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OLANZAPINE INTENSIFIES LIPID PEROXIDATION AND MODULATES CATALASE ACTIVITY IN LIVER OF SOCIAL ISOLATED RATS

A. Stanisavljević, I. Perić, N. Todorović, N. Đorđević and D. Filipović

Laboratory of Molecular Biology and Endocrinology, Vinča Institute of Nuclear Sciences, University of Belgrade,
Mike Petrovića Alasa 12-14, 11001 Belgrade, Serbia (dragana@vinca.rs)

ABSTRACT
Olanzapine is an antipsychotic proved to be effective in stress associated psychiatric diseases, but its effect on the liver, main site of drug metabolism, still remain unclear. We investigated the effects of chronic treatment of olanzapine (three-week) on the malondialdehyde (MDA) content and protein expression and activity of antioxidant enzyme catalase (CAT) in the liver of rats exposed to chronic social isolation (CSIS) (six-week), an animal model of depression. The increased cytosolic MDA content in both vehicle- and olanzapine-treated CSIS animals suggests oxidative stress. Increased CAT activity in vehicle-treated CSIS animals, which was not consistent with its protein expression, suggests induction of antioxidant defense mechanisms, while olanzapine significantly reduced CAT activity in CSIS group. Data revealed that although olanzapine treatment reversed the alterations in CAT activity, it has the ability to cause hepatotoxicity, as indicated by increased MDA content.

INTRODUCTION
Olanzapine, an atypical antipsychotic, is used to treat depressive disorder and schizophrenia [1]. Compared to older antipsychotics demonstrated to cause oxidative stress within the liver [2], the newer atypical antipsychotics, including olanzapine, may have protective properties against oxidative stress. Given that olanzapine is *extensively metabolized in the liver*, its effect on the liver is important. Recently, we have shown that chronic social isolation (CSIS), an animal model of depression, causes oxidative stress in the rat liver [2]. Oxidative stress may induce peroxidation of membrane lipids leading to the formation of malondialdehyde (MDA), where its increased production is associated with different pathological states. Moreover, oxidative stress may also affect protein expression and the activity of antioxidant enzymes, such as catalase (CAT). CAT is in the first line of cellular defense against oxidative damage and degrades hydrogen

peroxide (H_2O_2) into water and oxygen. Thus, we examined the effect of 3 weeks of olanzapine administration (7.5 mg/kg/day) on rat hepatic cytosolic MDA content and protein expression and activity of CAT following 6 weeks of CSIS, which causes depressive- and anxiety-like behavior in adult male Wistar rats.

EXPERIMENTAL

Adult male Wistar rats, 2.5 months old, were used at the onset of the experiment. Prior to CSIS exposure, the animals were housed in groups of four per cage and randomly divided into two groups. Control group consisted of four animals per cage, while rats that underwent CSIS, were individually housed for 6 weeks, during which they had normal auditory and olfactory experience, but were deprived of any visual and tactile contact. Following 3 weeks, both groups were subdivided into vehicle-treated rats receiving daily intraperitoneally (i.p.) injections of normal saline (0.9% NaCl) (Cont+Veh and CSIS+Veh) and olanzapine-treated receiving a 7.5 mg/kg/day of olanzapine-hydrochloride (Cont+Olan and CSIS+Olan). This selected dose of olanzapine in rats is within the therapeutic plasma concentration range in humans [3]. Lipid peroxidation was determined by estimating MDA content by the method which involves its reaction with thiobarbituric acid and spectrophotometric detection at 535 nm [4]. Cytosolic CAT activity was determined by spectrophotometric assays, monitoring the decrease in absorbance at 240 nm, and expressed as μmol of degraded H_2O_2 per minute per milligram of protein, using the extinction coefficient of 43.6 $M^{-1}cm^{-1}$ [5]. The results were analyzed using two-way ANOVA followed by Duncan's post hoc test and expressed as mean ± SEM of 6 rats per group.

RESULTS AND DISCUSSION

MDA content is presented in the Fig.1. Significant increase in hepatic MDA content in both vehicle- and olanzapine-treated CSIS rats as compared to vehicle-treated controls ($^{***}p<0.001$) suggests increased oxidative stress caused by CSIS alone and/or olanzapine treatment.. Interestingly, increased MDA in olanzapine-treated CSIS group compared to vehicle-treated CSIS group ($^{\wedge\wedge\wedge}p<0.001$) or olanzapine-treated controls ($^{\#\#\#}p<0.001$) suggests a synergistic action of olanzapine and CSIS on induction of lipid peroxidation. This synergism could be, at least in part, explained by the olanzapine oxidation to a pro-oxidant radical nitrenium ion [6], which may be produced in the CSIS-induced pro-oxidant environment and may further contribute oxidative damage in hepatic cells.

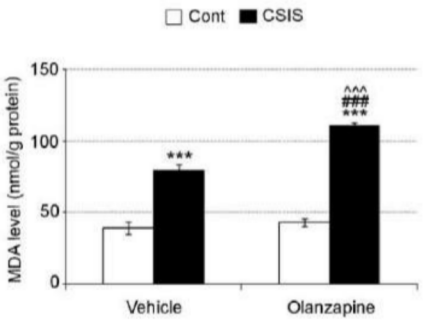

Figure 1. MDA content in liver cytosol of controls (Cont) and rats exposed to chronic social isolation (CSIS) treated with vehicle (0.9% NaCl) or olanzapine-hydrochloride (7.5 mg/kg/day). Symbols indicate differences as follow: $^{***}p<0.001$ CSIS+Veh or CSIS+Olan *vs.* Cont+Veh, $^{###}p<0.001$ CSIS+Olan *vs.* Cont+Olan; $^{\wedge\wedge\wedge}p<0.001$ CSIS+Olan *vs.* CSIS+Veh.

Protein expression and activity of hepatic cytosolic CAT are shown in Fig. 2 A and 2 B, respectively. Regard to CAT protein expression, two-way ANOVA followed by Duncan's post-hoc test revealed no significant differences between all tested groups of rats.

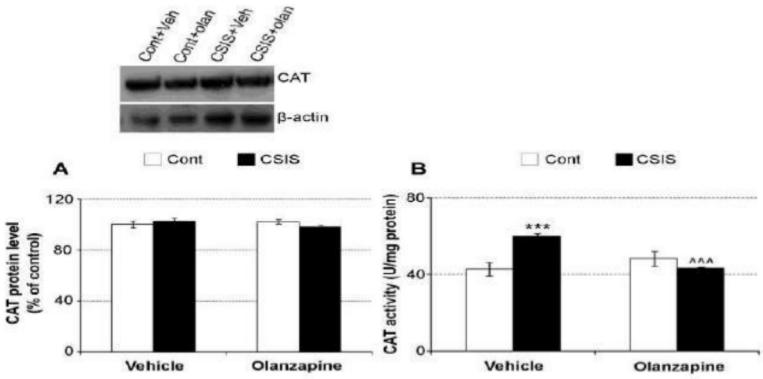

Figure 2. Hepatic cytosolic CAT protein levels (A) and activity (B) (U/mg protein) of controls (Cont) and rats exposed to chronic social isolation (CSIS) treated with vehicle (0.9% NaCl) or olanzapine-hydrochloride (7.5 mg/kg/day). Symbols indicate differences as follow: $^{***}p<0.001$ CSIS+Veh *vs.* Cont+Veh; $^{\wedge\wedge\wedge}p<0.001$ CSIS+Olan *vs.* CSIS+Veh.

Significant increases of CAT activity could be compensatory increase for meeting the demand to convert overloads H_2O_2 in the liver of CSIS rats. After olanzapine treatment in CSIS rats, CAT activity went back to the

normal level, which may indicate that olanzapine significantly reduces H_2O_2 and oxidative stress in the liver. Lack of correspondence between CAT protein expression and its activity in vehicle-treated CSIS group may be consequence of the presence of large amounts of its substrate, stress-induced H_2O_2.

CONCLUSION

Our results suggest increased oxidative stress in the liver of CSIS rats, judged by increased cytosolic MDA content and CAT activity. Olanzapine treatment had no any detrimental effect on the liver of control animals but it failed to alleviate CSIS-induced hepatic lipid peroxidation and this mechanism has yet to be worked out. We found that olanzapine down regulates the activity of CAT which reflects that olanzapine significantly reduced H_2O_2 and hepatic oxidative stress.

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