

Zabrze/ 20-09-2017

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Review of the PhD thesis of Marek Grosicki entitled "Evaluation of the effect of histamine and new histamine H₃ and H₄ receptor ligands on the human eosinophils adhesion to endothelium"

Histamine is a phylogenetically old amine which plays a role in many physiological and pathological conditions as a neurotransmitter and inflammatory mediator released by neurons, mast cells, basophils, gastric enterochromaffin-like cells and other cell types, including endothelial cells. Upon release, histamine acts locally as an intercellular hormone (autacoid). Its pleiotropic effects result from activation of four types of G-protein-coupled receptors (H₁-H₄). Histamine is able to influence many functions of the central nervous system as well as increases gastric secretion, induces vasorelaxation and increases vascular permeability. It is also involved in inflammatory processes, such as cellular chemotaxis, migration and trafficking into the site of inflammation.

The thesis addresses highly innovative area of the effects of histamine on the eosinophils adhesion to the endothelium and the role of H₃ and H₄ receptors in this process. The studies were carried out mainly at the Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University, Kraków and at the Jagiellonian Center of Experimental Therapeutics, Kraków under supervision of Prof. dr hab. Katarzyna Kieć-Kononowicz, who is a well known expert and authority in the field of histamine and of histamine H₃/H₄ receptor ligand synthesis.

This PhD thesis consists of 8 main chapters, including *Appendices* containing reprints of three papers and one textbook chapter which are the basis of the dissertation.

In the *Introduction*, the Author described histamine and its receptors, with the special role in the inflammatory processes, as well as the mechanisms of leukocytes adhesion to the endothelium.

The aim of the study was to develop a new *in vitro* pharmacological bioassay of human eosinophils adhesion to the endothelium which would be applied to investigate the effect of histamine and selected histamine receptor ligands on eosinophils adhesion to the endothelium.



The thesis contains a large body of high quality research work obtained with a variety of relevant, sophisticated techniques and models. To achieve the purposes of the study, highly purified eosinophils were isolated from the fresh human blood through the dextran sedimentation and Ficoll-Paque density gradient separation, followed by the negative immunomagnetic cell sorting. Isolated cells, prior to the adhesion experiments, were tested against their variability and functionality. This part of the experiment was performed based on tryptofan blue staining, MTS assay, apoptosis studies and eosinophil peroxidase release assay. The results of tested ligands on eosinophils adhesion to the endothelium were evaluated during eosinophils co-culture with human Ea.hy.926 nad hLMVEC endothelial cell lines. The original methods used in this thesis are described in the chapter by Grosicki and Kieć-Kononowicz *Techniques used in pharmacological evaluation of histamine H₄ receptor function on native human eosinophils* (pp209-232) In: *Histamine receptors as drug targets* (Eds. E. Tiligada and M. Ennis). Methods in pharmacology and toxicology. Humana press Springer, 2017.

The author obtained very interesting new data based on the described methodology. He isolated highly purified eosinophils population, which was characterized by preserved functionality. Eosinophil stimulation with histamine and fMLP resulted in a significant dose-dependent increase in the number of adherent cells to the endothelium. Studies with the standard histamine receptor ligands suggest that histamine-dependent eosinophils adhesion to the endothelium is dependent on histamine H₄ receptor activation. The studied adhesion assay was further used in the pharmacological examination of selected H₃ and H₄ receptor ligands.

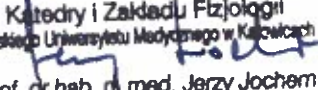
In his thesis, Marek Grosicki demonstrated that all of the tested compounds influence eosinophils adhesion. In addition, several compounds with the most potent action were selected. Finally, the Author performed preliminary studies concerning the evaluation of the mechanisms involved in histamine-dependent eosinophils adhesion to the endothelium. The results presented in the thesis have been published in three high-ranked international journals (*Current Medicinal Chemistry*, *European Journal of Pharmacology* and *European Journal of Medicinal Chemistry*) with Impact Factor 9.783.

The obtained results are exhaustively and critically discussed and the conclusion is supported by experimental data. The Author concludes that the newly developed assay will provide a valuable new tool in pharmacological studies on different receptor ligands on primary, non-modified human cells and could help in the development of new drugs used in the treatment of inflammatory diseases.

The thesis is very well presented – its clear and concise style and good English is worth noting. Performed studies were planned and performed very consistently. It should be mentioned at this point that for last few years I had an opportunity to observe Marek Grosicki, who presented his results during the Annual Meetings of the European Histamine Research Society (EHRS). He is an active member of the EHRS and he participated in the COST BM1007 Short Term Scientific Mission at the Department of Pharmacology and Allergy Unit 'D. Kalogeromitros', Medical School, University of Athens, Greece and in three COST training schools in Dublin (Ireland), Nottingham (UK) and Jerusalem (Israel).

Summing up, the thesis is of high scholarly and literary merit and leaves no doubts that the candidate is aware of the published literature. The thesis successfully demonstrates the competence of its Author to undertake independent research work and the work significantly contributes to the knowledge in the field of the study. Marek Grosicki, in my opinion, deserves to receive the degree of doctor of philosophy.

In addition, I recommend that the doctorate written by Marek Grosicki should be awarded with the distinction *Cum Laude* due to the following aspects: 1) excellent academic quality, 2) originality, 3) an extensive range of research, 4) very well prepared presentation and discussion of obtained results and 5) outstanding scientific activity confirmed by papers published.

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