

EFFECT OF HALOPERIDOL ON SELECTED OXIDATIVE STRESS PARAMETERS AND DOPAMINE LEVEL IN RATS BRAIN

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تأثير عقار الهالوبيريديل على بعض مؤشرات الإجهاد التأكسدي وعلى مستوى الدوبامين في دماغ الجرذان

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الخلاصة

اجريت الدراسة الحالية لتحديد تأثيرات عقار الهالوبيريديل (عقار نفسي) على بعض مؤشرات الإجهاد التأكسدي (المالونديهايدMDAكلوتاتيون GSH) وعلى مستوى الدوبامين في دماغ الجرذان .
أربعة وعشرون جرذا (ذكر) من نوع Sprague –Dawely ، حيث قسمت عشوائيا إلى ثلاث مجموعات، ثمان جرذان في كل مجموعة.
ضمت الدراسة المجموعة الأولى وأعطيت غذاء الجرذان الطبيعي لمدة ٤٥ يوم وعدت (مجموعة قياسية طبيعية)، المجموعة الثانية أعطيت ماء مقطر عن طريق أنبوب المعدة لمدة ٤٥ يوم وعدت (مجموعة سيطرة) ، اما المجموعة الثالثة فاعطيت عقار الهالوبيريديل (١ملغ /كغم) عن طريق أنبوب المعدة لمدة ٤٥ يوم.
بعد التضحية بالحيوان، تم فصل الدماغ ثم هرسه وقياس مستويات الدوبامين وMDA و GSH. تم التوصل من خلال الدراسة إلى النتائج الآتية :
١. عقار الهالوبيريديل (١ملغ/كغم) ذو قيمة احصائية عالية ($P<0.01$) في مستوى GSH و الدوبامين في دماغ الجرذان (انخفاض مستوياتهما).
٢. عقار الهالوبيريديل (١ملغ/كغم) ذو قيمة إحصائية عالية ($P<0.01$) في مستوى MDA في دماغ الجرذان (زيادة مستواها).
تم الاستنتاج بان عقار الهالوبيريديل يؤثر بشكل فعال على مؤشرات الإجهاد التأكسدي ومخفض عالي لمستوى الدوبامين في دماغ الجرذان .

Abstract

The present study was conducted to assess the effects of haloperidol (psychiatric drug) on some oxidative stress parameters (GSH and lipid peroxidation product MDA) and it's relation to the dopamine level in rats' brain.

Twenty-four male Sprague- Dawely rats were enrolled in this study. They were randomly separated into three groups, eight rats in each group: Group 1, (normal control group), eight rats were put on normal chow diet for 45 days from which the baseline value of selected parameters were measured. Group 2 (self control group) on distilled water orally administered by stomach tube for 45 days. Group 3 on Haloperidol 1 mg/kg orally administered by stomach tube for 45 days.

After decapitation, brain were removed from rats and homogenized from which GSH, MDA, and dopamine levels were measured. This study revealed the following results.

- 1- Administration of haloperidol was highly significant (P value < 0.01) decreased the GSH and dopamine level in rats' brain.
- 2- Administration of haloperidol was highly significant (P value < 0.01) increased the MDA levels in rats' brain.

It was concluded that haloperidol is a potent inducer of oxidative stress and highly decreased the levels of dopamine in rats' brain.

Introduction

Oxidative stress:

It defines as an imbalance between oxidants and antioxidants in favour of oxidation that could lead to cellular and molecular damage. {1} It has consequences for cells as a result of one of three factors {2}

- 1- An increase in oxidants generation.
- 2- A decrease in antioxidants production.
- 3- A failure to repair oxidative damage.

Oxidative stress - mediated cell damage occur, in part, via reactive oxygen species (ROS). They are produced continually in most tissue as intermediates in cellular process and respiration, also in the degradation and generation of fatty acids by phagocytes during the destruction of bacteria and virally infected cells {2,3}, Which leads to speculate that increased oxidative stress occurs during inflammatory immune responses.

Reactive oxygen species are highly reactive entities with unpaired electron in their outer orbital {4}. They include molecule like hydrogen peroxide, ions like hypochlorite ions, radicals like hydroxyl radical, and both radicals and ions like super oxide anion {5}. They have extremely unstable configuration and quickly react with other molecules to achieve the stable configuration. Once formed, they participate in a number of reactions, yielding additional free radicals such as peroxynitrite, and hypochlorous acid.

These ROS can cause damage to proteins, lipids, membrane, DNA and alteration in the intracellular environment {6}. ROS are believed to be involved in xenobiotics toxicity, aging of the skin, atherosclerosis, cataract, cognitive dysfunction, neoplastic diseases, diabetics retinopathy, shock, organ dysfunction, neurodegeneration disorder like Alzheimer's' disease and Parkinson's disease. {6,7}

Antioxidant systems:

Antioxidants are molecules that act as free radical scavengers. Most antioxidants are electron donors and react with the free radicals to form innocuous end products such as water. Thus, they protect against oxidative stress and prevent damage to cells {8} **Types of antioxidants:**

- 1- Exogenous antioxidants.
- 2- Endogenous antioxidants.

Some aspects of the tested drugs: Haloperidol:

Haloperidol is a typical potent neuroleptic drug or major tranquilizer. It has been used clinically in psychiatry, obstetrics and anesthesiology {9}. Chemically haloperidol belongs to the butyrophenone series of neuroleptic compound. Neuroleptic drugs or major tranquilizer or mood regulating are used extensively in the treatments of schizophrenia and other affective disorders {9}

Commonly used antipsychotic drugs are:

- 1- Typical antipsychotic.
- 2- A typical antipsychotic.
- 3- Partial dopamine

agonists. Dopamine:

Is one of the principal modulatory neurotransmitters in the brain {10}. Dopamine systems from two primary midbrain clusters, the ventral tegmental area (A 10) and the substantia nigra (A9), which have discrete projections to mesolimbics, mesocortical and striatal regions of the brain. A separate tuberoinfundibular pathway runs from hypothalamus neurons to the pituitary glands {10}

Dopamine pathways in the brain {11} include:

- 1- Mesolimbic-mesocortical pathway.
- 2- Nigrostriatal pathway.
- 3- Tuberoinfundibular pathway.
- 4- Medullary- periventricular pathway.
- 5- Incertohypothalamic pathway.

Dopamine receptors in brain:

There are two main families of dopamine receptors, D1 and D2 which are linked respectively by activation and inhibition of adenylate cyclase. The original D1 family now includes D1 and D5, while D2 family, which is pharmacologically more important in the central nervous system.

Materials and Methods

Preparation of animals:

Twenty four male Spague- Dawely rats were enrolled in this study. The animals were randomly separate into three groups, eight rats in each one. Group 1 (normal control group), eight rats were put on normal chow diet for 45 days from which the baseline value of experimental parameters were measured. Group 2 (self control group) on distilled water orally by stomach tube fore 45 days. Group 3 (haloperidol group) on haloperidol(1 mg/kg) (23), orally by stomach tube for 45 days.

Preparation of

drug: Haloperidol:

It was used in a dose of 1/mg/kg/day {12} P.O.A 5 mg tablet (HALDOL-5, SOBHAN PHARM.CO. IRAN. B.No. 0063000RLS) was dissolved in distilled water and the dose was given to the rats according to body weight once daily through stomach tube for 45 days.

Preparation of sample:

On the 46th day of treatment, the animals were sacrificed by decapitation. The brains were removed after dissection and exposure of skull from foramen magnum posteriorly, olfactory bulbs and cerebellum were dissected and the brain were removed gently from skull and rinsed in isotonic saline and weighted. The sample divided into two equal parts. One of these parts put in ice cool 6 ml of 0.4 perchlorid

acid containing 1% EDTA- 2 NA and 0.1 % sodium metabisulfite (a buffer for dopamine estimation) {114} Other part put in 0.1 M phosphate buffer, pH 7.4 (a buffer for MDA and GSH estimation){13,14}

Reading of sample:

Measurement of brain dopamine level.

Dopamine was determined by the tryhydroxyindole method of change {15}, all the chemical were supplied by Merck Co.Ltd.

Measurement of brain GSH level.

Determination of tissue homogenate glutathione (GSH) level depends on the action of sulfhydryl groups. The used reagents were supplied by Biochemical's Co.Ltd for EDTA and GSH, Sigma Co.Ltd. For DTNB.

Measurements of brain MDA level

Tissue homogenate malondialdehyde (MDA) was determined according to the method of Tomotsu *et al* {14} all the chemicals were supplied by Merck Co.Ltd.

Statistical analysis:

The data expressed as means $\pm$ SEM unless otherwise stated. Statistical analysis had been done by using paired t- test and ANOVA. Significant different was set at a =0.05.

Results

Effect of haloperidol on brain GSH

Brain GSH level was not significantly changed in control group, but highly significant decreased in haloperidol group.

Table (1) Effect of haloperidol (1mg/kg orally for 45 days) on brain GSH level (Micro mol/ mg tissue) in rats. (No=8 in each group).

	GSH level Before treatment	GSH level After treatment	P value
Control group	0.9300 $\pm$ 0.0487	0.9550 $\pm$ 0.04392	P > 0.05
Haloperidol group	0.9300 $\pm$ 0.0487	0.0775 $\pm$ 0.01031	PO.01

The value expressed as
mean $\pm$ SEM

Effect of haloperidol on brain MDA

Brain MDA was not significantly changed in control group, but highly significant increased in haloperidol group.

Table (2) Effect of haloperidol (1mg/kg orally for 45 days) on brain MDA level (nmol/ mg tissue) in rats. (No =8 in each group).

	MDA level Before treatment	MDA level After treatment	P value
Control group	0.4350±0.01861	0.4350±0.01532	P > 0.05
Haloperidol group	0.4350±0.01861	1.2863±0.02232	PO.01

The value expressed as mean ± SEM

Effect of haloperidol on brain Dopamine

Brain Dopamine was not significantly changed in control group, but highly significant decreased in haloperidol group.

The value expressed as mean ± SEM

Table (3) Effect of haloperidol (1mg/kg orally for 45 days) on brain dopamine level (nmol/ mg tissue) in rats. (No =8 in each group).

	Dopamine level Before treatment	Dopamine level After treatment	P value
Control group	0.1238±0.00263	0.1213±0.00259	P > 0.05
Haloperidol group	0.1238±0.00263	0.0231±0.00138	P<0.01

Discussion

Oxidative stress is an important risk factor for the development and progression of various neurological disorders [16]. It occurs due to several factors such as trauma, infection, medication, and radiation [17]. The existing evidence indicates that an imbalanced production of free radicals, decreased antioxidant mechanisms, reduction in dopamine release, and increased dopamine turnover are associated with chronic neuroleptic treatment and might contribute to the onset of extrapyramidal symptoms [18]. Chronic haloperidol-treated animals showed a decrease in brain level of (GSH) and an increase in lipid peroxidation product (MDA) [19] and a decrease in dopamine level [20]. Balijepalli et al (2001) had found that chronic treatment with neuroleptics increases free radical production and oxidative stress [21]. Ichikawa and Meltzer (1991) had found that in freely moving animals, chronic haloperidol administration reduces dopamine release and increases its turnover. [22] The present study attempts to evaluate the effect of chronic haloperidol treatment on selected markers of oxidative stress (GSH and MDA) and dopamine level in rat's brain.

Effects of chronic haloperidol treatment on (GSH and MDA) level in rat's brain.

In the present study, brain GSH level was highly significantly decreased ($p < 0.01$) and MDA level was highly significantly increased ($p < 0.01$) in haloperidol (1 mg/kg, orally, for 45 days) group as compared to control (distilled water, orally, for 45 days) treated group. That is the same finding by Bangalore et al (2002) [23], and Pattipati S. Naidu et al [24]. The molecular mechanism by which haloperidol increases oxygen free radical production is that neuroleptics act by blocking dopamine receptors [25]. Such blockage could result in increased dopamine turnover, which in turn leads to an increased production of hydrogen peroxide. Resulting in oxidative stress [26]. Dopamine is primarily metabolized by oxidation by monoamine oxidase (MAO) to 3, 4-dihydroxyphenyl-acetic acid (DOPAC). This reaction produces hydrogen peroxide radicals [27]. Dopamine is also metabolized by auto-oxidation yielding superoxide radicals [24]. Hydrogen peroxide can further react with iron or copper ions to produce the hydroxyl radicals, which are the most toxic free radicals [28]. Neuroleptics may also have a direct cytotoxic effect via the production of toxic metabolites (Pyridinium metabolites RHPP +) [29]. Also these metabolites may

inhibit (complex 1) of the electron transport chain and may contribute in the production of free radicals. {30}.

Effects of chronic haloperidol treatment on (Dopamine) in rats' brain.

The present study, brain dopamine level was highly significant ($P < 0.01$) in haloperidol (1 mg / kg, orally, for 45 days) group as to control (distilled water, orally, for 45 days) group. These are consistent with Darakhshan J. *et al* (2002){31} and Junghyun Hahn *et al* (2003) {27}. The molecular mechanisms by which chronic haloperidol treatment decreases the level of brain dopamine are that, Dz and Ds autoreceptors (which are responsible for negative feed back inhibition of dopamine release) were blocked by haloperidol, so, increased synthesis and turn over of dopamine by MAO and COMT to its metabolites (HVA and OOP AC) which lead to decrease its level in mid and forebrain. A reduction in glutathione level could prevent the clearance of hydrogen peroxide (H_2O_2) generated from normal dopamine metabolism, so, insufficient clearance of H_2O_2 and the production of hydroxyl free radicals may cause damage to dopamine containing cells {31,27}.

Conclusions:

From the obtained results of this study, the following can be concluded:

Haloperidol significantly induced oxidative stress and decreased the dopamine level in rats' brain.

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Recived (11/5/2008)
Accepted (3/9 /2008)