Modulation of Antipsychotic-induced Oxidative Stress by Selective and Non Selective COX, Nonsteroidal Anti-inflammatory Drugs

Sally A. El-Awdan and Omar M. Abdel-Salam
Department of Pharmacology, National Research Centre, Dokki, Cairo, Egypt

Abstract: Oxidative stress has been implicated in the pathogenesis of diverse disease states and may be a common pathogenic mechanism underlying many major disorders. Use of typical antipsychotics like haloperidol in treatment of schizophrenia is associated with a high incidence of oxidative stress in different body tissues. An inflammatory hypothesis has been suggested for haloperidol-induced tissue disorders which deemed of importance to evaluate these effects. The use of NSAIDs has been advocated as a possible therapeutic intervention aiming to decrease tissue inflammation. Thus, this study aimed at investigating the effect of NSAIDs on haloperidol-induced oxidative stress in different tissues. The treatment groups were as follows: control group (1% v/v of tween 80), haloperidol (2 mg kg⁻¹), haloperidol (2 mg kg⁻¹)+aspirin(100 mg kg⁻¹), haloperidol (2 mg kg⁻¹)+nimestide (10 mg kg⁻¹) and haloperidol (2 mg kg⁻¹)+celecoxib (10 mg kg⁻¹). Drugs were orally administered for 21 days. The results show that haloperidol itself impairs cellular antioxidant status because of the decreased brain, liver and stomach levels of GSH and increased levels of MDA in brain and liver tissues as well as increased NO levels in brain and depleted levels in liver and stomach tissues. In addition, findings in the present study suggest that nimestide and celecoxib in haloperidol-treated rats significantly improved the affected oxidative stress in different tissues.

Key words: Haloperidol, oxidative stress, inflammation, NSAIDs, lipid peroxidation

INTRODUCTION

Recent studies have focused on the possible role of reactive oxygen species in the pathogenesis of several brain disorders e.g., schizophrenia, autism, parkinson's disease. “Oxidative Stress” is defined as a state of higher cellular levels of reactive oxygen species (ROS, e.g., O²⁻, OH, O², NO and ONOO⁻). ROS include both free radical and non free radical molecules (Beal, 1995). Under normal circumstances, ROS are eliminated by cellular enzymatic (Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Glutathione (GSH) and Catalase (CAT)) and non-enzymatic (GSH and uric acid) antioxidant defenses and dietary antioxidants such as vitamins A, E and C, β-carotene, quinones and flavones (Halliwell and Gutteridge, 1989). If ROS are not effectively eliminated, they can cause oxidative cell injury i.e., peroxidation of cell membrane phospholipids, proteins (receptors and enzymes) and DNA (Shiripaa et al., 1992). Thus an imbalance between free-radical generation and radical scavenger systems results in “oxidative Stress” (Coyle and Puttfareken, 1993).

ROS are very reactive molecules which are oxidizing in nature, including free radicals and free radicals are chemical species that have single unpaired electrons in their outer orbit. They may be initiated with in cells in several ways such as Radiant energy (UV light, X-rays (Ravindranath and Reed, 1990). Haloperidol is a widely prescribed typical antipsychotic for treatment of schizophrenia and other effective disorders (Granger and Albu, 2005). Haloperidol belongs to a class of antipsychotics known as the first generation antipsychotics, sometimes referred to as conventional or typical antipsychotics. It is classified as a highly potent neuroleptic which has pro-oxidant properties (Jeding et al., 1995; Arvindakshan et al., 2003). Chronic treatment with haloperidol produces serious side effects such as Tardive Dyskinesias (TD), catalepsy, acute dystonia (Naidhi et al., 2003) akathasia and cognitive effects (Waddington, 1990).

The use of haloperidol is therefore limited by its tendency to produce a range of extrapyramidal side effects. Haloperidol has high affinity for the dopamine receptor (D₂ receptor) and administration of haloperidol results in blockade of D₂ receptor (Granger and Albu, 2005). Haloperidol results in the increased turnover of dopamine leading to increased production of hydrogen peroxide following metabolism of dopamine by monoamine oxidase (Spirvak et al., 1992). The generation of reactive oxygen species due to increased turnover of dopamine is a source of oxidative stress (Singh et al., 2003). In addition, haloperidol is metabolized by an

Corresponding Author: Sally Abdul Wanees El-Awdan, National Research Centre, Dokki, Cairo, Egypt Tel: +202-3335963
oxidase and generates large quantities of oxy radicals and a potent toxic pyridinium like metabolite which contribute to oxyradical injury in schizophrenic patients (Jyothi et al., 2011).

Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease. Neuroinflammatory mechanisms probably also contribute to the cascade of events leading to neuronal degeneration (Ansari and Scheff, 2010). These mechanisms comprise microglial activation, astrogliosis and lymphocytic infiltration. The neuroinflammatory processes have been evidenced in Parkinson's disease as well as the cellular and molecular events associated with neuroinflammation involved in the degeneration of dopaminergic neurons in animal models (Hirsch and Humot, 2009).

The present research was designed to study the effect of haloperidol administration for one month on oxidative stress in brain of mice and its possible modulation by aspirin, nimesulide or celecoxib. In addition, this study has investigated the effect of haloperidol in the stomach and liver.

MATERIALS AND METHODS

Drugs: Haloperidol (Kahira Pharmaceutical and Chemical Industrial Company, Cairo, A.R.E.), aspirin (Industrial Arabic Company, Cairo, A.R.E.), nimesulide (Alkan, Cairo, A.R.E.) and celecoxib (Pfizer Egypt, Cairo, A.R.E).

Animals: A pathogen free male mice weighing 20-25 g were used. The animals were housed in cages at room temperature with 12/12 h light and darkness and were fed with a balanced diet and tap water. Animals were obtained from the animal colony house in the National Research Center.

Study design: Rats were randomly divided into different groups of six animals each. The treatment groups were as follows: control group (1% w/v of tween 80), haloperidol (2 mg kg⁻¹), haloperidol (2 mg kg⁻¹) + aspirin (100 mg kg⁻¹), haloperidol (2 mg kg⁻¹) + nimesulide (10 mg kg⁻¹) and haloperidol (2 mg kg⁻¹) + celecoxib (10 mg kg⁻¹). Drugs were administered simultaneously for two weeks.

Animals were sacrificed and brain, liver and stomach tissues were removed and used for the determination of reduced glutathione (GSH), malondialdehyde (MDA) and Nitric Oxide (NO) which are essential indicators of the oxidative stress in the tissue homogenates.

Biochemical studies

Determination of reduced glutathione content: Reduced glutathione (GSH) was determined according to Ellman (1959). The procedure is based on the reduction of Ellman's reagent by -SH a group of GSH to form 2-nitro-5-mercaptobenzoic acid and the nitromercaptobenzoic acid anion has an intense yellow color which can be determined spectrophotometrically. A mixture was directly prepared in a cuvette containing 2.25 mL of 0.1 M K phosphate buffer, pH 8.0, 0.2 mL of the sample, and 25 mL of Ellman’s reagent (10 mM 5,5-dithio-bis-2-nitrobenzoic acid in methanol). After 1 min, the assay absorbance was measured at 412 nm and the GSH concentration was calculated by comparison with the standard curve.

Determination of lipid peroxidation: Measuring thiobarbituric acid-reactive substances (TBARS) including lipid hydroperoxides and aldehydes, in biological samples is a method widely used for screening and monitoring lipid peroxidation. Malondialdehyde (MDA) forms a 1:2 adduct with thiobarbituric acid which can be measured by spectrophotometry. In practice, TBARS are expressed in terms of malondialdehyde (MDA) equivalents (Collins, 2005). Malondialdehyde was determined by measuring thiobarbituric-reactive species using the method of Ruiz-Larrea et al. (1994), in which the thiobarbituric acid-reactive substances react with thiobarbituric acid to produce a red-colored complex having peak absorbance at 532 nm.

Determination of nitric oxide metabolites: Stomach NO metabolites were determined according to the method described by Miranda et al. (2001) and expressed as μM g⁻¹ wet tissue. Nitric oxide has a short biological half-life and is rapidly converted into its stable metabolites, nitrite and nitrate. Determination of nitrite and nitrate (NOx) in body fluid and tissues is widely used as a marker of NO⁺ production Miranda et al. (2001). Nitric oxide measured as nitrite was determined by using Griess reagent, according to the method of Moshage et al. (1995), where nitrite, stable end product of nitric oxide radical, is mostly used as an indicator for the production of nitric oxide.

Statistical analysis: Data are expressed as means ± S.E. The results of the present experiments were analyzed using one way ANOVA followed by tukey multiple comparisons test. p < 0.05 was accepted as being significant in all types of statistical tests. Statistical analysis of results, were done using software SPSS 17.

RESULTS

Reduced glutathione: The results in Table 1 show the effect of drugs on brain, liver and stomach GSH level.
Table 1: Effect of drugs on GSH content in brain, liver and stomach of rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glutathione content (mg g⁻¹ tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs (dose)</td>
<td>Brain</td>
</tr>
<tr>
<td>Normal Haloperidol (2 mg kg⁻¹)</td>
<td>7.96±0.102*</td>
</tr>
<tr>
<td>Haloperidol+Aspirin (2 mg kg⁻¹)</td>
<td>5.51±0.701</td>
</tr>
<tr>
<td>(2 mg kg⁻¹)</td>
<td>7.98±0.132</td>
</tr>
<tr>
<td>Haloperidol+Nimesulide (2 mg kg⁻¹)</td>
<td>14.42±0.874*</td>
</tr>
<tr>
<td>(2 mg kg⁻¹)</td>
<td>12.48±1.148*</td>
</tr>
</tbody>
</table>

*Significantly different from normal group at p<0.05, **Significantly different from haloperidol group at p<0.05

Table 2: Effect of drugs on MDA content in brain, liver and stomach of rats

<table>
<thead>
<tr>
<th>Drugs and doses</th>
<th>MDA content (µM g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>25.62±3.738</td>
</tr>
<tr>
<td>Haloperidol (2 mg kg⁻¹)</td>
<td>253.6±16.88*</td>
</tr>
<tr>
<td>(2 mg kg⁻¹)</td>
<td>208.1±7.85*</td>
</tr>
<tr>
<td>Haloperidol+Nimesulide (2 mg kg⁻¹)</td>
<td>132.7±13.47*</td>
</tr>
<tr>
<td>(2 mg kg⁻¹)</td>
<td>135.9±12.39*</td>
</tr>
</tbody>
</table>

*Significantly different from normal group at p<0.05, **Significantly different from haloperidol group at p<0.05

Table 3: Effect of drugs on NO content in brain, liver and stomach of rats

<table>
<thead>
<tr>
<th>Drugs and doses</th>
<th>NO content (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>490.8±20.26</td>
</tr>
<tr>
<td>Haloperidol (2 mg kg⁻¹)</td>
<td>820.0±38.49*</td>
</tr>
<tr>
<td>(2 mg kg⁻¹)</td>
<td>528.3±26.56</td>
</tr>
<tr>
<td>Haloperidol+Aspirin (2 mg kg⁻¹)</td>
<td>928.2±37.34*</td>
</tr>
<tr>
<td>(2 mg kg⁻¹)</td>
<td>957.7±24.81</td>
</tr>
</tbody>
</table>

*Significantly different from normal group at p<0.05, **Significantly different from haloperidol group at p<0.05

Nimesulide (10 mg kg⁻¹) and celecoxib (10 mg kg⁻¹) increased the GSH level in brain by 140% and 108%, respectively when compared to the haloperidol group.

Haloperidol (2 mg kg⁻¹) treated rats showed significant decrease in liver and stomach GSH levels by 33.05 and 23.91%, respectively when compared to the normal group. The haloperidol-induced decline in GSH content was significantly inhibited by nimesulide (10 mg kg⁻¹) or celecoxib (10 mg kg⁻¹) by 17.25 and 23.25%, respectively when compared to the haloperidol group.

Test drugs were suspended in 1% Tween 80 and orally administered to the corresponding groups over 14 days. Normal group received 1% Tween 80. Animals were sacrificed by cervical dislocation under ether anesthesia on the last day. The liver, brain and stomach were collected and used after homogenization for the determination of gastric GSH content.

Lipid peroxidation: Following the oral administration of all drugs, a significant elevation of the brain MDA content was observed in the haloperidol (2 mg kg⁻¹) and haloperidol (2 mg kg⁻¹) with aspirin (100 mg kg⁻¹) by 756.17 and 602.56%, respectively when compared to the normal group. Nimesulide (10 mg kg⁻¹) and celecoxib (10 mg kg⁻¹) significantly reversed the brain MDA elevation elicited by haloperidol as drugs decreased MDA level by 49.25 and 46.41%, respectively when compared to the haloperidol group. In liver, haloperidol significantly elevated the MDA content by 42.15% when compared with the normal group. However, coadministration of either aspirin, nimesulide or celecoxib significantly decreased the MDA content by 40.91, 28.22 and 28.79%, respectively. In addition, haloperidol (2 mg kg⁻¹) with aspirin (100 mg kg⁻¹) significantly elevated stomach MDA content when administered with haloperidol (Table 2).

Nitrite: Haloperidol (2 mg kg⁻¹) administration resulted in a significant elevation of the brain nitrite content by 67.34% when compared to the normal group. However, aspirin (100 mg kg⁻¹) significantly normalized the elevated brain nitrite level by decreasing nitrite level by 35.6% when compared with the haloperidol group. On the other hand, liver nitrite level was significantly reduced by haloperidol (2 mg kg⁻¹) by 52.27% when compared to the normal group. Both nimesulide (10 mg kg⁻¹) and celecoxib (10 mg kg⁻¹) elevated that depletion when administered with haloperidol (2 mg kg⁻¹) by 102.76 and 124.39%, respectively. No significant change was noticed in the stomach nitrite level (Table 3).

DISCUSSION

Oxidative stress is defined as a breach in the balance between free radical production and antioxidant defense mechanisms. The present study provides the evidence that haloperidol increases oxidative stress in the brain, liver and stomach of mice.

The reduced form of glutathione, a tripeptide of glycine, glutamic acid and cysteine and a major antioxidant in tissue defense against oxidative stress, is decreased in brain tissue by the administration of haloperidol in brain, liver and stomach tissues indicating generation of oxidative stress by the drug. This is in accordance with other studies that indicated that administration of haloperidol to rats led to a decrease in reduced glutathione (GSH) levels in the striatum (Abdel-Raheem, 2010).

Glutathione (GSH) has a very important role in protecting against oxygen free radical damage by providing reducing equivalents for several enzymes; GSH
is also a scavenger of hydroxyl radicals and singlet oxygen radicals. Other studies revealed the role of reactive oxygen species in inducing oxidative stress as one of the mechanisms of haloperidol-induced neurotoxicity (Ferera et al., 2011). The present study showed that nimesulide and celecoxib reversed the depleted GSH content in all tissues selected by haloperidol administration.

The free radical oxidation of polyunsaturated fatty acids in biological systems is known as lipid peroxidation. The detection and measurement of lipid peroxidation is the evidence most frequently cited to support the involvement of free radical reactions in toxicology and diseases (Gutteridge, 1995). Lipid peroxidation evaluated by the measurement of thiobarbituric acid-reactive substances (TBARS) was increased in brain after haloperidol administration.

Haloperidol is found to increase oxidative stress mediated neuronal damage in animals (Kane and Smith, 1982). This neuronal damage by pro-oxidant actions of haloperidol has led to suggest that the side effects of haloperidol resulted from induced oxidative injury.

Existing evidence indicates that an excessive production of free radicals is associated with chronic haloperidol use and its side effects (Cadet et al., 1986) which is in harmony with the results of the present study. Haloperidol act by blocking dopamine receptors (Creese et al., 1976) and this blockade increases catecholamine turnover, which leads to excessive production of free radicals, especially in catecholamine rich areas such as basal ganglia. Because of high oxidative metabolism in these regions, neurons are particularly vulnerable to membrane lipid peroxidation and cell death. Free radicals are highly reactive with specific cellular components and have cytotoxic properties (Rang et al., 2003) and neuronal loss in striatum has been reported in animals treated chronically with haloperidol (Nielson and Lyon, 1978).

Increased generation of nitric oxide can be deleterious to brain functions, where synthesis of nitric oxide by both the inducible and constitutive nitric oxide synthase isoforms contributes to the activation of apoptotic pathways in the brain during systemic inflammation.

Nitric oxide itself is a free radical and can react with many other free radicals, e.g., superoxide radical, generating peroxynitrite radical, capable of causing oxidative changes to macromolecules (Moncada and Bolanos, 2006). The brain may be particularly vulnerable to oxidative stress because of its high metabolic oxidative activity and the relative deficiency of antioxidants (Halliwell, 1996) compared with the abundance of glutathione in the liver.

CONCLUSION

In summary, haloperidol itself impairs cellular antioxidant status because of the decreased brain, liver and stomach levels of GSH and increased levels of MDA in brain and liver tissues as well as increased NO levels in brain and depleted levels in liver and stomach tissues. In addition, findings in the present study suggest that nimesulide and celecoxib-in haloperidol-treated rats significantly improved the affected oxidative stress in different tissues.

REFERENCES


