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RESEARCH ARTICLE

Chronic treatment of haloperidol induces pathological changes in striatal neurons of guinea pigs: a light and electron microscopical study

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Abstract

In the present work, we investigated whether there would be any change in histological structure of striatal neurons after haloperidol applications at different doses. Adult male guinea pigs were treated once-daily with saline (group 4, control) or haloperidol during 6 weeks, and the dose was 1, 2, or 3 mg/kg (groups 1, 2, and 3, respectively). After treatment, all animals were anesthetized and striata were dissected and examined. When striata were evaluated histologically, dark neurons and some degenerating striatal neurons had distinctive morphological changes consistent with cell death, including reduced neuronal size with nuclear and cytoplasmic shrinkage. Also, in sections of striata in groups 1 and 2, but not in group 3, more glial cells were observed than in those of the control group. In all treated groups, fibrous content of intersititium was paralelly increased by increasing dose. Ultrastructural investigation of striatal neurons in haloperidol-treated rats showed notched nuclei and many lysosomes. Moreover, degeneration of myelin, scarce microglial macrophages, expansion of nuclear intermembranous space, degenerated mitochondria, and vacuoles were found. Also, cytoplasmic swelling, lysosomes, and apoptotic bodies were present. These results suggest that haloperidol treatment may lead to damage in neurons via the necrotic process in both low- and high-dose applications.

Keywords: Haloperidol, striatum, histology, electron microscopy, guinea pig

Introduction

Antipsychotic medications have been available since the mid-1950s. They have greatly improved the outlook for individual patients. These medications reduce the psychotic symptoms of schizophrenia and usually allow the patient to function more effectively (Lacasse et al., 2006). Antipsychotic drugs are often very effective in treating certain symptoms of schizophrenia, particularly hallucinations and delusions. The drugs may not be as helpful with other symptoms, such as reduced motivation and emotional expressiveness (Lacasse et al., 2006).

The older antipsychotics, medicines such as haloperidol (Haldol), in the treatment of acute psychotic states may even produce side effects that resemble the more difficult-to-treat symptoms. Behl et al. reported that chronic high-dose haloperidol was neurotoxic and caused striatal oxidative stress in rodents (Behl et al., 1996).

In the study of Andreassen et al. (2003), haloperidol decanoate was administered to rats, and the development of vacuous chewing movements (VCMs) was observed. Also, rats with high and low levels of VCM shown reduced nerve cell number and atrophic neurons and prominent features in the substantia nigra rats with high levels of VCM. Some alterations were also present in the substantia nigra of the old rats.

Thus, the toxic effects of haloperidol on different brain sides, such as the prefrontal cortex, hippocampus, striatum, and cerebral cortex, are well known (Martins et al.,

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2008). But, dose levels provoking these side effects and mechanisms of neuronal injury are not clear (Shivakumar and Ravindranath, 1992).

In our study, some common hypotheses were tested histopatologically regarding the potential effects of chronic haloperidol treatment at different doses.

Methods

Experimental design and applying the drug

In the present study, 20 adult male guinea pigs (from the Ataturk University Experimental Researching and Applying Center) were kept on a 12-hour light-dark cycle, with food and tap water available *ad libitum*. The animals (5 guinea pigs for each experimental group) were intraperitoneally (i.p.) treated once-daily (at 9:00 a.m.) with saline or haloperidol in different doses for 6 weeks, according to the following schedule: 1) haloperidol 1 mg/kg i.p. (low-dose group); 2) haloperidol 2 mg/kg i.p. (medium-dose group); 3) haloperidol 3 mg/kg i.p. (high-dose group); and 4) saline vehicle i.p. (control group). All experimental protocols were approved by the Ethical Committee of Ataturk University.

Drugs and chemicals

HALDOL Decanoate 100 was obtained from Ortho-McNeil Pharmaceutical (San Bruno, California, USA).

Perfusion and fixation

Subsequent to drug treatment, all animals were anesthetized by a short inhalation of ether, then perfused intracardially. Initially, a 0.9% saline (30-mL) solution was given, followed by a mixture of 2% paraformaldehyde and 2% gluteraldehyde (150 mL) in 0.1 M of phosphate buffer (pH 7.4) for approximately 30 minutes at room temperature. Brains were removed and stored in the same fixative overnight at 4°C (Unal et al., 2002).

Histological procedure

On the following day, the striatum in each brain was dissected out, as described previously (Ozbek et al. 2010), and striatal tissue samples were postfixed in 1% osmium tetraoxide for 1 hour, dehydrated through a graded acetone series, and embedded in Araldite CY212. Each Araldite-embedded sample was cut into semithin sections (1 μm) with the Nova Ultratome (LKB, Bromma, Sweden), and slides were stained with toluidine blue. Then, ultrathin sections, at 70–80 nm in thickness, were taken onto cupper grids and were contrasted with uranyl acetate and lead citrate. Semithin sections were observed under a light microscope (Nikon Eclipse 600; Nikon Co., Tokyo, Japan), and ultrathin sections were evaluated under an electron microscope (Jeol 100 SX; Jeol, Tokyo, Japan).

Results

Light microscopic results

The normal neuronal view was observed in the striatal regions from the toluidine-blue–dyed sections of the control group by the light microscope; when evaluating neuronal structure, the nucleus was large in size, with dispersed chromatin and prominent nucleoli, and the neuroplasm was metachromatic because of extensive ribosomal RNA. Nisll substance (GER), as dark purple material, revealed a granular appearance; nuclear DNA had loose staining properties (Figure 1A). In the low-dose group, there were dark neurons among healthy ones (Figure 1B). Light microscopic investigation of the medium-dose group showed that, proximately, all neurons were dark colored. In the light microscopic section of high-dose-haloperidol–administred rats, degenerating striatal neurons had distinctive morphological changes consistent with cell death, including reduced neuronal size with nuclear and cytoplasmic shrinkage (Figure 1D). Also, in sections of striata in groups 1 and 2, but not in group 3, more glial cells were observed according to those of the control group. In all treated groups, fibrous content of intersititium was paralelly increased by the risen dose (Figure 1B–D).

Electron microscopic results

Corpus-striatum sections of the all groups were examined under a transmission electron microscope. The ultrastructural appearance of the cytoplasmic organelles and nuclear components of striatal neurons was normal in the control group (Figure 2A and B). In the ultrastructural evaluation of group 1, striatal neurons had notched nuclei. In neurons of this group, more lysosomes were observed than those in the control group. In addiditon to these findings, mild degeneration of myelin and scarce microglial macrophages were found. The microglial macrophages contained electron-dense nuclei, dilated cistarnae of the endoplasmic reticulum, and many lysosomes (Figure 2C and D).

When sections of group 2 were ultrastructurally evaluated, expansion of the nuclear intermembranous space was detected, whereas the outer nucleus membrane was blebbed to the cytoplasm and severe myelin degenerations had occured. In the cytoplasm, degenerated mitochondria and vacuoles were present. Also, in the striata of group 2, there were edematous changes in the perivascular and -nuclear sites (Figure 3A–D).

In the ultrathin sections of the high-dose-haloperidol–admistred group (group 3), the nuclei of neurons were smaller than those of the previous groups, their chromatine was electron dense, and boundaries were irregular. In the striatal neurons of this group, cytoplasmic swelling, secondary lysosomes, and degenerations of myelin were observed. The areas of perivascular edema cuffs surrounding the capillaries were enlarged according to group 2. Moreover, apoptotic bodies and active microglial macrophages (i.e., phagocyting ones) were present in the sections of group 3. Nuclei of astrocytes were acutely notched and the cytoplasmic membrane of the cells was degenerated by budding (Figure 4A–D).

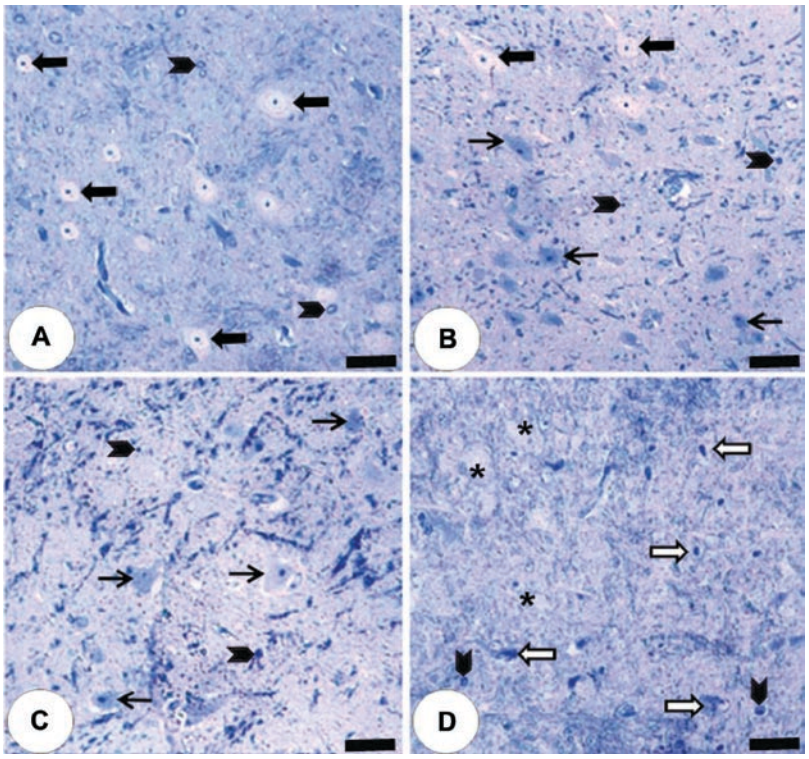

Figure 1. Light micrographs of control (A), low- (B), medium- (C), and high-dose (D) groups. Thick arrows, neurons with healthy appearance; thin arrow, dark neurons; black arrowheads, glial cells; arrows with white filling, degenerated neurons; asterisks show fibrous content of intersititium. Dye: toluidine blue; magnification bars: 20 μm.

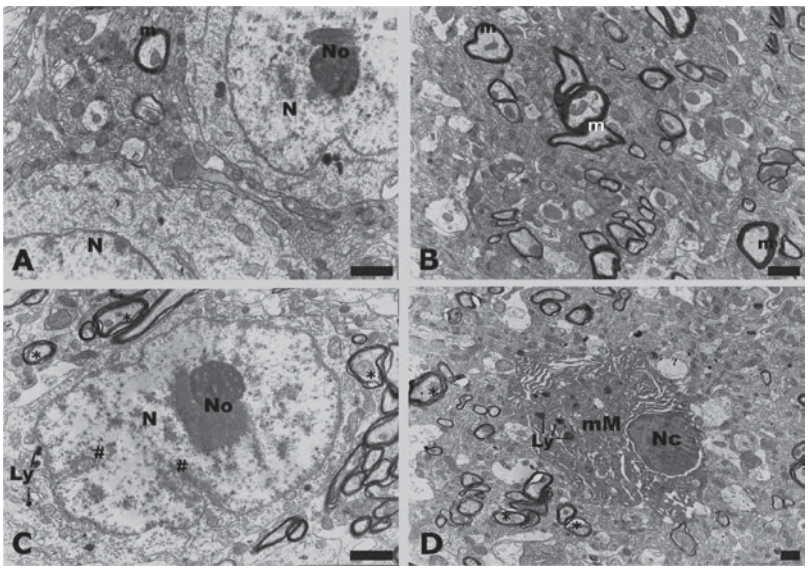

Figure 2. Electron micrographs of control (A,and B) and low-dose (C and D) groups. N, nucleus; No, nucleolus; m, myelin with healthy appearance; Ly, lysosome; #, deep notch of nuclear membrane in striatal neurons; mM, microglial macrophage; Nc, nucleus of microglial macrophage; asterisks show degenerated myelin. Dye: uranyl acetate, lead citrate; magnification bars: 1 μm.

Discussion

Neuroleptic drugs are used in the treatment of severe psychiatric disorders, especially schizophrenia. However, their long-term use may be limited by unwanted motor side effects, such as tardive dyskinesia, parkinsonism, and akathisia (Geptiremen et al., 2004).

Although the mechanism of haloperidol-induced cell degeneration is not well understood, our previous report indicated that one of the possible reasons for cause of damage is the vasoconstrictor effects of haloperidol administration (Kane et al., 1995). Moreover, Merchant et al. pointed out that low-dose haloperidol might be benefical, and the number of cells did not appear to be affected from this treatment (Merchant et al., 1991).

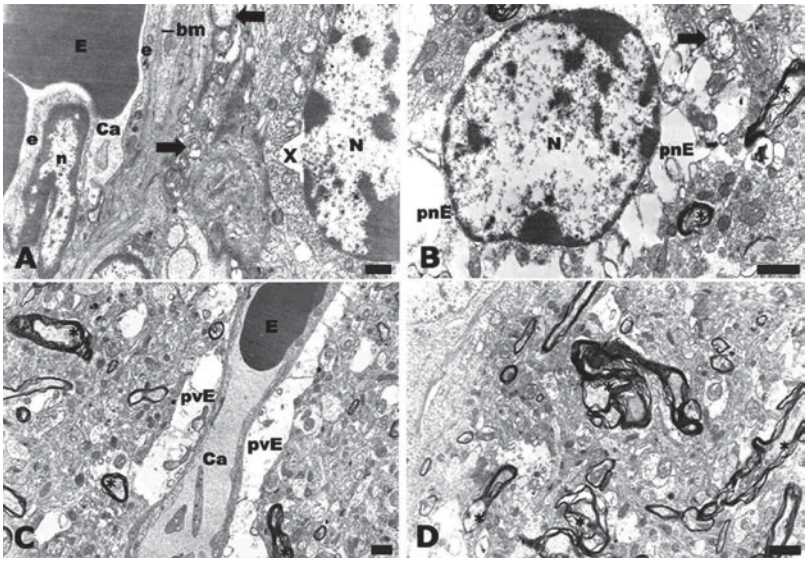

Figure 3. Electron micrographs of the medium-dose group (A–D). E, erythrocyte; e, endothelial cell line; n, nucleus of endothelial cell; N, nucleus of neuron; Ca, capillary; bm, basal membrane; arrow, degenerated mitochondrion; x, expansion of nuclear intermembranous space; pvE, perivascular edema; pnE, perinuclear edema; arrows, degenerated mitochondria; asterisks show degenerated myelin. Dye: uranyl acetate, lead citrate; magnification bars: 1 μm.

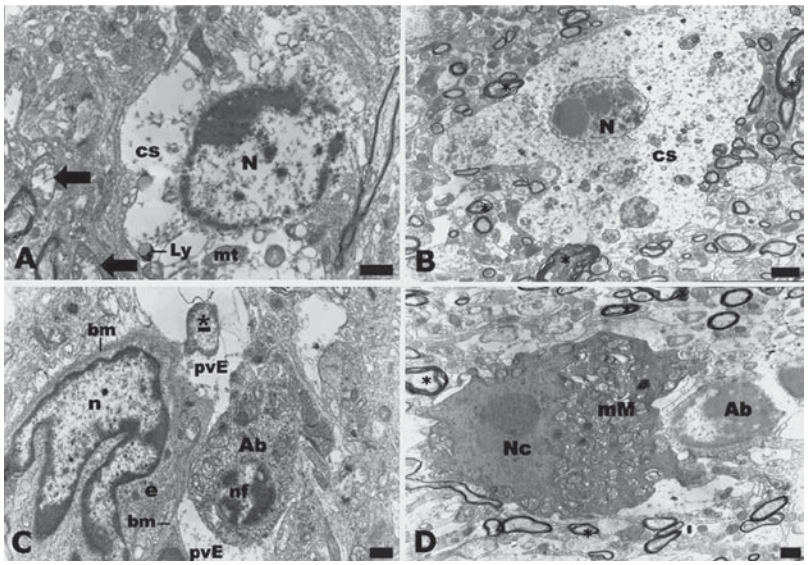

Figure 4. Electron micrographs of the high-dose group (A–D). N, nucleus of neuron; Ly, lysosome; mt, mitochondrium; cs, cytoplasmic swelling; arrows, degenerated mitochondria; bm, basal membrane; pvE, perivascular edema; n, notched nucleus of astrocyte; mM, microglial macrophage; Ab, apoptotic body; nf, nuclear fragment; Nc, nucleus of microglial macrophage; underlined asterisks, budding cytoplasm of astrocyte; asteriks show degenerated myelin. Dye: uranyl acetate, lead citrate; magnification bars: 1 μm.

Cesario reported that intravenous injection of haloperidol caused a serious uneasiness with muscular rigor, perspiration, high blood pressure, and serious shortness of breath with cyanosis (Cesario, 2008). According to the view of Seitz and Gill, neuroleptic malignant syndrome (NMS) is a potentially fatal adverse event associated with the use of antipsychotics. Also, they said that NMS was developed in cases that involved men with agitated delirium who received relatively high doses of parenteral haloperidol (Seitz and Gill, 2009).

In this study, first, it was limited to a 42-day period. In rats, a 42-day chronic treatment period in patients corresponds to approximately 6 years of treatment and this study may have imitated chronic haloperidol treatments in humans (Wakade et al., 2002), and, second, this study investigated the effects of different doses of haloperidol on the general and fine structure of the corpus striatum of guinea pigs.

According to our results, there were dark neurons, reduced neuronal size, and more glial cells, suggesting a neuronal death process, and that both more glial cells and a dose-paralell increase in the fibrous content of the intersititium may have been a sign of scar formation, instead of the dead cells. Ultrastructurally detected notched nuclei

and greater lysosome degeneration of myelin supported our opinion regarding cell degeneration in the low-dose group. Already, the presence of microglial macrophages may point to the phagocytosis of degenerating cells. As we have shown in electron microscopical results, myelin degeneration was advanced in the high-dose group. Also, in the striatal neurons of this group, small nuclei, with their electron-dense chromatine, cytoplasmic swelling, secondary lysosomes, perinuclear edema, degenerated mitochondria, and vacuoles, were clearly showing a mean cellular death cascade. Likewise, detected apoptotic bodies in this group proved that haloperidol caused cell death at the higher dose. Also, both light microscopically detected dark neurons and electron microscopically observed apoptotic bodies indicated that haloperidol may lead to cell death by the apoptotic pathway.

The presence of perivascular edema, notched nuclei of endothelial cells, and astrocytes may indicate damage in the blood-brain barrier.

Reports of some studies have confirmed our data that treatment with haloperidol may have side effects on neurons (Kondo and Iwatsubo, 1980; Post et al., 2002). It has also been reported that treatment with these agents may have side effects, such as neurodegeneration or death of neorons, in the hippocampus (Unal et al., 2004; Lezoualc'h et al., 1996), striatum (Altunkaynak et al., 2011), and medial prefrontal cortex (Bardgett et al., 2002). Results of the study of Zhuravliova et al. (2007) suggest that haloperidol induces neuronal cell death by interaction with the *N*-methyl-*D*-aspartate receptor.

The pathway of haloperidol-induced cell death has been discussed previously. Some researchers have reported that haloperidol increases p53 expression, leading to apoptosis (Post et al., 2002). A preliminary study was accompanied by an increase in the number of apoptotic cells in the striatum (Mitchell et al., 2002). Other studies have reported on haloperidol-induced cell death by apoptosis (Crawford and Bowen, 2002).

Cell loss in the treated groups could have been caused by excitotoxic cell death, because long-term haloperidol administration has been reported to increase striatal glutamatergic activity (Yamamoto and Cooperman, 1994; Andreassen et al., 1996). In a separate study, Skoblenick et al. (2006) reported that cellular DNA was damaged after haloperidol treatment in both humans and rats. Oxidative stress could also play a role in toxic response. Haloperidol induces free radicals *in vitro* (Behl et al., 1996), and clinical studies have shown increased markers of oxidative stress in schizoprenic patients (Tsai et al., 1998) and beneficial effects of antioxidative treatment (Post et al., 2002).

We thought, on this subject, that all the possible mechanisms mentioned above were triggered by arterial vasoconstriction, as pointed out previously (Geptiremen et al., 2004). Present histological data show that haloperidol causes neurodegeneration through apoptosis in the striatum, as previously reported (Post et al., 2002). Thus, haloperidol may lead to neuronal death by means of both ischemic necrosis caused by vasoconstriction (Geptiremen et al., 2004) and apoptosis by caspase activation (Pillai et al., 2008).

Conclusion

In conclusion, haloperidol is a substance that is toxic to neurons, and it is possible that a cognitive impairment might be expected as a result of striatal neuron loss after not only high-dose, but also low-dose chronic haloperidol treatment. Consequently, in clinical settings, neuroleptic treatment with haloperidol should be avoided, even in a lower dose.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

References

Altunkaynak, B. Z., Ozbek, E., Aydin, N., Aydin, M. D., Altunkaynak, M. E., Vuraler, O., et al. (2011). Effects of haloperidol on striatal neurons: relation to neuronal loss (a stereological study). Folia Neuropathol 49:21–27.

Andreassen, O. A., Aamo, T. O., Jùrgensen, H. A. (1996). Inhibition by memantine of the development of persistent oral dyskinesias induced by long-term haloperidol treatment of rats. Br J Pharmacol 119:751–757.

Andreassen, O. A., Ferrante, R. J., Aamo, T. O., Beal, M. F., Jørgensen, H. A. (2003). Oral dyskinesias and histopathological alterations in substantia nigra after long-term haloperidol treatment of rats. Neuroscience 122:717–725.

Bardgett, M. E., Humphrey, W. M., Csemansky, J. G. (2002). The effects of excitotoxic hippocampal lesions in rats on risperidone- and olanzapine-induced locomotor suppression. Neuropsychopharmacology 27:930–938.

Behl, C., Lezoulac'h, F., Widmann, M., Rupprecht, R, Holsboer, F. (1996). Oxidative stressresistant cells are protected against haloperidol toxicity. Brain Res 717:193–195.

Cesario, V. (2008). Acute extrapyramidal syndrome and neuroleptical malignant syndrome. A case report. [Article in Italian]. Recenti Prog Med 99:146–148.

Crawford, K. W., Bowen, W. D. (2002). Sigma-2 receptor agonists activate a novel apoptotic pathway and potentiate antineoplastic drugs in breast tumor cell lines. Cancer Res 62:313–322.

Geptiremen, A., Aydin, N., Halici, Z., Sahin, O., Unal, B., Aydin, M. D., et al. (2004). Chronic treatment of haloperidol causes vasoconstriction on basilar arteries of rats, dose dependently. Pharmacol Res 50:569–574.

Kane, J. M. (1995). Tardive dyskinesia: epidemiology and clinical presentation. In: Bloom, F. E., Kupfer, D. J. (Eds.), Psychopharmacology: the fourth generation of progress (vol. 39, pp. 1485–1495). New York: Raven Press.

Kondo, Y., Iwatsubo, K. (1980). Diminished responses of nigral dopaminergic neurons to haloperidol and morphine following lesions in the striatum. Brain Res 181:237–240.

Lacasse, H., Perreault, M. M., Williamson, D. R. (2006). Systematic review of antipsychotics for the treatment of hospital-associated delirium in medically or surgically ill patients. Ann Pharmacother 40:1966–1973.

Lezoualc'h, F., Rupprecht, R., Holsboer, F., Behl, C. (1996). Bcl-2 prevents hippocampal cell death induced by the neuroleptic drug haloperidol. Brain Res 738:176–179.

Martins, M. R., Petronilho, F. C., Gomes, K. M., Dal-Pizzol, F., Streck, E. L., Quevedo, J. (2008). Antipsychotic-induced oxidative stress in rat brain. Neurotox Res 13:63–69.

Merchant, K. M., Miller, M. A., Ashleigh, E. A., Dorsa, D. M. (1991). Haloperidol rapidly increases the number of neurotensin mRNA-expressing neurons in neostriatum of the rat brain. Brain Res 540:311–314.

Mitchell, I. J., Cooper, A. C., Griffiths, M. R., Cooper, A. J. (2002). Acute administration of haloperidol induces apoptosis of neurones in the striatum and substantia nigra in the rat. Neuroscience 109:89–99.

Ozbek E, Altunkaynak BZ, Aydin MD, Aydin N, Vuraler O, Unal B (2010). Haloperidol-Induced Neuronal Damage in Guinea Pig Hippocampus: A Microscopic Study. J. Neurol Sci [Turk], 27: 438–445.

Pillai, A., Dhandapani, K. M., Pillai, B. A., Terry, A. V., Jr., Mahadik, S. P. (2008). Erythropoietin prevents haloperidol treatment-induced neuronal apoptosis through regulation of BDNF. Neuropsychopharmacology 33:1942–1951.

Post, A., Rucker, M., Ohl, F., Uhr, M., Holsboer, F., Almeida, O. F., et al. (2002). Mechanisms underlying the protective potential of alpha-tocopherol (vitamin E) against haloperidol-associated neurotoxicity. Neuropsychopharmacology 26: 397–407.

Seitz, D. P., Gill, S. S. (2009). Neuroleptic malignant syndrome complicating antipsychotic treatment of delirium or agitation in medical and surgical patients: case reports and a review of the literature. Psychosomatics 50:8–15.

Shivakumar, B. R., Ravindranath, V. (1992). Oxidative stress induced by administration of the neuroleptic drug haloperidol is attenuated by higher doses of haloperidol. Brain Res 595:256–262.

Skoblenick, K. J., Castellano, J. M., Rogoza, R. M., Dyck, B. A., Thomas, N., Gabriele, J. P., et al. (2006). Translocation of AIF in the human and rat striatum following protracted haloperidol, but not clozapine treatment. Apoptosis 11:663–672.

Tsai, G., Goff, D. C., Chang, R. W., Flood, J., Baer, L., Coyle, J. T. (1998). Markers of glutamatergic neurotransmission and oxidative stress associated with tardive dyskinesia. Am J Psychiatry 155:1207–1213.

Unal, B., Bradley, P. M., Sahin, B., Canan, S., Aslan, H., Kaplan, S. (2002). Estimation of numerical density and mean synaptic height in chick hippocampus 24 and 48 hours after passive avoidance training. Dev Brain Res 136:135–144.

Unal, B., Özbek, M. E., Aydın, M. D., Aydın, N., Bulucu, Z., Vuraler, Ö. , et al. (2004). Effect of haloperidol on the numerical density of neurons and nuclear height in the rat hippocampus: a stereological and histopathological study. Neurosci Res Commun 34:1–9.

Wakade, C. G., Mahadik, S. P., Waller, J. L., Chiu, F. C. (2002). Atypical neuroleptics stimulate neurogenesis in adult rat brain. J Neurosci Res 69:72–79.

Yamamoto, B. K., Cooperman, M. A. (1994). Differential effects of chronic antipsychotic drug treatment on extracellularglutamate and dopamine concentrations. J Neurosci 14:4159–4166.

Zhuravliova, E., Barbakadze, T., Natsvlishvili, N., Mikeladze, D. G. (2007). Haloperidol induces neurotoxicity by the NMDA receptor downstream signaling pathway, alternative from glutamate excitotoxicity. Neurochem Int 50:976–982.