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Co-administration of nitric oxide (NO) donors prevents haloperidol-induced orofacial dyskinesia, oxidative damage and change in striatal dopamine levels

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ABSTRACT

Tardive dyskinesia (TD) has been considered as a major clinical issue in the treatment of schizophrenia. Various animal studies have indicated the role of oxidative stress and nitric oxide pathway in haloperidol-induced TD. The present study investigated the effect of NO donors (molsidomine and L-arginine) in haloperidol-induced TD in rats. Chronic administration of haloperidol (1 mg/kg i.p. for 21 days) significantly increased vacuous chewing movements (VCMs), tongue protrusions, and facial jerking in rats which was dose dependently inhibited by NO donors. Besides, haloperidol also increased striatal superoxide anion levels and decreased striatal NO and citrulline levels which were prevented by molsidomine and L-arginine. On chronic administration of haloperidol, there was a decrease in the striatal levels of dopamine, which was again reversed by treatment with NO donors. The findings of the present study suggested for the involvement of NO in the development of neuroleptic-induced TD and indicated the potential of NO donors as a possible therapeutic option. Furthermore, a sub-study on a possible schizophrenic phenotype, i.e. a possible clinical worsening in the animals receiving NO donors and neuroleptics will substantiate the clinical utility of the study.

1. Introduction

Tardive dyskinesia (TD) is a painful and disfiguring motor side effect of long-term anti-psychotic use. TD presents months or years after initiation of anti-psychotic treatment, persists after drug withdrawal and may be irreversible. Although, new generation atypical agents have a lower incidence and risk of TD, the induction of TD remains a constant risk (Naidu and Kulkarni, 2001). A better understanding of the neurodevelopment of TD is vital for the development of newer preventive or treatment strategies. Brain imaging studies in TD have demonstrated the presence of long-lasting structural change in striatal neurons. Structural changes at the synaptic and neuronal level are invariably associated with glutamatergic activity (Marino and Conn, 2002). Chronic haloperidol administration provokes the release of glutamate in the striatum (See and Chapman, 1994), while long-term administration of haloperidol to rats increases striatal hydrogen peroxide and lipid radical formation *in vivo* (Yokoyama et al., 1998).

Nitric oxide (NO) is a product of the enzyme, NO synthase (NOS), of which three isoenzymes exist, namely inducible (NOS II) and the constitutively expressed endothelial (NOS III) and neuronal NOS (NOS I or nNOS) isoforms (Steuhr, 1999). NO is an important brain messenger released upon activation of the glutamate *N*-methyl-D-aspartate (NMDA) receptor and the subsequent Ca^{2+}-dependent activation of nNOS (Kiss and Vizi, 2001). NO has been accredited with both pro-oxidant and anti-oxidant actions (Lipton et al., 1993; Wink et al., 1999). Thus, while NO has been implicated in various neurodegenerative pathologies (Dawson and Dawson, 1998), it has also been found to reduce the generation of reactive oxygen species, such as hydrogen peroxide and superoxide, and to prevent lipid peroxidation (Kanner et al., 1992). However, this molecule also has important neuromodulatory roles as it has been found to exert significant effects on extracellular dopamine (DA) levels by inhibitory actions on DA transport (Kiss and Vizi, 2001; West et al., 2002). Although studies have described both inhibitory and facilitatory effects for NO on DA release, possibly due to the use of different *in vitro* or *in vivo* methods (Kiss and Vizi, 2001; Silva et al., 2003) or the state of oxidative stress associated with the tissue (West et al., 2002), the data are nonetheless emphatic that NO is an important modulator of basal ganglia output and nigrostriatal dopaminergic activity.

Classic anti-psychotic drugs elevate neuronal NO synthase (NOS) expression in the rat striatum. Given that NO signaling potently modulates the membrane excitability of striatal projection neurons, it is plausible that up-regulation of NOS activity after DA D2 receptor blockade contributes to the therapeutic efficacy and/or motor side

Abbreviations: FJ, Facial Jerkings; NO, Nitric Oxide; NOS, Nitric Oxide Synthase; NMDA, *N*-Methyl D-Aspartate; SOD, Super Oxide Dismutase; TD, Tardive Dyskinesia; TP, Tongue Protrusions; VCM, Vacuous Chewing Movements.

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effects associated with anti-psychotic drugs (Morris et al., 1997; Sammut et al., 2007). In contrast there are other studies also that haloperidol inhibits neuronal NOS activity *in vitro* (Hu et al., 1994; Iwahashi et al., 1996). Behavioural studies have found that NOS inhibitors induce or exacerbate catalepsy (Del Bel and Guimaraes, 2000), while oral dyskinesia and reduced striatal cyclic guanosine monophosphate (cGMP), as well as plasma nitrogen oxides (Harvey and Bester, 2000), are evoked by long-term (17 weeks) haloperidol treatment. The above data strongly suggest that NOS inhibition may predict the later development of TD, possibly by interfering with the neuromodulatory role of NO in the striatum, or by compromising an NO-dependent neuroprotective mechanism. There are several ways to increase the NO concentration in tissues. L-arginine is a precursor for the synthesis of NO. Molsidomine is a NO-releasing prodrug. Liver esterases convert molsidomine to the active metabolite, SIN-1 (half-life in plasma is 1–2 h), which then releases NO. Both L-arginine and molsidomine increase the concentration of NO in several tissues (Nitz and Fiedler, submitted for publication).

The present study has, therefore, been designed to confirm the inhibitory action of haloperidol on striatal NO levels using an animal model of orofacial dyskinesia, and to establish whether co-administration of NO donors, L-arginine and molsidomine has any effect on haloperidol-induced orofacial dyskinesia, an animal model of tardive dyskinesia.

2. Methods

2.1. Animals

Male Wistar rats (180–220 g; 10–12 rats/group) bred in the Central Animal House facility of Panjab University were used. The animals were housed under standard laboratory conditions, maintained on a normal light–dark cycle and free access to food and water. Animals were acclimatized to laboratory conditions before the test. Each animal was used only once in the experiment. All the experiments were carried out between 0900 and 1500 h. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the guidelines of Indian National Science Academy (INSA) for the use and care of experimental animals.

2.2. Drugs and treatment schedule

The following drugs were used in the present study. Haloperidol (Serenace, Searle, India) was diluted with distilled water and administered intraperitoneally in a constant volume of 0.5 ml per 100 g of bodyweight of rat for 21 days.

Group	Treatment (mg/kg, i.p. for 21 days)
1	Control
2	Haloperidol (H) (1) alone
3	Molsidomine (M) (4) *per se*
4	Molsidomine (2)+haloperidol (1)
5	Molsidomine (4)+haloperidol (1)
6	L-arginine (LA) (100) *per se*
7	L-arginine (50)+haloperidol (1)
8	L-arginine (100)+haloperidol (1)

In combination studies, haloperidol and NO donors (molsidomine and L-arginine) were administered simultaneously once daily at 0900 for a period of 21 days and behavioural assessments were done 24 h after the last dose (Naidu et al., 2003).

2.3. Behavioural assessment of orofacial dyskinesia

On the test day (on day 22), rats were placed individually in a small (30×20×30 cm) Plexiglas cage for the assessment of oral dyskinesia. Animals were given 10 min to get acclimatized to the observation cage before behavioural assessments. To quantify the occurrence of oral dyskinesia, hand operated counters were employed to score tongue protrusion (TPs), facial jerkings (FJs) and vacuous chewing movements (VCMs). In the present study, VCM is referred to as single mouth openings in the vertical plane not directed toward physical material. TPs are referred to stereotypically turning movements of the tongue with protrusions (fly catching tongue) and jerky movements of face in either direction were referred as FJs. If tongue protrusion or VCM occurred during a period of grooming, they were not taken into account. Counting was stopped whenever the rat began grooming, and restarted when grooming stopped. Mirrors were placed under the floor and behind the back wall of the cage to permit observation when the animal faced away from the observer. The behavioural parameters of oral dyskinesia were measured continuously for a period of 5 min. In all the experiments, the scorer was unaware of the treatment given to the animals (Naidu et al., 2003).

2.4. Dissection and homogenization

After behavioural assessment, animals were divided in 2 groups on the basis of severity and susceptibility (high vs low orofacial dyskinetic movements) of behavioural assessments. Animals from each of the two groups were randomized and equal numbers of animals with high and low orofacial dyskinetic movements in each group were distributed in two groups. In one group of animals, the brains were removed; striatum was dissected out and weighed. A 10% (w v^{-1}) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). Homogenates were centrifuged at 2500 ×g for 10 min, at 4 °C and supernatant was used for estimation of superoxide anion, total nitric oxide and citrulline levels. In another set of animals, the brains were removed; striatum was dissected out and was stored at −80 °C for HPLC studies to estimate dopamine.

2.5. Biochemical assessment

2.5.1. Estimation of superoxide anion

The superoxide anion levels were measured by method devised by Babior et al. (1973). The brain samples were homogenized in Tris KCl buffer to produce 10% homogenate. From each homogenized sample 25 µl of homogenate was taken and mixed with 0.05 mM cytochrome *C* solution in Tris KCl buffer to make up the volume to 2 ml. This mixture was then incubated for 15 min at 37 °C. The reaction was terminated by placing the mixture in ice. It was then centrifuged at 700 ×g for 10 min. The supernatant was taken and absorbance was measured at 550 nm with Shimadzu UV spectrophotometer. Results were calculated as nanomoles of cytochrome *c* reduced/minute using molar extinction coefficient of chromophore (2.1×10^4 M^{-1} cm^{-1}) and expressed as percentage of control taking control values as 100%.

2.5.2. Total no estimation

The quantification of total nitric oxide was done by the help and instructions provided by R&D Systems Total nitric Oxide Assay Kit which involves the conversion of nitrate to nitrite by the enzyme nitrate reductase. The detection of total nitrite is then determined as a colored azo dye product of the Griess Reaction. The Griess reaction is based on the two step diazotization reaction in which acidified NO_2-produces a nitrosating agent which reacts with sulphanilic acid to produce the diazonium ions. This ion is then coupled to *N*-(1-naphthyl) ethylenediame to form the chromophoric azo derivative which absorbs light at 540–570 (User guide: R&D systems Total nitric oxide estimation kit).

2.5.3. Citrulline estimation

Citrulline was estimated by the method of Boyde and Rahmatullah (1980). The citrulline assay is based on its reaction with diacetylmonoxime and absorbance was measured at 530 nm. To the 200 µl of sample, 50 µl of 30% $ZnSo_4$ solution was added and mixed well to precipitate proteins and the tube was then centrifuged. 20 µl of the

supernatant was diluted to 480 µl with 0.1 N HCl and 1.5 ml of freshly prepared chromogenic solution was added and vortexed. Mixture was boiled in water bath for 5 min at 100 °C and absorbance was measured at 530 nm. Results were expressed as percentage of control taking control values as 100%.

2.5.4. Protein estimation

The protein content of the brain tissue was measured according to the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.5.5. Measurement of dopamine levels by HPLC

Striatal dopamine was estimated with Waters standard system consisting of a high pressure isocratic pump, a 20 µl sample injector valve, C18 reverse phase column and electrochemical detector. Data were recorded and analyzed with the help of empower software. Mobile phase consisted of 2% citric acid, 2% $KHPO_4$, 1 mM EDTA, 1.2% MeOH, and 70 mg/ml of sodium octyl sulphate. pH of the mobile phase was adjusted to 3 with the help of HCl (6 N). Electrochemical conditions for the experiment were +0.800 V, sensitivity ranges from 5–50 nA. Separation was carried out at a flow rate of 1 ml/min. Samples (20 µl) were injected manually. On the day of experiment, frozen striatum samples were thawed and they were homogenized in homogenizing solution containing 0.1 M perchloric acid. After that the samples were centrifuged at 12,000 ×g for 5 min. The supernatant was further filtered through 0.25 µm nylon filters before injecting in the HPLC injection pump. Data were recorded and analyzed with the help of empower software (Church et al., 2005; Bishnoi et al., 2008).

2.7. Statistical analysis

All data are expressed as means S.E.M. The data were analyzed by using one way analysis of variance (ANOVA) followed by Fischer LSD test for multiple comparison in between groups. In all tests, the criterion for statistical significance was $P<0.05$. Pearson correlation analysis was used to examine the correlation between two independent factors. The criterion for significance in correlation analysis was $P<0.005$.

3. Results

3.1. Behavioural assessment

3.1.1. Assessment of orofacial dyskinesia

Haloperidol (H) (1 mg/kg, i.p.) treatment resulted into a significant increase in VCMs, tongue protrusion and facial jerking. Molsidomine (M) (4 mg/kg) and L-arginine (LA) (100 mg/kg) *per se* did not cause any significant change in VCMs, tongue protrusions and facial jerking as compared to control (Fig. 1(a), (b), (c). Co-administration with molsidomine (2 and 4 mg/kg) and L-arginine (50 and 100 mg/kg) dose dependently inhibited the increase of haloperidol-induced VCM ($dF=59$, $F=20.33$), tongue protrusions ($dF=59$, $F=18.43$) and facial jerking ($dF=59$, $F=24.03$) (Fig. 2(a), (b), (c), haloperidol (H), molsidomine (M), L-arginine (LA)).

3.2. Biochemical assessment

3.2.1. Estimation of total nitric oxide

Chronic haloperidol treatment (1 mg/kg, i.p.) resulted in significant decrease in striatal total nitric oxide levels as compared to control animals. Co-administration with molsidomine (2 and 4 mg/kg) and L-arginine (50 and 100 mg/kg) dose-relatedly prevented the decrease in striatal total nitric oxide levels ($dF=35$, $F=14.33$). Molsidomine (4 mg/kg) and L-arginine (100 mg/kg) *per se* did not cause any significant change in striatal total nitric oxide levels as compared to control (Fig. 3(a), haloperidol (H), molsidomine (M), L-arginine (LA)).

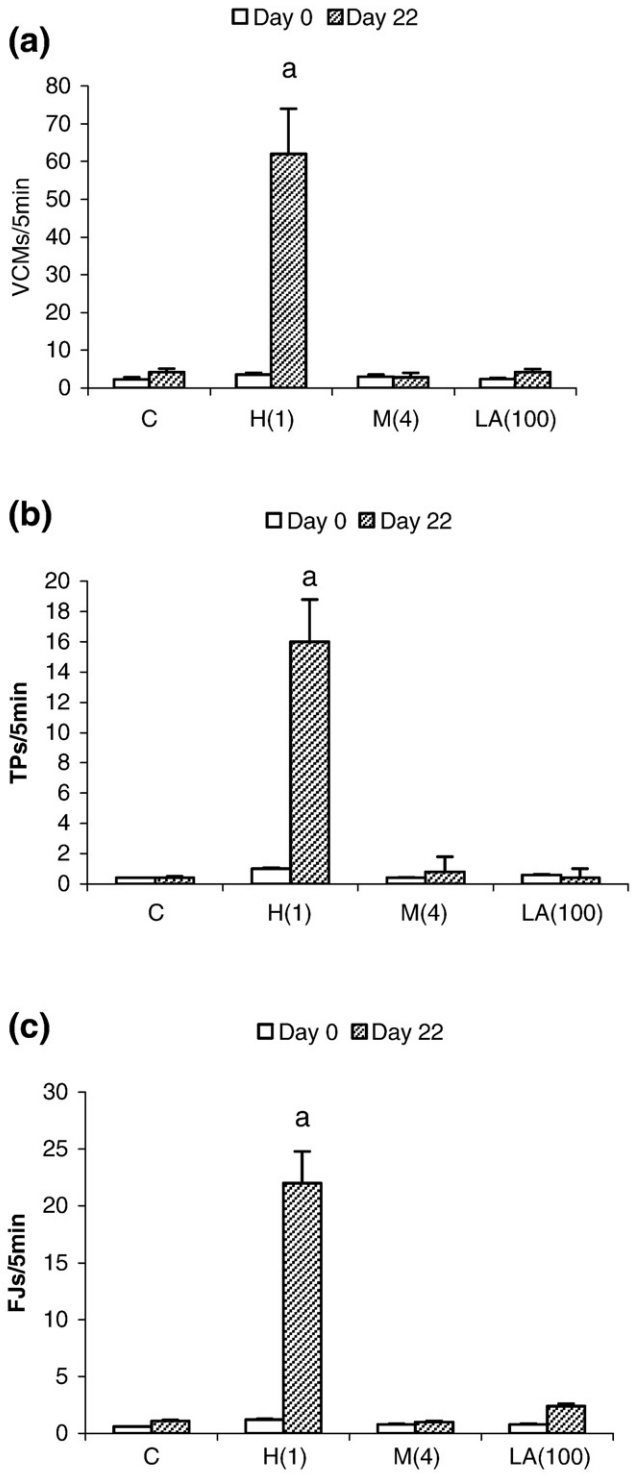

Fig. 1. Effect of haloperidol (1 mg/kg, i.p. 21 days), molsidomine (4 mg/kg) *per se* and L-arginine (100 mg/kg) *per se* on (a) vacuous chewing movements (VCMs), tongue protrusions (TPs) and facial jerkings (FJs) in rats ($n=10$–12) on day 0 and day 22. $^{a}p\leq0.05$ as compared to control group (haloperidol (H), molsidomine (M), L-arginine (LA).

3.2.2. Superoxide anion levels

Chronic haloperidol treatment (1 mg/kg, i.p.) resulted in a significant increase in striatal superoxide anion as compared to control animals. Co-administration with molsidomine (2 and 4 mg/kg) and L-arginine (50 and 100 mg/kg) dose- relatedly inhibited the increase striatal superoxide anion levels ($dF=35$, $F=16.49$). Molsidomine (4 mg/kg)

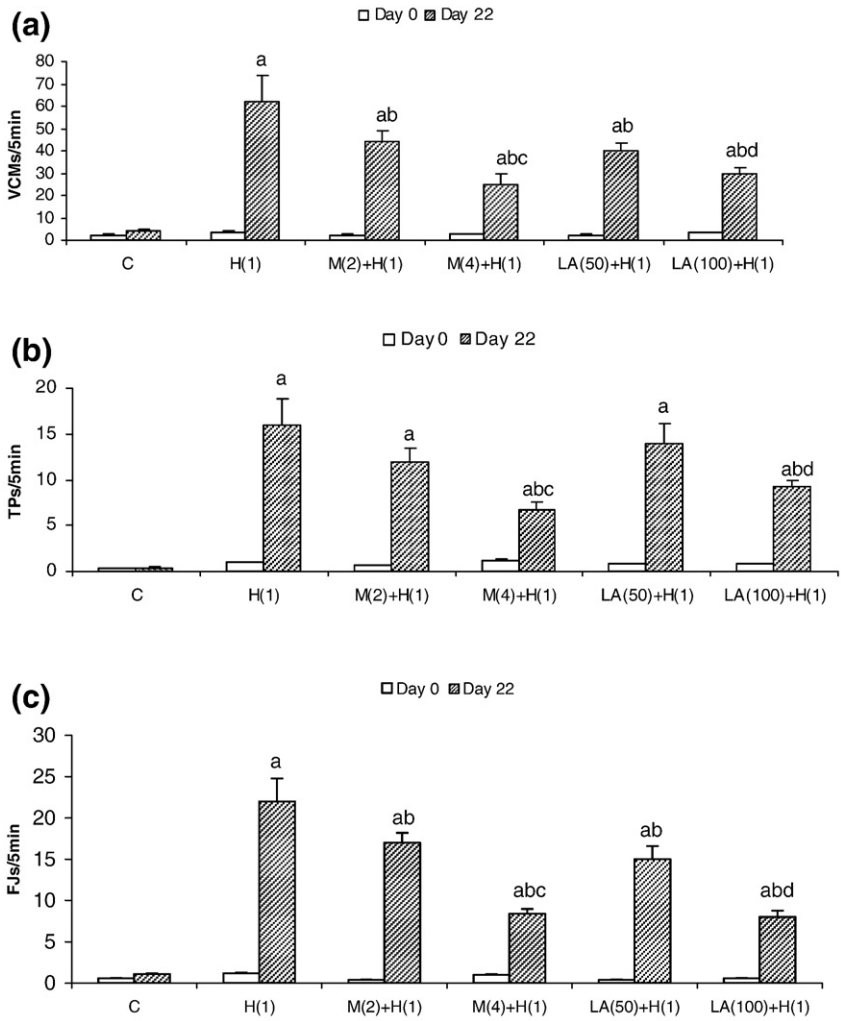

Fig. 2. Effect of haloperidol (1 mg/kg, i.p. 21 days) and co-administration of different doses of molsidomine (2 and 4 mg/kg) and L-arginine (50 and 100 mg/kg) on (a) vacuous chewing movements (VCMs), tongue protrusions (TPs) and facial jerkings (FJs) in rats (n=10–12) on day 0 and day 22. [a]$p \leq 0.05$ as compared to control group, [b]$p \leq 0.05$ as compared to haloperidol group, [c]$p \leq 0.05$ as compared to molsidomine (2 mg/kg)+haloperidol(1) group, [d]$p \leq 0.05$ as compared to L-arginine (50 mg/kg)+haloperidol (1) group (haloperidol (H), molsidomine (M), L-arginine (LA).

and L-arginine (100 mg/kg) *per se* did not cause any significant change in striatal superoxide anion levels as compared to control (Fig. 3(b), haloperidol (H), molsidomine (M), L-arginine (LA)).

3.2.3. Estimation of citrulline levels

Chronic haloperidol treatment (1 mg/kg, i.p.) resulted in a significant decrease in striatal citrulline levels as compared to control animals. Co-administration with molsidomine (2 and 4 mg/kg) and L-arginine (50 and 100 mg/kg) inhibited the decrease in striatal citrulline levels at higher doses only (dF=35, F=21.33). Molsidomine (4 mg/kg) and L-arginine (100 mg/kg) *per se* did not cause any significant change in striatal citrulline levels as compared to control (Fig. 3(c), haloperidol (H), molsidomine (M), L-arginine (LA)).

3.3. Neurochemical assessment

3.3.1. Striatal dopamine levels

Chronic administration of haloperidol resulted in significant decrease in striatal dopamine levels which was prevented by co-administration with molsidomine (2 and 4 mg/kg) (only at higher doses) and L-arginine (50 and 100 mg/kg) (dose related) (dF=35, F=18.81). Molsidomine (4 mg/kg) and L-arginine (100 mg/kg) *per se* did not cause any significant change in striatal dopamine levels as compared to control (Fig. 4, haloperidol (H), molsidomine (M), L-arginine (LA)).

3.3.2. Pearson's correlation analysis

Pearson correlation analysis revealed that the decrease in total nitric oxide induced by haloperidol has negative correlation with VCMs ((−) 0.917) and superoxide anion levels ((−) 0.870) whereas positive correlation with dopamine levels (0.938). Further, the decrease in citrulline levels also has negative correlation with VCMs ((−) 0.888) and superoxide anion levels ((−) 0.810) whereas positive correlation with dopamine levels (0.901) (Table 1).

4. Discussion

The most important finding of the present study is that VCMs and other orofacial dyskinetic movements evoked by chronic treatment with haloperidol are causally related to striatal NO levels. Chronic administration of haloperidol significantly increased the VCMs and other orofacial movements and superoxide anion levels while significantly decreasing striatal NO and citrulline levels, thereby suggesting decreased NOS activity. Further, this decrease in NO levels can also be related to haloperidol-induced decrease in striatal dopamine levels. However, co-administration of NO donors (L-arginine and molsidomine) dose dependently prevented all the behavioural, biochemical and neurochemical alterations.

Earlier, several *in vitro* studies have noted the ability of haloperidol to inhibit neuronal NOS activity (Hu et al., 1994; Iwahashi et al., 1996)).

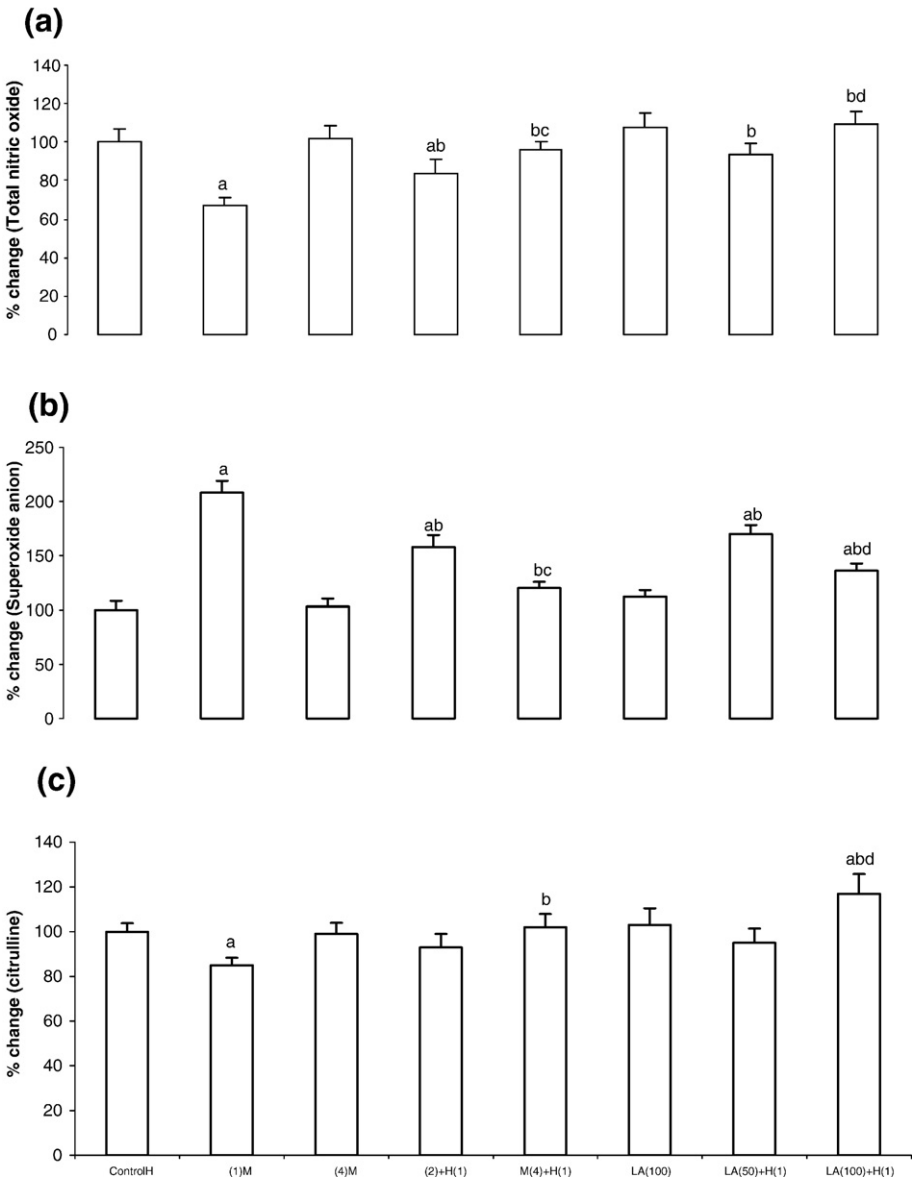

Fig. 3. Effects haloperidol (1 mg/kg, i.p. 21 days), molsidomine (4 mg/kg) *per se*, L-arginine (100 mg/0kg) *per se* and co-administration of different doses of molsidomine (2 and 4 mg/kg) and L-arginine (50 and 100 mg/kg) with haloperidol on striatal (a) total NO levels (b) superoxide anion and (c) citrulline levels in rats ($n=6$). [a]$p \leq 0.05$ as compared to control group, [b]$p \leq 0.05$ as compared to haloperidol group, [c]$p \leq 0.05$ as compared to molsidomine (2 mg/kg) + haloperidol (1) group, [d]$p \leq 0.05$ as compared to L-arginine (50 mg/kg) + haloperidol (1) group (haloperidol (H), molsidomine (M), L-arginine (LA). Baseline value for citrulline: 36.5 ± 1.5 pmol/mg protein. Baseline value for total nitric oxide: 101.06 ± 8.2 μmol/l. Baseline value for superoxide anion: 0.04 ± 0.003 cyt C reduced/min/mg proteins.

Chronic administration of haloperidol followed by withdrawal, has been found to lower striatal cGMP levels and peripheral NO *ex-vivo* (Harvey and Bester, 2000). The current study extends support to these observations by determining the striatal NO and citrulline levels and correlating the same with striatal superoxide anion and dopamine levels. Tardive dyskinesia is a neurodegenerative disorder characterized by striatal oxidative stress as well as reduced circulating levels of Superoxide Dismutase (SOD) (Bishnoi et al., 2008; Su et al., 2007; Zhang et al., 2003; Hori et al., 2000; Yamada et al., 1997). The resulting excessive production of superoxide anion together with reduced NO synthesis induced by chronic administration of haloperidol will further provoke oxidative stress and cell damage. We observed a positive linear correlation between the decrease in striatal NO levels and the increase in generation of superoxide anion. This observation is corroborated by other workers who have demonstrated that NO exerted a neuroprotective effect on striatal neurons by quenching the release of superoxide anion (Lancelot et al., 1995). Excessive inhibition of NOS activity and resultant decrease in NO levels also potentiates NMDA receptor-mediated excitotoxicity which under normal conditions is prevented by nitrergic inactivation of NMDA receptors (Connop et al., 1995; West and Galloway, 1997).

Progressively reduced NOS activity or NO release in striatum during aging, a prominent risk factor for TD, confirms the involvement of NO in haloperidol-induced orofacial dyskinesia, an animal model of tardive dyskinesia (Andreassen and Jorgensen, 2000; Casey, 2000). Interestingly, suppression of the NOS-cGMP by the combination of haloperidol and methylene blue, a NOS-guanylate cyclase inhibitor, has been found to evoke a reactive increase in nitrogen oxides (Bester and Harvey, 2000). Our data suggest that chronic haloperidol treatment by removing NO-dependent neuroprotective mechanisms increases the superoxide anion levels. Co-administration of molsidomine but not L-arginine dose dependently increased the NO levels and was able to restore the normal physiological condition. Citrulline is a co-product of NOS catalyzed reaction. Chronic administration of

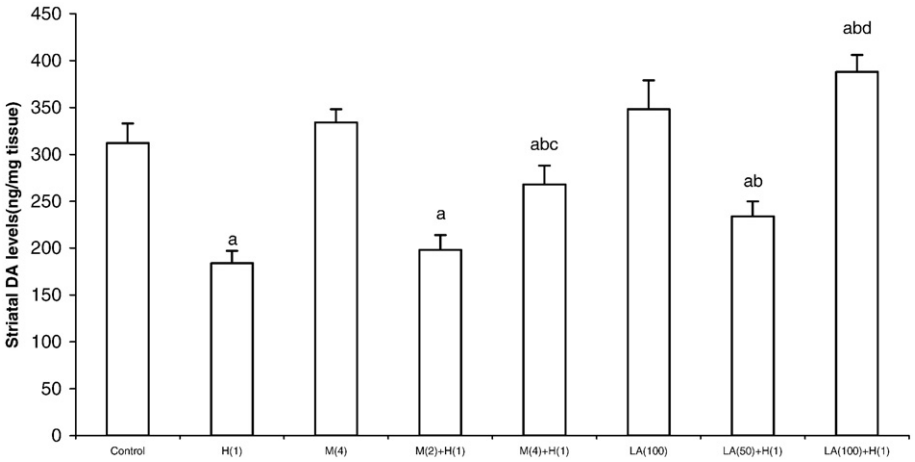

Fig. 4. Haloperidol (1 mg/kg, i.p. 21 days), molsidomine (4 mg/kg) *per se*, L-arginine (100 mg/kg) *per se* and co-administration of different doses of molsidomine (2 and 4 mg/kg) and L-arginine (50 and 100 mg/kg) with haloperidol on striatal levels of dopamine in rats ($n=6$). [a]$p \leq 0.05$ as compared to control group, [b]$p \leq 0.05$ as compared to haloperidol group, [c]$p \leq 0.05$ as compared to molsidomine (2 mg/kg)+haloperidol (1) group, [d]$p \leq 0.05$ as compared to L-arginine (50 mg/kg)+haloperidol (1) group (haloperidol (H), molsidomine (M), L-arginine (LA).

haloperidol resulted in a significant decrease in the level of citrulline. Citrulline can be recycled to arginine in a pathway known as arginine–citrulline pathway (Morris, 2007, 2004). Similar is the case of citrulline–NO cycle, some of the citrulline produced by NOS can be recycled to arginine (Morris, 2007). Decrease in the levels of citrulline further decreases the amount of substrate arginine hence resulting in a further decrease in NO levels. Co-administration of NO donors prevented this decrease in the levels of citrulline, thereby enhancing its availability for its conversion into L-arginine. Citrulline can also substitute for arginine to some degree in supporting NO synthesis (Morris, 2004, 2007). L-arginine treatment did not show dose dependent effect against striatal NO and citrulline levels. According to recent reviews nitric oxide, due to its dual nature, has an array of functions in CNS. Particularly interesting is the role of nitric oxide as a Janus molecule in the cell death or survival mechanisms in brain cells. In fact, it all depends on the concentration of NO. Physiological amounts of this gas are neuroprotective, whereas higher concentrations are clearly neurotoxic. It might be possible that at higher doses concentration of NO tends to be neurotoxic. The increased concentration of NO has been rapidly reacted with superoxide anion to form peroxynitrite hence the levels of NO are less. Due to resultant effect the levels of citrulline (citrulline–NO pathway) are also less. This concentration dependent effect can be possible explanation for NO donor (molsidomine) not causing oxidative injury due to formation of peroxy nitrite.

Neurochemically, the present study suggests that chronic haloperidol administration significantly decreased the levels of dopamine in striatum. However, the decrease was prevented by co-administration of molsidomine and L-arginine. Considerable evidences support the regulatory role of NO in the striatum (Kiss and Vizi, 2001; West et al., 2002). A well recognized effect of NO is to promote striatal dopamine release by promoting dopamine efflux or by inhibiting dopamine transporters and thus decreasing dopamine reuptake (Kiss and Vizi, 2001). Further, dysfunctional cortico-striatal NO-dependent signalling will disrupt dopaminergic transmission at the level of terminal synapse as well as at the cell body (West et al., 2002). NO also increases striatal glutamate increase both *in vitro* and *in vivo*, while glutamate mediated NMDA-receptor activation plays a critical role in the release of dopamine by NO (West and Galloway, 1997; West et al., 2002). It is thus important to note that haloperidol, by inhibiting NO-dependent facilitation of dopamine release in striatum, will further attenuate striatal dopaminergic function already compromised by chronic D_2 receptor blockade. Previous studies reported from our laboratory suggested the decrease in the levels of biogenic amines after chronic administration of haloperidol (Bishnoi et al., 2007). Inhibition of NOS activity and the decrease in the levels of NO could be an accentuating mechanism along with oxidative stress and dopamine receptor supersensitivity to explain the decrease in dopamine levels. Moreover, newer atypical anti-psychotics have recently been found to be devoid of any effect on NOS with lower risks of producing TD (Harvey et al., 1999).

The mechanism of haloperidol-induced NOS suppression requires further investigation, but may follow a direct inhibitory action on enzyme activity (Hu et al., 1994; Iwahashi et al., 1996). However, given the relatively higher K_i for NOS inhibition for haloperidol *in vitro*, it is unlikely to occur at clinically relevant doses (Hu et al., 1994). Reduced enzyme activity may also ensue after oxidative modification of NOS evoked by chronic haloperidol treatment, leading to altered response to substrates and/or allosteric effectors (Chao et al., 1997). Indeed NO donors (molsidomine and L-arginine) directly increase the NO levels and this will have significant impact on the important physiological functions of NO as a neuromodulators.

Recent clinical and preclinical studies showed the overactivity of the NO system in schizophrenia. Behavioural and biochemical effects of PCP in rodents had been blocked by nitric oxide synthase (NOS) inhibitors, suggesting that NO plays an important role in at least the pharmacological effects of PCP (Klamer et al., 2004). It is concluded that the NOS-sensitive behavioural effects of phencyclidine in rodents are dependent on neuronal NOS. Findings also suggest that regulatory polymorphisms of NOS1 contribute to the genetic risk for schizophrenia (Reif et al., 2006). Hence co-administration of NO donors with haloperidol or other neuroleptic drugs may worsen the clinical situation of tardive dyskinesia. Although in our animal study NO donors were devoid of any such effects but further inclusion of substudy on a possible schizophrenic phenotype is advisable to find out the exact status of NO donors in clinical situation.

Table 1
Pearson's correlation analysis for total NO levels and citrulline levels with behavioural, biochemical and neurochemical parameters in the haloperidol-treated animal

Parameters measured	NO Levels	Citrulline levels
	Pearson correlation factor (r)	
VCMs/5 min	(−)0.917	(−)0.888
Superoxide anion levels	(−)0.870	(−)0.810
Dopamine levels	0.938	0.901

[a]$p \leq 0.005$ in all the groups. The pair(s) of variables with positive correlation coefficients tends to increase together.

In conclusion, the present study supports the fact that NO has a potential involvement in haloperidol-induced orofacial dyskinesia and NO donors (molsidomine and L-arginine) are capable of attenuating it.

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References

Andreassen OA, Jorgensen HA. Neurotoxicity associated with neuroleptic-induced oral dyskinesias in rats, implications for tardive dyskinesia. Prog Neurobiol 2000;61:525–31.
Babior BM, Kipner RS, Cerutte JT. Biological defense mechanism. The production by leukocytes of superoxide, a potential bacterial agent. J Clin Invest 1973;52:741–4.
Bester A, Harvey BH. Early suppression of striatal cyclic GMP may predetermine the induction and severity of chronic haloperidol-induced vacuous chewing movements. Metab Brain Dis 2000;15:273–83.
Bishnoi M, Kumar A, Chopra K, Kulkarni SK. Comparative neurochemical changes associated with chronic administration of typical and atypical neuroleptics: implications in tardive dyskinesia. Indian J Exp Biol 2007;45:175–9.
Bishnoi M, Chopra K, Kulkarni SK. Protective effect of *Curcumin*, the active principle of turmeric (*Curcuma longa*) in haloperidol-induced orofacial dyskinesia and associated behavioural, biochemical and neurochemical changes in rat brain. Pharmacol Biochem Behav 2008;88:511–22.
Boyde TR, Rahmatullah M. Optimization of conditions for the colorimetric determination of citrulline, using diacetyl monoxime. Anal Biochem 1980;107:424–31.
Casey DE. Tardive dyskinesia: pathophysiology and animal models. J Clin Psychiatry 2000;61:5–9.
Chao CC, Ma YS, Stadtman ER. Modification of protein surface hydrophobicity and methionine oxidation by oxidative systems. Proc Natl Acad Sci U S A 1997;94:2969–74.
Church WH. Column chromatography analysis of brain tissue: an advanced laboratory exercise for neuroscience majors. J Undergrad Neurosci Educ 2005;3:A36–41.
Connop R, Boegman R, Jhamandes K, Beninger R. Excitotoxic action of NMDA agonists on nigrostriatal dopaminergic neurons: modulation by inhibition of nitric oxide synthesis. Brain Res 1995;676:124–32.
Dawson VL, Dawson TM. Nitric oxide in neurodegeneration. Prog Brain Res 1998;118:215–29.
Del Bel EA, Guimaraes FS. Sub-chronic inhibition of nitric oxide synthesis modifies haloperidol-induced catalepsy and the number of NADPH-diaphorase neurones in mice. Psychopharmacology 2000;147:356–61.
Harvey BH, Stein DJ, Emsley RA. The new-generation anti-psychotics: integrating the neuropathology and pharmacology of schizophrenia. S Afr Med J 1999;89:661–72.
Harvey BH, Bester A. Withdrawal-associated changes in peripheral nitrogen oxides and striatal cyclic GMP after chronic haloperidol treatment. Behav Brain Res 2000;111:203–11.
Hori H, Ohmori O, Shinkai T, Kojima H, Okano C, Suzuki T, et al. Manganese superoxide dismutase gene polymorphism and schizophrenia: relation to tardive dyskinesia. Neuropsychopharmacology 2000;23(2):170–7.
Hu J, Lee JH, El-Fakahany E. Inhibition of neuronal nitric oxide synthase by anti-psychotic drugs. Psychopharmacology 1994;114:161–6.
Iwahashi K, Yoneyama H, Ohnishi T, Nakamura K, Miyatake R, Suwaki H, et al. Haloperidol inhibits neuronal nitric oxide synthase activity by preventing electron transfer. Neuropsychobiology 1996;33:76–9.
Kanner J, Harel S, Granit R. Nitric oxide, an inhibitor of lipid peroxidation by lipoxygenase. Lipids 1992;27:46–9.
Kiss JP, Vizi ES. Nitric oxide: a novel link between synaptic and non-synaptic transmission. Trends Neurosci 2001;24:211–5.
Klamer D, Engel JA, Svensson L. The neuronal selective nitric oxide synthase inhibitor, Nomega-propyl-L-arginine, blocks the effects of phencyclidine on prepulse inhibition and locomotor activity in mice. Eur J Pharmacol 2004;503(1–3):103–7.
Lancelot E, Callebert J, Lerouet D, Revaud M, Boulu R, Plotkine M. Role of L-arginine-nitric oxide pathway in the basal hydroxyl radical production in the striatum of awake rats as measured by brain microdialysis. Neurosci Lett 1995;202:21–4.
Lipton SA, Choi YB, Pan ZH, Lei SZ, Chen HSV, Sucher NJ, et al. A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature 1993;364:626–32.
Lowry OH, Rosebrough NJ, Farr AL. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
Marino MJ, Conn PJ. Direct and indirect modulation of the *N*-methyl-D-aspartate receptor: potential for the development of novel antipsychotic therapies. Curr Drug Target 2002;1:1-16.
Morris Jr SM. Enzymes of arginine metabolism. J Nutr 2004;134:2743S–7S.
Morris Jr SM. Arginine metabolism: boundaries of our knowledge. J Nutr 2007;137 (6):1602S–9S.
Morris BJ, Simpson CS, Mundell S, Maceachern K, Johnston HM, Nolan AM. Dynamic changes in NADPH-diaphorase staining reflect activity of nitric oxide synthase: evidence for a dopaminergic regulation of striatal nitric oxide release. Neuropharmacology 1997;36(11–12):1589–99.
Naidu PS, Kulkarni SK. Excitatory mechanisms in neuroleptic-induced vacuous chewing movements (VCM): possible involvement of calcium and nitric oxide. Behav Pharmacol 2001;12:209–16.
Naidu PS, Singh A, Kulkarni SK. Quercetin, a bioflavonoid, attenuates haloperidol-induced orofacial dyskinesia. Neuropharmacology 2003;44:1100–6.
Nitz, R.E., Fiedler, V.B.: . submitted for publication Molsidomine: alternative approaches to treat myocardial ischemia. Pharmacotherapy. 7(1):28–37.
Reif A, Herterich S, Strobel A, Ehlis AC, Saur D, Jacob CP, et al. A neuronal nitric oxide synthase (NOS-I) haplotype associated with schizophrenia modifies prefrontal cortex function. Mol Psychiatry 2006;11(3):286–300.
Sammut S, Bray KE, West AR. Dopamine D2 receptor- dependent modulation of striatal NO synthase activity. Psychopharmacology (Berl) 2007;191(3):793–803.
Su JM, Tan YL, Zhou DF. Relationship between tardive dyskinesia and the polymorphism of superoxide dismutase val9Ala and efficacy of chaihu taoren capsules on it. Zhongguo Zhong Xi Yi Jie He Za Zhi 2007;27(8):700–3.
See RE, Chapman MA. Chronic haloperidol, but not clozapine, produces altered oral movements and increased extracellular glutamate in rats. Eur J Pharmacol 1994;263:269–76.
Silva MT, Rose S, Hindmarsh JG, Jenner P. Inhibition of neuronal nitric oxide synthase increases dopamine efflux from rat striatum. J Neural Transm 2003;110:353–62.
Steuhr DJ. Mammalian nitric oxide synthases. Biochim Biophys Acta 1999;1411:217–30.
West AR, Galloway MP. Endogenous nitric oxide facilitates striatal dopamine and glutamate efflux in vivo: role of ionotropic glutamate receptor-dependent mechanisms. Neuropharmacology 1997;279:1571–81.
West AR, Galloway MP, Grace AA. Regulation of striatal dopamine neurotransmission by nitric oxide: effector pathways and signaling mechanisms. Synapse 2002;44:227–45.
Wink DA, Vodovotz Y, Grisham MB, DeGraff W, Cook JC, Pacelli R, et al. Antioxidant effects of nitric oxide. Methods Enzymol 1999;301:413–24.
Yamada K, Kanba S, Anamizu S, Ohnishi K, Ashikari I, Yagi G, et al. Low superoxide dismutase activity in schizophrenic patients with tardive dyskinesia. Psychol Med 1997;27(5):1223–5.
Yokoyama H, Kasai N, Ueda Y, Niwa R, Konaka R, Mori N, et al. In vivo analysis of hydrogen peroxide and lipid radicals in the striatum of rats under long-term administration of a neuroleptic. Free Radic Biol Med 1998;24:1056–60.
Zhang XY, Zhou DF, Cao LY, Chen DC, Zhu FY, Wu GY. Blood superoxide dismutase level in schizophrenic patients with tardive dyskinesia: association with dyskinetic movements. Schizophr Res 2003;62(3):245–50.