Summary

The most common cause of dementia among people over 65 is Alzheimer's disease (AD) that is an irreversible and progressive neurological disease. Despite substantial efforts to understand its exact causes and biological mechanisms the etiology of AD remains unknown due to heterogeneous background. There are several concepts describing factors contributing to the development of AD, the leading ones being the cholinergic hypothesis, the β-amyloid (Aβ) aggregation hypothesis, the *tau* protein propagation hypothesis, and the oxidative stress and inflammation hypothesis. Many attempts have been made to develop anti-AD drugs based on these pathomechanisms underlying AD, but for many years only five drugs have been approved for its treatment. AD is characterized by memory loss and behavioral disturbances, often accompanied by neuropsychiatric symptoms such as agitation and aggressiveness. For this reason antipsychotic drugs are also permanently included in the pharmacotherapy of AD in addition to esterase inhibitors (AChEIs, BuChEIs) and memantine.

Drug repositioning strategy has become increasingly popular in the recent years. It enables the introduction of new therapies much faster and cheaper. This strategy uses already registered medicines with a known pharmacological profile, for which the existence of new biological activities has been demonstrated by the results of *in silico* tests and *in vitro* bioanalysis.

A research hypothesis was formulated regarding the influence of antipsychotic drugs on the selected biochemical aspects of AD as part of this doctoral thesis. This research hypothesis was formulated on the basis of literature data from clinical observations of patients with AD and the results of *in silico* studies on the repositioning of antipsychotics. The aim of this *in vitro* research was to evaluate the effect of 7 selected antipsychotic drugs: haloperidol, bromperidol, benperidol, penfluridol, pimozide, quetiapine and promazine, belonging to the N 05 group according to the ATC classification and various chemical groups, on the elements of the three main hypotheses of the development of AD.

• Cholinergic hypothesis - the impact of selected drugs on the enzymatic activity of AChE and BuChE were assessed as well as the type of enzymatic inhibition and potential

interactions occurring *in vitro* between the tested drugs and AChE inhibitors routinely used in AD therapy.

• Aβ aggregation hypothesis - the effect of antipsychotic drugs on three phases of Aβ1-42 aggregation was assessed.

• Oxidative stress hypothesis - the potential antioxidant properties of the tested antipsychotic drugs were assessed under the conditions of induced oxidative stress. The research was conducted *in vitro* in the erythrocyte model and in cell culture using human endothelial cells (*HUVEC*) and astrocytes.

Based on the results obtained from experimental studies, it can be concluded that the tested compounds in the therapeutic concentration range did not demonstrate any significant inhibitory effect on AChE activity, which is contrary to the predictions of *in silico* analyses. However, three of the tested compounds: bromperidol, quetiapine and promazine significantly inhibited BuChE activity according to the *in silico* predictions. Within the tested range of therapeutic concentrations, IC_{50} values could be determined only for quetiapine and promazine relative to BuChE. In the assessed concentration range, the remaining tested compounds inhibited AChE activity by approximately 5 - 10%, and BuChE activity by 4 to 32%, therefore IC_{50} values were not determined for these compounds. The kinetic parameters of enzymatic reactions were determined for standard inhibitors and compounds that most strongly inhibit BuChE activity, thus confirming that donepezil inhibits both AChE and BuChE in a mixed manner (competitive and non-competitive). A mixed type of inhibition was also demonstrated for rivastigmine with regard to AChE, while the inhibition of BuChE activity by rivastigmine turned out to be non-competitive. For the first time, a mixed type of inhibition was demonstrated for quetiapine and promazine in relation to BuChE. The researcher conducted in a binary mixture, assessing the interactions of the tested antipsychotics and routinely used ChEIs, showed the existence of interactions, both synergistic and antagonistic. Mathematical analysis using the Chou-Talalay model and the CompuSyn program also confirmed the existence of these interactions. All tested antipsychotic compounds inhibited the early (10 - 60 min) and late (24 h and 48 h) phases of Aβ aggregation, in a statistically significant manner. The tested antipsychotic drugs showed significant antioxidant potential in both cellular models of antioxidant activity, carried out under conditions of induced oxidative stress.

The results of the research conducted as part of this doctoral dissertation suggest that the use of antipsychotic drugs in patients with AD may bring some clinical benefits, but on

the other hand they may weaken or uncontrollably intensify the effects of ChEIs routinely used in AD therapy. Demonstrating the inhibitory effect of antipsychotics on the formation of Aβ deposits and their antioxidant effects may also provide some benefit to non-AD patients treated with antipsychotics in preventing or slowing the progression of this disease. In conclusion, the obtained *in vitro* test results can be a start-point for in-depth *in vivo* research in an animal model, as well as further clinical observations.