading to the awarding of a doctoral degree of Agata Barzowska in the field of life sciences, the discipline of biological sciences.

Finally, taking into account a broad array of results received, a therapeutically important field of investigations, the direct perspective of the potential application of received results, involvement in the important social problem, and good publication history of the Candidate I am confident that the doctoral thesis of Agata Barzowska fulfil all requirements and merits to receive the distinction.

Sincerely:



prof. dr hab. Piotr Suder