Oral administration of haloperidol at clinically recommended doses elicits smaller parkinsonian effects but more tardive dyskinesia in rats

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Abstract: The present study was designed to monitor extrapyramidal symptoms (EPS) elicited by the oral administration of haloperidol at clinically recommended doses and to compare it with EPS produced when the drug is injected intraperitoneally at doses used in animal research. Rats injected with haloperidol at a dose of 1 mg/kg daily for 5 weeks exhibited akinesia in an open field and impaired motor coordination. Effects of the drug on motor coordination but not on open field akinesia were attenuated gradually from 2-5 weeks of treatment. Oral administration of haloperidol in drinking water at clinically recommended dose exhibited decreased exploratory activity without producing akinesia. Motor coordination was impaired maximally after 3 weeks and tolerance was developed in the drug induced motor impairment after 5 weeks of treatment. Intensity of vacuous chewing movements (VCMs) and tardive VCMs was greater by oral administration than intraperitoneal injections of haloperidol. The present results showed that oral administration of haloperidol expected to produce sustained effect may result in tolerance in acute parkinsonian like effects but more intensity of tardive dyskinesia. We suggest that drugs which may helpful in alleviating tardive dyskinesia may be more useful if person is on oral drug therapy.

Keywords: haloperidol, extrapyramidal symptoms, motor coordination, vacuous chewing movements, oral drug therapy

INTRODUCTION

Schizophrenia is a chronic, severe mental illness characterized by disturbed form and content of thoughts (Rupp and Keith, 1993; Cohen *et al*, 2006). Typical neuroleptic drugs such as haloperidol are widely prescribed for the treatment of schizophrenia, their therapeutic effects are associated by movement disorder (Grohman *et al*., 1990) such as parkinsonian like effects and tardive dyskinesia often described as extrapyramidal side effects (EPS) (Casey, 2000; Kulkarni and Naidu., 2001; Haleem and Khan., 2003; Haleem *et al*., 2004, 2007a, b). Tardive dyskinesia, a syndrome of potentially irreversible, involuntary hyperkinetic movements in the orofacial region which develops in patients during chronic neuroleptic treatment, is a major limitation of neuroleptic therapy (Egan *et al*., 1997; Casey, 2000; Haleem *et al*, 2007a, b).

It has been reported that single administration of haloperidol suppressed exploratory locomotor activity and impaired motor coordination leading to a state of catalepsy (Haleem *et al*., 2002, 2004; Karl *et al*., 2006). On the other hand, long term administration of haloperidol develops orofacial movements in rats described as vacuous chewing movements (VCMs) accompanied with the twitching of facial musculature. These dyskinetic orofacial parameters are widely used for quantification in the animal model of tardive dyskinesia (TD) (Egan *et al*., 1996; Casey, 2000; Kulkarni and Naidu, 2001). It has been reported that chronic haloperidol administration is responsible for the shrinkage of dopaminergic cell bodies in the substantia nigra and striatum. These morphological changes were reinstated after the drug withdrawal (Marches *et al*, 2002; Glenthoj, 1993) indicating that long term changes in brain function are produced following chronic use of haloperidol (Meshul *et al*, 1994; Marches *et al*, 2002).

Studies on animal models of tardive dyskinesia have used oral administration via drinking water, long acting depot formulations, intracerebroventricular (ICV) injections, daily intraperitoneal injections, and long acting subcutaneous implants (Waddington, 1990). Most patients take their antipsychotic medication via the oral routes on a daily basis. Therefore, administration of antipsychotic to rats via drinking water may be seen as more appropriate route of drug administration. The present study was designed to monitor EPS elicited by the oral administration of haloperidol at therapeutically recommended doses and to compare it with EPS produced when the drug is injected intraperitoneally. On the other hand, studies on animal models largely used haloperidol injections at high doses (Haleem *et al*., 2002, 2003; Casey, 2000; Naidu *et al*., 2002). It was hypothesized that slow and sustained effect of oral administration of haloperidol may not elicit EPS in rats.

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METHODS

Animal and treatment

Twenty four locally bred male albino Wistar rats weighing 180-220g purchased from HEJ research institute Karachi were housed individually under a 12-hours light/dark cycle (lights on at 06:00 A.M) in a quiet room with free access of cubes of standard rodent diet and tap water for 3 days before experimentation. The protocol for experimentation was approved and performed in strict accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, US National Research Council, 1996) and the Institutional Animal Ethics Committee (IAEC).

Drugs

Haloperidol (Serenace Drops, Searle; USA) purchased as oral drops of 2.0 mg/ml was given orally in drinking water in clinically recommended (10 mg/70 kg/day) dose. The drug was added in the drinking water at a concentration of 0.3 mg/100 ml. Average intake of water was 23 + 0.2 ml/rat/day and drug was 0.069 mg + 0.02/rat/day.

Haloperidol (Serenace; Searle, USA), purchased as injectable ampoules of 5 mg/ml, was injected intraperitoneally at a dose of 1.0 mg/kg body weight. Control animals were injected with saline in volumes of 1 ml/kg body weight.

Experimental protocol

Administration of haloperidol at clinically recommended dose via oral drops

Twelve animals divided into water and haloperidol treated groups were treated accordingly. Haloperidol drops were given in drinking water for 5 weeks at clinically recommended dose. Behavioral assessments of motor activity, motor coordination, VCMs and tardive VCMs were carried out weekly.

A*dministration of haloperidol (1 mg/kg) via intra-peritoneal injections*

Twelve animals divided into saline and haloperidol injected groups were injected accordingly with saline (1 ml/kg) or haloperidol (1mg/kg). The animals were injected twice a day at 9:00-9:30 and 17:00-17:30h for 5 weeks. Behavioral assessments were carried out weekly. Motor activity and motor coordination respectively were 60 min and 75 post injection whereas VCMs and tardive VCMs were quantified 1h before the drug administration. This was done to minimize that cataleptogenic effects of haloperidol may interfere with the behavioral assessment of VCMs.

Activity in an open field activity

To monitor activity in a novel environment, open field apparatus was used. The open field apparatus used in the present investigation consisted of a square area 76x76 with walls 42 cm high. The floor was divided by lines into 25 equal squares. To determine activity a rat was placed in the center square of the open field. The number of squares crossed with all four paws was scored for 5 minutes as described earlier (Haleem *et al.*, 2007a).

Quantification of orofacial dyskinesia

Animals were placed individually in a rectangular Perspex activity cage (26×26×26 cm) with a saw dust covered floor and were allowed to adapt to the observation cage for a period of 15 minutes orofacial dyskinesia were quantified as VCMs and tardive VCMs; that is, burst of purposeless chewing and opening of mouth in the vertical plane not directed towards physical materials respectively, during 10-min observation period.

Motor coordination

Motor coordination was assessed for all rats on a Rota-Rod (UGO BASEILE, Biological research apparatus, COMERIO, Varese, Italy). The Rota-Rod with a drum of 7-cm diameter was adjusted to a speed of 2-20 revolution/minute during the training session and a fixed speed of 20.revolution/minute during the test session. A day before the treatment rats were trained in a single session until they attained 150 seconds on Rota-Rod. The latency to fall in a teat session of 150s was taken as measure of motor coordination.

STATISTICAL ANALYSIS

Data were analyzed by two-way analysis of variance (ANOVA). Post-hoc comparisons were carried out by Newman-Keuls test. *P* values <0.05 were taken as significant.

RESULTS

Fig. 1: Effects of oral administration of haloperidol on exploratory activity in an open field (1A), motor coordination (1B), tardive vacuous chewing movements (1C) and vacuous chewing movements (1D) in rats.

Fig. 1A shows the effects of oral administration of haloperidol on the weekly changes of exploratory activity in an open field. Two way ANOVA showed significant effect of haloperidol ($F = 135$ $p<0.01$) and weeks ($F = 7.0$ $p<0.01$). The interaction between haloperidol x weeks was not significant ($F = 0.4$ N.S). Post hoc analysis showed that administration of haloperidol for one week decreased open field exploration. The decreases were smaller after two weeks of administration. The effects were attenuated after 3 weeks of drug administration.

Fig.1B shows the effects of oral administration of haloperidol on the weekly changes of motor coordination on a Rota-Rod. Two way ANOVA revealed significant

effect of haloperidol ($F = 17$ $p<0.01$) and weeks ($F = 15$ $p<0.01$). The interaction between haloperidol and weeks was also significant ($F = 22$ $p<0.01$). Post-hoc analysis by Newman-Keuls test showed that administration of haloperidol impaired motor coordination after 1st week. The impairment of motor coordination was maximum after 3rd weeks and progressively normalized during 5 weeks of drug administration of drug administration.

Fig. 1C shows the intensity of tardive VCMs following oral administration of haloperidol. Two way ANOVA revealed significant effect of haloperidol ($F = 217$ $p<0.01$) and weeks ($F = 232$ $p<0.01$). The interaction between haloperidol and weeks was also significant ($F = 284$ $p<0.01$). Post-hoc analysis by Newman-Keuls test showed that administration of haloperidol elicited tardive VCMs after 3 weeks of administration that increased in a time dependent manner during the subsequent weeks of drug administration.

Fig. 1D shows the intensity of VCMs following oral administration of haloperidol. Two way ANOVA revealed significant effect of haloperidol ($F = 247$ $p<0.01$) and weeks ($F = 292$ $p<0.01$). The interaction between haloperidol and weeks was also significant ($F = 262$ $p<0.01$). Post-hoc analysis by Newman-Keuls test showed that administration of haloperidol elicited VCMS after 1 week of administration which increased in a time dependent manner during 5 weeks of treatment.

Fig. 2. Effects of administration of haloperidol via intraperitoneal injection (1 mg/kg) on exploratory activity in an open field (2A), motor coordination (2B), tardive vacuous chewing movements (2C) and vacuous chewing movements (2D) in rats.

Fig. 2A shows the effects of haloperidol injections on the weekly changes of exploratory activity in an open field. Two way ANOVA showed significant effect of haloperidol ($F = 53$ $p<0.01$) and weeks ($F = 27$ $p<0.01$). The interaction between haloperidol x weeks was not significant ($F = 14$ N.S). Post hoc analysis showed that administration of haloperidol produced complete akinesia in an open field during 5 weeks of treatment.

Fig. 2B shows the effects of haloperidol injections on the weekly changes of motor coordination on a Rota-Rod. Two way ANOVA revealed significant effect of haloperidol ($F = 115$ $p<0.01$) and weeks ($F = 10$ $p<0.01$). The interaction between haloperidol and weeks was also significant ($F = 2$ N.S). Post-hoc analysis by Newman-Keuls test showed that administration of haloperidol impaired motor coordination after 1st week. The deficits were attenuated in a time dependent manner during 5 weeks of treatment.

Fig. 2C shows the intensity of tardive VCMs in haloperidol injected treated rats. Two way ANOVA revealed significant effect of haloperidol ($F = 130$ $p<0.01$) and weeks ($F = 32$ $p<0.01$). The interaction between haloperidol and weeks was also significant ($F = 44$ $p<0.01$). Post-hoc analysis by Newman-Keuls test showed that administration of haloperidol elicited tardive VCMs after 4 weeks of administration that increased in a time dependent manner during 5 weeks of treatment.

Fig. 2D shows the intensity of VCMs in haloperidol injected rats. Two way ANOVA revealed significant effect of haloperidol ($F = 462$ $p<0.01$) and weeks ($F =21$ $p<0.01$). The interaction between haloperidol and weeks was also significant ($F = 33$ $p<0.01$). Post-hoc analysis by Newman-Keuls test showed that administration of haloperidol elicited VCMs after 1st week of administration which increased in a time dependent manner during 5 weeks of treatment.

DISCUSSION

The present study shows that oral administration of haloperidol at clinically recommended dose elicited smaller parkinsonian like symptoms and more VCMs and tardive VCMs (Fig.1) that increased in a time dependent manner as the treatment continued for 5 weeks as compared to intraperitoneal injection (Fig. 2).

The dopamine system is known to be involved in the control of motor activity (Clausing *et al*, 1995). Striatum is a region of brain involved in the control of motor behavior. It is well established that administration of haloperidol suppressed spontaneous exploratory locomotor activity (Karl *et al*, 2006; Haleem *et al*, 2002, 2007a, b; Shireen and Haleem, 2011) and prevents hyperactivity induced by amphetamine (Moore, 1999). This effect of haloperidol is explainable in terms of its antagonist activity at dopamine D-2 receptor. In the present study, administration of haloperidol at a dose of 1mg/kg via i.p injection route decreased exploratory locomotor activity in an open field leading to akinesia (fig. 2). The effects remained constant during 5 weeks of treatment. Activity reducing effects of haloperidol are also relevant that the administration of drug for 5 weeks does not release motor activity from the inhibitory influence of neuroleptic. Thus, activity reducing effects of haloperidol are thought to be mediated via the blockade of postsynaptic DA D-2 receptor (Haleem *et al*., 2002; 2007a). An important finding of the present study was that the administration of haloperidol via oral route at clinically recommended doses although decrease activity (fig. 1) but akinesia was not produced. Moreover, tolerance was produced in haloperidol-induced reduction in motor activity on long term drug administration. The results suggest that tolerance is produced in the

parkinsonian like effects of haloperidol when the drug is administered orally in clinically recommended doses.

Haloperidol-induced impairment in motor coordination in rats is also used as an animal model of parkinsonism (Haleem *et al.*, 2007b; Shireen and Haleem, 2011). The present results show that i.p injection of haloperidol at a dose of 1 mg/kg elicited a large decrease in motor coordination (fig. 2). The deficits gradually attenuated on repeated administration but complete tolerance was not produced. On the other hand, oral administration of haloperidol decreases motor coordination after 1st and 2nd weeks but maximum after 3rd week (fig. 1). This effect attenuated after 4 weeks treatment were no more significant after 5th week of treatment. Tolerances in haloperidol induced motor deficits by long term oral drug administration however can be explained in terms of slow and sustained dopamine D-2 receptor occupancy with little variation over 24 hrs because drug was continuously given in drinking water. Conversely, if the drug is given via i.p. injections then possible temporary occupancy of dopamine D2 receptor occurred that is expected to be deceased at the end of the day.

Other authors have reported that repeated (chronic and subchronic) administration of haloperidol-induced orofacial dyskinesia (Naidu and Kulkarni, 2001; Naidu *et al*, 2002; Tamminga *et al*, 1990). In the present study, treatment with haloperidol at a dose of 1 mg/kg twice a day (fig. 2) and oral drug administration induced VCMs (fig. 1) within two weeks that increased in a time dependent manner as the treatment continued for 5 weeks. Intensity of vacuous chewing movements (VCMs) and tardive VCMs were greater by oral administration than intraperitoneal injections of haloperidol. It may be noted that time at which VCMs are induced is same when tolerance is produced in the cataleptic response. It supports the notion that DA D-2 receptor upregulation may have a role in the elicitation of TD. It has been reported that chronic administration of haloperidol induces VCMs in a dose dependent manner and more than 70-63% DA D-2 receptor occupancy is required to develop VCM (Turrone *et al*, 2002). Therefore, DA receptor supersensitivity due to the upregulation of DA D-2 receptor is responsible for the development of TD following chronic neuroleptics therapy (Klawans and Rubovits, 1972).

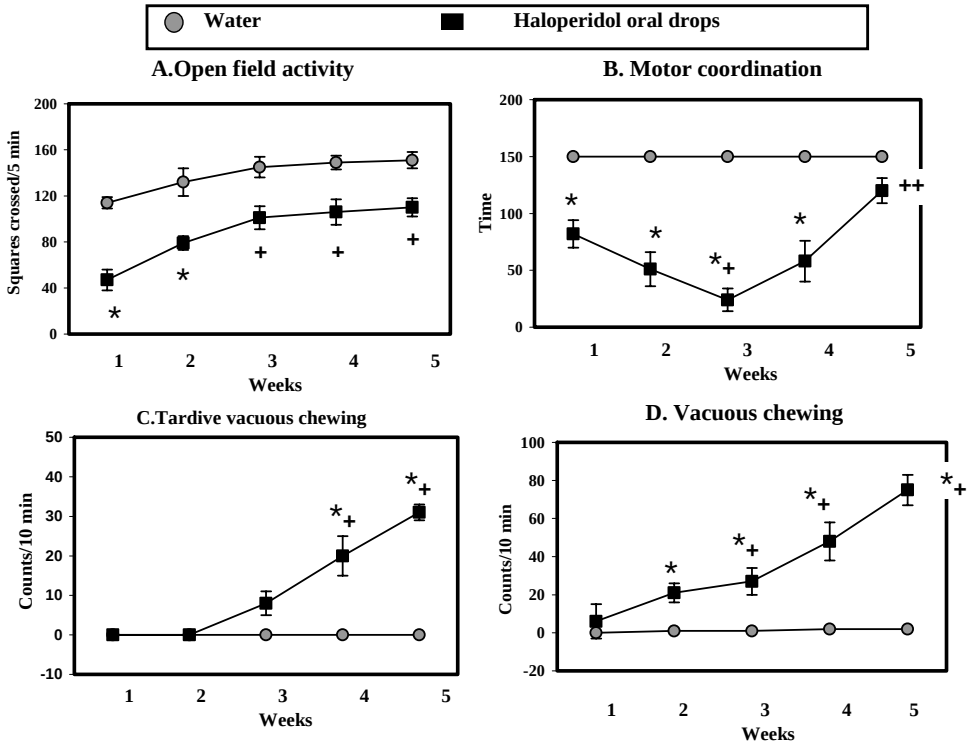

Fig. 1: Effects of oral administration of haloperidol on exploratory activity in an open filed (1A), motor coordination (1B), tardive vacuous chewing movements (1C) and vacuous chewing movements (1D) in rats. Values are mean + (n=6) 60 min (open field) and 75 min (motor coordination). Significant differences by Newman-Keuls test: *P<0.05, **P <0.01 from water treated and haloperidol treated animals. :+P<0.05, ++P<0.01 from first week values of water or haloperidol treated animals.

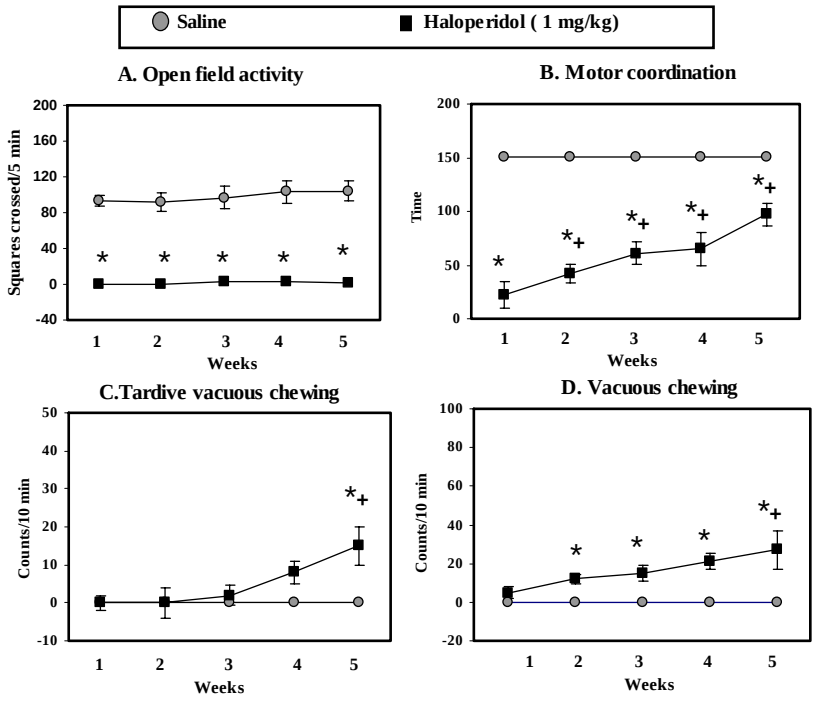

Fig. 2: Effects of administration of haloperidol via intraperitoneal injection (1 mg/kg) on exploratory activity in an open filed (2A), motor coordination (2B), tardive vacuous chewing movements (2C) and vacuous chewing movements (2D) in rats. Values are mean + (n=6) 60 min (open field) and 75 min (motor coordination). Significant differences by Newman-Keuls test: *P<0.05, **P <0.01 from saline treated and haloperidol treated animals. :+P<0.05, ++P<0.01 from first week values of saline or haloperidol treated animals

5-HT-1A receptor is involved in the etiology of VCMs. Long term (2 weeks) administration of haloperidol elicited VCMs and supersensitized 5-HT-1A receptors (Haleem and Khan, 2003). The Serotonergic system is known to inhibit dopamine neurotransmission at the level of the origin of dopamine system in the midbrain as well as in the terminal regions. 5-HT-2A/2C receptor antagonists such as mianserin could release DA neurotransmission from the inhibitory influence of 5-HT in order to minimize neuroleptics-induced parkinsonian effects (Kapur and Ramington, 1996; Millan *et al*, 1998; Shireen and Haleem, 2011). Stimulation of somatodendritic 5-HT-1A receptor decreases the availability of 5-HT possibly at 5-HT-2C receptors could release DA neurotransmission from the inhibitory influence of 5-HT to alleviate haloperidol-induced parkinsonian like effects (Haleem *et al*, 2004; Haleem, 2006). Recent studies from our laboratory showed that an increase in the effectiveness of somatodendritic 5-HT-1A receptors are involved in the elicitation of haloperidol induced VCMs (Haleem *et al*, 2007a, b). The present study suggests that haloperidol-induced increase in the sensitivity of somatodendritic 5-HT-1A receptors may be greater when the drug is administered orally in drinking water.

In conclusion, the present study showed that administration of haloperidol in drinking water may produces less parkinsonian like effects because occupancy of DA D-2 receptors and/or 5-HT-1A receptors is smaller than the receptor blockade by acute administration. It shows that an upregulation of dopamine D-2 receptors is known to have a role in the elicitation of tardive VCMs is greater when the drug is administered orally in drinking water.

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