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

Study of some atypical antipsychotic`s effect on adipogenesis: contributions to the elucidation of central and peripheral mechanisms.

Scientific coordinator:

Prof. Dr. Imre Zoltán Kun

PhD student:

László-István Bába

Tîrgu Mureș

2021

Introduction: Atypical antipsychotics (AAPs) are drugs that are used in psychiatry being indispensable in the treatment of psychotic diseases. The AAPs are distinct from the classical antipsychotics, their most important adverse events (AE) being of metabolic nature, including dyslipidemia, hyperglycemia, weight gain. The incidence and severity of these AE vary greatly between the different AAPs. The most severe AE are seen after olanzapine (Ola) and clozapine, while paliperidone, risperidone, quetiapine are considered AAPs with AE of medium severity. Aripiprazole (Ari), ziprazidone and lurasidone are considered to bear the least risk for such effects.

Although Ari is considered an AAP, recently it was reconsidered as member of the third generation antipsychotics (also known as *`dopamine stabilizers/modulators`*). This classification takes into account the fact that this drug is no longer thought a neutral antagonist, but rather a partial agonist of the D2/D3 receptors. A recently approved drug with similar mechanism of action is cariprazine (Car). The clinical data regarding this novel AAP`s metabolic adverse effects were sparse at the moment of authorisation, but indicated that it might have favourable adverse effects profile (similar to that of Ari).

The endocannabinoid system (ECS) is a physiological signalling system that has relative recently been described, having a key role in energy homeostasis, both at central (CNS) and peripheral levels (in the different organs and tissues). Activation of this system generally leads to enhancement of energy-sparing mechanisms, including stimulation of adipo- and lipogenesis, increase of appetite and energy intake and concurrent lowering of energy expenditure by various mechanisms. Considering the mentioned characteristics of ECS, it was postulated, that it might play a role in the metabolic adverse effects (mAE) of the AAPs. It was not surprising to see that a number of AAPs induced the characteristic mAE (at least partially) by means of ECS. Important to note, at the time of our experiments, there was no data available in the literature in this regards with Car.

Aims and scope: In this PhD thesis I aimed to study the mechanisms that are involved in the mAE of three AAPs, namely: Ola, Ari and Car. Car represents a new molecule with limited clinical experience. Comparison of its metabolic and behavioral effects with those of Ola, characterized with very intensive metabolic advers effects, and with those of Ari, known for negligible metabolic actions, may contribute with new data to pharmacological and clinical characterisation of Car.

No doubt, the mechanisms involved are complex and include the interaction of numerous central (regulation of hunger/satiety, spontaneous motricity) and peripheral phenomena (the influence on adipose tissue, muscles, and endocrine pancreas), thus I aimed to separately examine these two, applying an experimental design that offers this separation.

Having in mind the importance of the adipose tissue as an endocrine organ, I also aimed to study the effects of the mentioned three AATs on the adipo- and lipogenesis. Another important aim was to investigate the role of peripheral ECS (within the adipose tissue), that might contribute to the metabolic/endocrine mAE of the mentioned drugs.

Materials and methods: This thesis is comprised of four studies. The first study focused on the metabolic and endocrine effects of AAPs, using a rat experimental model of chronic administration. 42 white, adult, female Wistar rats were chosen, in an auto-administration paradigm, to avoid the stress caused by forced administration. After 6 weeks of treatment with AAPs, the animals were sacrificed and the blood lipid levels (serum total cholesterol – TotCHOL, triglycerides - TG) and prolactin levels were measured. To assess the effects of drugs on the white, visceral (perirenal) adipose tissue, the vacuolar area of adipocytes and the expression of SREBP-1 (*sterol response element binding protein-1)*, and UCP-1 (*uncoupling protein-1*) were quantified using immunohistochemistry. In the second experiment I studied the behavioral (anxiolytic and sedative/activating) actions after 4 weeks treatment with the mentioned drugs, using the *‚elevated plus maze'* test. The third study was made to explore the influence of the AAPs on the expression of three key components of the ECS (*cannabinoid receptor 1* – CB1, *fatty acid amide hydrolase* – FAAH and *monoacylglycerol lipase* – MAGL) at the level of adipose tissue. The fourth experiment was designed to observe, under *in-vitro* conditions, the effects of the three AAPs on the process of adipogenesis (maturation of preadipocytes resulting the mature adipocyte) and lipogenesis, *using a mouse embrional fibroblasts* culture. We also determined the concentration of adiponectin (AN) in the cell culture supernatants and the expression levels of peroxisome proliferator activated receptor-γ (PPARγ).

Results: The body weight gain of the animals was accelerated by Ola, compared to the vehicle treated group (Ctr), while the animals treated with Ari and Car showed slower body weight gain, compared to Ctr. Car administered in the higher dose, led to an increase (although not statistically significant) of serum total cholesterol levels. Similarly, the prolactin values were increased by Car, but not significantly, compared to Ctr. All three drugs significantly increased the vacuolar area of adipocytes in perirenal adipose tissue, the greatest changes were seen in the case of the lower dose (1.5 mg/kg) of Ari. Upon the frequency analysis of the vacuole size measurements, we found out, that the reason for the great dispersion of values in case of Ola 1.5 mg/kg treated group was that a great number of cells had small vacuoles. The SREBP-1 tissue levels showed an increasing tendency in the case of Ola and Car, while the administration of the higher dose (3 mg/kg) of Ari resulted in a marked decrease of this paramether. Car significantly increased the tissue level of UCP-1.

Ola influenced the behaviour of the animals by decreasing the time spent on the open arm, in the head dip zone, and also the number of entrances and the preference for the open arm. In the same time, we observed a decreasing tendency of the variables reflecting the interest for the environment, and the general activity of the animals. Concerning the moved distance, significant decrease can be seen after the greater dose of Ola compared to Ctr. Ari displayed an opposite effect to that seen with Ola, leading to increasing tendency of the variables describing the exploratory behaviour and anxiolytic effect. The time spent on-, and the preference for the open arms, time spent in the head dip zone and distance moved, all increased. The mentioned results were not statistically significant, compared to the Ctr, because of the great dispersion of the values and the reduced number of subjects per treatment group (*n=6*). Car induced behavioral effects that resemble that of Ola: slight, insignificant reduction in open arm time, moved distance and head dip time.

Regarding the expression of CB1 receptors, we observed an increasing tendency in the case of animals treated with Ola 1.5 mg/kg, and to a lesser extent with Ari 3 mg/kg and with both doses of Car. The enzymes involved in the breakdown of the cannabinoid mediators were influenced by the all three AAPs. In the case of Car 0.1 mg/kg, the expression level of FAAH was signifficantly increased, compared to Ctr, while the expression of MAGL, was influenced clearly by none of the treatments applied. It is worth to mention, that the values displayed great dispersion (similar to that seen in the case of CB1). Nonetheless, an increasing tendency was evidenced in the case of Ari 1.5 mg/kg and Car 0.25 mg/kg.

The studied substances had marked effects on the adipo- and lipogenesis under *in-vitro* conditions. All three AAPs stimulated the accumulation of TG by the adipocytes, evidenced by the increased optical density of extracted Oil Red O. As regarding the supernatant AN concentration, we observed a significant increase of this adipokine (adipocyte-derived hormone) after treatment with the higher concentration of Ari. This action may be a fundamental contributor to the advantageous metabolic effects. No other treatment regimens did have a clear effect on this parameter. Somewhat surprising, the expression of PPARγ decreased significanty in the cell lines treated with Car 0,1 µM.

Conclusion: In our studies the metabolic effects of the three AAPs were compared, each representing a special subgroup. Car had no pronounced metabolic effects, attenuating the weight gain of the subjects, lowering serum TG levels. In the same time it caused an increase of TotCHOL, and an increasing tendency in PRL levels. The effects on ECS are not clear in the case of this AAP, but the up-regulation of FAAH expression might contribute to the favourable mAE of this drug. It was also observed a possible sedating effect, that might influence the mAE of this AAP.

Ari influenced the weight gain of the animals favourably, in a similar way to Car, but without the effects on the lipid profile. This AAP also displayed a possible anxiolytic effect without sedation. The lowering of SREBP-1 expression in the adipose tissue could be an advantage (in the context of mAE), that has not been described previously.

Ola accelerated the weight gain, and resulted a fall in serum TG levels. The increasing tendency of CB1 receptor expression could offer explanation (to some extent) for the mAE seen with this AAP. All three AAPs increased significantly the adipocyte vacuole size, indicating that these all induce stimulation of the adipo- and/or lipogenesis, depending on the drug.

Under *in-vitro* conditions, Car and Ari have some clear advantages over Ola, that increased the cellular TG content and led to a decreasing tendency of AN content in the cell culture supernatant. All these effects could contribute to the mAE of the studied drugs.

Our results showed that AAPs might interfere with the metabolism in multiple ways: by means of central (altering general activity) and peripheral (adipose tissue-related) mechanisms, too.