UNIVERSITATEA DE MEDICINĂ,
FARMACIE, ȘTIINȚE ȘI TEHNOLOGIE
„GEORGE EMIL PALADE"
DIN TÂRGU MUREȘ

Iatrogenic pseudoparkinsonism - an animal model of oxidative stress for the evaluation of pharmacological neuroprotection strategies

PhD Coordinator

Prof. dr. Maria Titica Dogaru

PhD Student

George Jîtcă

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Introduction

The side effects of first-generation antipsychotics are well known. Newer compounds, although with an improved pharmacotoxicological profile, have a higher acquisition cost, so the use of conventional antipsychotics remains the first option in many cases. Studies to date have aimed at the effectiveness of treatment and establishing the pharmacotoxicological profile of compounds in the two classes of antipsychotics, without focusing on the possibilities of reducing the central toxic effects of the first generation (extrapyramidal symptoms, dyskinesia, anticholinergic effects). Any intervention that could reduce these side effects of classic antipsychotics is welcome because it increases patient compliance and adherence to treatment, without affecting the therapeutic effect.

The purpose of the doctoral thesis

The general objective of this thesis is to conceive, implement and analyze through the prism of oxidative stress, a model of iatrogenic pseudoparkinsonism, induced by chronic treatment with haloperidol, classic antipsychotic, alone or in combination with metformin. Assuming that the beneficial effects of metformin therapy, presented in various literature studies, extend beyond the scope of metabolic disorders explained by its molecularly mediated antioxidant properties by activating AMPK, combination therapy should reduce the motor side effects of haloperidol.

In order to verify the previously formulated hypothesis, the following studies were performed:

- Development of an animal model of iatrogenic pseudoparkinsonism by administration of haloperidol. Study of the neuroprotective effect of metformin;
- Conducting behavioral tests (catalepsy, rotarode, open field) to confirm the induction of pseudoparkinsonism, respectively to evaluate the beneficial effect of concomitant treatment with metformin;
- Assessment of cognitive function (via the Novel object recognition test and Morris Water Maze) in order to assess the degree of impairment caused by haloperidol, respectively the influence of metformin on working memory;
- Determination of markers of oxidative stress (MDA, GSH / GSSG) and BDNF from biological samples (brain and plasma, respectively). HPLC / DAD analytical methods were developed for the quantitative determination of MDA, GSH and GSSG and BDNF was determined by an ELISA method;
- Histopathological analysis of brain samples to correlate the degree of neuronal destruction with the biochemical values of oxidative stress;

Material and method

Animals and treatment

Haloperidol (Haloperidol Richter 2 mg/ml, Gedeon Richter, Târgu Mureș, Romania), metformin (Glucophage 500 mg, Merck Santé, Semoy, France) were purchased from the Romanian pharmaceutical market. The protective layer of the tablets was removed and the tablets were crushed. The powder was then added to the solvent distilled water to give an extemporae suspension.

Rodents were randomly divided into 4 groups: CTR (control, distilled water, n=10), HAL (haloperidol, n=10), METF (metformin, n=10), HALMETF (haloperidol + metformin, n=10). The treatment was administered for a period of 34 days, daily, between 8:00 and 10:00, using an oral feeding cannula, in a volume of 1 ml/kg, in a separate chamber. Doses, route of administration and duration of treatment were established on the basis of

literature. Also, the sample size for each group was chosen by consulting other similar experiments on rats from the literature.

At the end of the experiment, all animals were decapitated under anesthesia with ketamine/xylazine in a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) to collect biological samples (blood and brain).

2.1. Development of the animal model of pseudoparkinsonism. Behavioral and motor studies

The aim of this study was to investigate the influence of metformin on haloperidol-induced behavioral changes and its ability to prevent or delay the occurrence of Parkinson's symptoms induced by neuroleptic medication in rats by conducting a battery of behavioral tests on motor activity in rats.

The animals were randomly divided into 4 groups, the total duration of the experiment being 34 days, according to Table I. The doses used in this study (Table I) were calculated taking into account both body mass and body surface area ratio to achieve a better correlation with human subjects.

Table I. Groups of animals and doses administered

Abbreviation	Group	Dose
CTR	Control (n=10)	Distiled water
METF	Metformin (n=10)	500 mg/kg
HAL	Haloperidol (n=10)	2 mg/kg
HAL+METF	Haloperidol+Metformin (n=10)	2 mg/kg corp+500 mg/kg

2.2. Assessment of cognitive and memory abilities in relation to oxidative stress

This study was designed to assess and correlate the long-term effects of using haloperidol concomitantly with metformin on cognitive, memory and learning abilities. To assess cognitive abilities, memory and learning ability, the Morris Water Maze and the Novel Object Recognition test were used. These assessment methods were chosen because these methods require an intact hippocampal function, involving several important processes for memorization and learning (encoding, consolidating, preserving, and reusing information). All tests were analyzed using EthoVision XT software (Noldus IT, Wageningen, the Netherlands, version 11.5), by monitoring the distance traveled, swimming speed, latency until reaching the platform, time spent in each dial, etc.

2.3. Determination of oxidative status markers (MDA, GSH/GSSG) and BDNF in biological samples (brain and plasma, respectively)

The method for determining the markers of oxidative status in the brain involves quantifying the level of MDA and the GSH/GSSG ratio (reduced glutathione/oxidized glutathione). For this, two HPLC/DAD methods were validated using a Merck HPLC system. In the present study, validation methods were performed in accordance with regulatory guidelines (FDA 2018). The validation parameters followed were linearity, selectivity, accuracy, precision, lower limit of quantification, stability, and robustness. The methods have been validated so that they can be used to determine oxidative stress in the neural matrix, given that most data in the literature relate to a method of spectrophotometric determination, which has some shortcomings in terms of specificity. At the same time, the study provides insight into the neurotrophic potential of metformin through BDNF. Plasma BDNF quantification was performed using the conventional sandwich ELISA technique according to the manufacturer's instructions (0266F0750, Sigma-Aldrich), using an Elisa Dynex DSX automatic analyzer. In order to evaluate the morphology of the hippocampus, following the applied treatments, a histopathological analysis of the sections in question was performed to correlate the degree of neuronal destruction with the biochemical values of oxidative stress.

General conclusions

In the light of the studies carried out in this doctoral thesis, we managed to draw an image of the oxidative status at the central level, as well as how it can be influenced by the administration of dopaminergic antagonists (haloperidol), respectively substances considered neuroprotective (metformin). This has been possible due to the development of methods for determining the main markers of oxidative stress, such as MDA and GSH, respectively GSSG. The studies described provided information on possible central antioxidant interventions. At the same time, through behavioral diagnostic tests we obtained results that confirm the influence of oxidative stress on motor and cognitive functions.

The originality of the thesis

The subject of this thesis highlights the problem of one of the most common side effects of treatment with classical antipsychotics, but this time from the perspective of medication-induced oxidative stress. At the same time, the reports regarding the neuroprotective effects of metformin, it was desired to develop a model of pseudoparkinsonism induced by haloperidol treatment and to evaluate the main parameters of oxidative stress, respectively behavioral and cognitive evaluation in case of co-administration of metformin.

The paper is a synthesis of how oxidative stress influences brain activity, mainly dopaminergic neurotransmission in the context of neuroleptic medication. The thesis reveals aspects related to pharmacology, biochemistry, toxicology and analytical chemistry, information that can be used for the design of future studies and for the research of other compounds with neuroprotective effect.