

Haloperidol-induced extra pyramidal symptoms attenuated by imipramine in rats

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Abstract: Effects of administration of imipramine (IMI) are determined on haloperidol-induced extrapyramidal symptoms (EPS). Haloperidol is administered orally at a dose of 0.2 mg/rat/day in rats for a period of 5 weeks, by this treatment rats developed vacuous chewing movements (VCMs) after 2 weeks, which increased in a time dependent manner as the treatment continued for 5 weeks. Motor coordination (assess on rota rod activity) impaired maximally after 3 weeks and tolerance was developed in the haloperidol induced motor impairment after 5 weeks of treatment. Motor activity in an open field or activity box was not altered. The administration of IMI (intraperitoneally, for 5 weeks) did not affect motor activity or motor coordination. Co-administration of IMI at a dose of 5 mg/ml/kg/day attenuated the induction of haloperidol elicited VCMs (Quantitative orofacial dyskinesia) as well impairment of motor coordination. Results are discussed in the context of the mechanism involved by which imipramine attenuated haloperidol-induced EPS.

Keywords: Haloperidol, imipramine (IMI), extrapyramidal symptoms (EPS), vacuous chewing movement (VCMs).

INTRODUCTION

Anti-psychotics medication is the mainstay of treatment in schizophrenia and other disorders (Achilla and McCrone, 2013). Treatment with atypical anti-psychotics such as haloperidol often causes adverse movement disorders termed as extrapyramidal symptoms including tardive dyskinesia (Creed and Nobrega, 2013). In rats, chronic haloperidol treatment leads to a syndrome of vacuous chewing movements (VCMs) which are considered analogous to human TD (Turrone *et al.*, 2002).

Several lines of evidence implicate that role of 5-hydroxytryptamine (5-HT; serotonin) and particularly 5-HT-1A receptors is important in the etiology of schizophrenia and the elicitation of extrapyramidal symptoms (EPS) (Meltzer, 2012). In various animal researches, role of 5-HT-1A receptor is shown for the treatment of TD, because haloperidol induced dyskinesia in a rat model of TD was reversed by sarizoton (a 5-HT-1A agonist/D3/D4 ligand) (Rosengarten *et al.*, 2006), in another study reserpine-induced dyskinetic movements in a rat model of TD were reversed by the co-administration of buspirone (Queiroz *et al.*, 1999), a partial agonist at 5-HT-1A receptor and also an anxiolytic and antidepressant (Gobert *et al.*, 1999). 8-Hydroxy -2- (di-n-propylamino) tetraline (8-OH-DPAT), a selective 5-HT-1A agonist, attenuated haloperidol-induced VCMs (Naidu and Kulkarni, 2001).

Previously we have reported that repeated administration of buspirone inhibited the EPS-induced by haloperidol by

desensitization of somatodendritic 5-HT-1A receptors (Haleem *et al.*, 2007a). Therefore, the present study was designed to examine the effect of imipramine (IMI) a tricyclic antidepressant (TCAs) on haloperidol-induced EPS at a dose (5mg/ml/kg body weight) intraperitoneally for 5 weeks, cumulative studies showed that IMI produced strong antidepressant effects (Rogoz *et al.*, 2003) and behavioral stimulation (Borocco *et al.*, 2002) following repeated administration.

MATERIALS AND METHODS

Animals

24 male albino Wistar rats (locally bred) weighing 170-220 g purchased from HEJ Research Institute, University of Karachi, Pakistan were housed individually with free access to cubes of standard rodent diet and tap water 3 days before starting the experiment.

Drugs

Haloperidol (Serenace, Searl; USA) purchased as oral drops of 2.0 mg/ml was given orally in drinking water at a dose of 0.2 mg/rat/day in clinically recommended dose. IMI purchased from Sigma, was dissolved in saline and injected subcutaneously at a dose of 5 mg/ml/kg/day.

Experimental Protocol

Twenty-four animals were divided into four groups (i) water+saline; (ii) water+IMI (iii) haloperidol+saline; (iv) haloperidol+IMI, were received respective treatment for 5 weeks. Haloperidol as oral drops of 2.0mg/ml was given orally in drinking water at a dose of 0.2mg/kg/day. IMI was injected at a dose of 5 mg/ml/kg once daily. VCMs,

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motor coordination, exploratory activity in an open field and in home cage were monitored weekly during 5 weeks as described by Merchese *et al.*, 2002; Haleem *et al.*, 2007a,b.

Behavioral analysis

Open field activity

To monitor activity in a novel environment, open field apparatus was used. The procedure was similar as described by Haleem *et al.*, 2002. The open field apparatus used in the present study consisted of a square area 76x76 cm with walls 42 cm high. The floor was divided by lines into 25 equal squares. To determine activity Animals were placed in the central square of the open field and numbers of square crossed with all four paws were counted for 5 minutes.

Home cage activity

Locally made transparent (Perspex) activity box consisted of small square area (26x26x26 cm) with sawdust-covered floor were used to monitor activity. Before monitoring the activity an animal was placed in it for 15 minutes for habituation. Numbers of crossings across the box were monitored for 10 minutes as described by Haleem *et al.*, 2007a,b.

Rota-rod activity

Motor coordination was assessed for all rats on a rota-rod (UGO BASILE, Biological research apparatus, COMERIO, Varese, Italy). The rota-rod with a drum of 7 cm diameter was adjusted to a speed of 16 revolutions/minute during the training session and fixed speed of 16 revolutions/minute during the test session. A day before the treatment rats were trained in a single session until they attained 150s on the Rota Rod. The latency to fall in a test session of 150s was taken as a measure of motor coordination as described by Haleem *et al.*, 2007a,b.

Vacuous chewing movements (VCMs) quantification

Animals were placed individually in a rectangular Perspex activity cage (26x26x26 cm) with sawdust-covered floor and allowed to adapt the observation cage for a period of 15 minutes. *Orofacial dyskinesias* were quantified as tardive VCMs; that is, opening of the mouth in the vertical plane not directed towards physical materials, during a 10 minutes observation. For calculation purposes, each burst of purposeless chewing was counted as one, if its duration was at least 3 seconds as described by Haleem *et al.* (2007a,b).

STATISTICAL ANALYSIS

Data analyzed by three-way ANOVA. Post-hoc comparison was done by Newman-Keuls test: P<0.05 taken as significant.

RESULTS

Fig. 1 illustrates weekly changes of activity in the activity box in the four groups of rats. Three-way ANOVA showed significant effects of haloperidol (F=34.21 df=1,20 p<0.01), IMI (F=31.70 df=1,20 p<0.01) and weeks (F=4.68 df=4,80 p<0.05). Interactions between IMI and haloperidol (F=2.87 df=1,20 p>0.05), weeks and haloperidol (F=1.56 df=4,80 p>0.05), IMI and weeks (F=0.13 df=4,80 p>0.05) and haloperidol*IMI*weeks (F=0.017 df=4,80 p>0.05) were not significant. Differences by Newman-Keuls test were not significant.

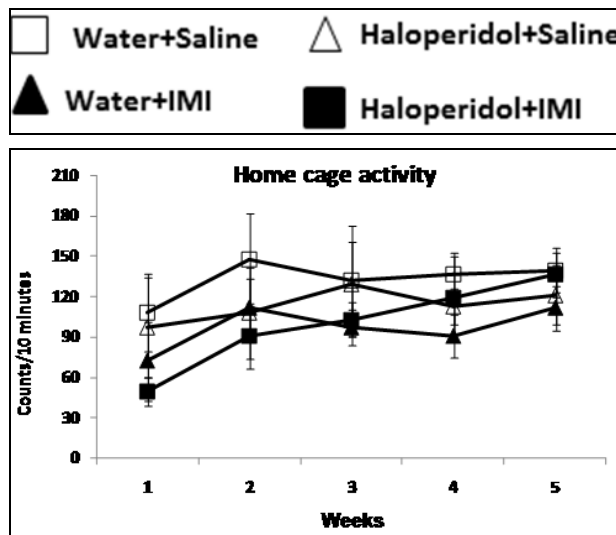


Fig. 1: Effects of administration of haloperidol on activity in activity box in animals treated with saline and IMI. Values are means ±S.D. (n=6).

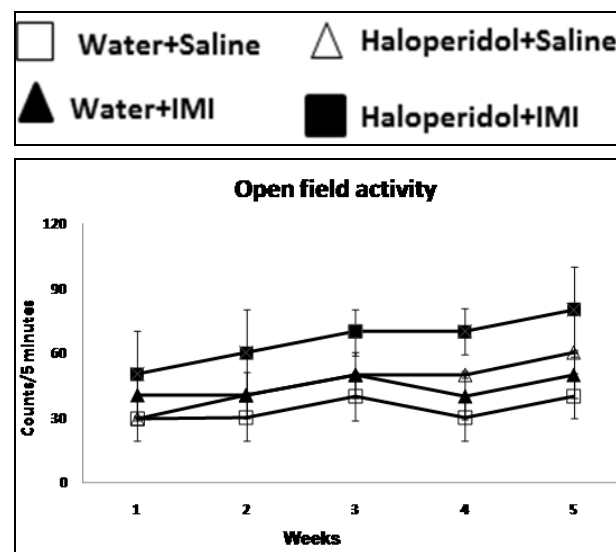


Fig. 2: Effects of administration of haloperidol on open field in animals treated with saline and IMI. Values are means ±S.D. (n=6).

Fig. 2 illustrates weekly changes of exploratory activity in an open field in the four groups of rats. Three-way

ANOVA showed significant effects of IMI ($F=29.64$ $df=1,20$ $p<0.01$) and weeks ($F=11.14$ $df=4,80$ $p<0.05$), but not significant for haloperidol ($F=3.11$ $df=1,20$ $p<0.01$). Interaction between IMI and haloperidol ($F=5.71$ $df=1,20$ $p<0.05$) was significant. Interactions between weeks and haloperidol ($F=2.14$ $df=4,80$ $p>0.05$), IMI and weeks ($F=1.64$ $df=4,80$ $p>0.05$) and haloperidol*IMI*weeks ($F=1.60$ $df=4,80$ $p>0.05$) were not significant. Differences by Newman-Keuls test were not significant.

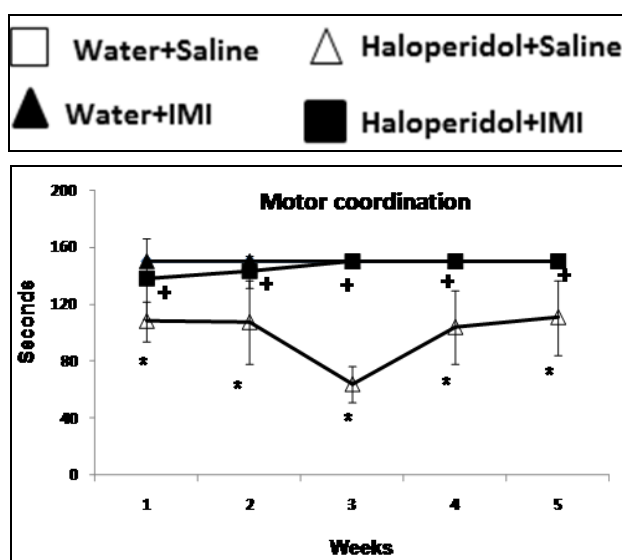


Fig. 3: Effects of administration of haloperidol on motor coordination in animals treated with saline and IMI. Values are means \pm SD ($n=6$). Significant differences by Newman-Keuls test: * $p<0.01$ from water +saline treated animals, + $p<0.01$ from haloperidol +saline treated animals following three-way ANOVA.

Fig. 3 illustrates motor coordination on a Rota-Rod in the four groups of rats. Three-way ANOVA showed significant effects of haloperidol ($F=27.63$ $df=1,20$ $p<0.01$), IMI ($F=63.01$ $df=1,20$ $p<0.01$). Effects of weeks ($F=2.39$ $df=4,80$ $p>0.05$) were not significant. Interactions between IMI and haloperidol ($F=83.15$ $df=1,20$ $p<0.05$) and IMI and weeks ($F=4.48$ $df=4,80$ $p<0.05$) were significant. Interactions between weeks and haloperidol ($F=2.55$ $df=4,80$ $p>0.05$) and haloperidol*IMI* weeks ($F=2.64$ $df=4,80$ $p>0.05$) were not significant. Post-hoc analysis showed that administration of haloperidol impaired motor coordination in saline injected animals after 1st week. The impairment of motor coordination was maximum after 3rd week and gradually returned to 1st week value. Administration of IMI attenuated haloperidol-induced impairment of motor coordination.

Fig. 4 illustrates the intensity of VCMs in saline and IMI injected animals. Three-way ANOVA revealed significant effects of haloperidol ($F=400.85$ $df=1, 20$ $p<0.01$), IMI

($F=130.56$ $df=1,20$ $p<0.01$) and weeks ($F=59.67$ $df=4,80$ $p<0.01$). Interactions between IMI*haloperidol ($F=147.23$ $df=1,20$ $p<0.01$), weeks*haloperidol ($F=41.96$ $df=1,100$ $p<0.01$), weeks*IMI ($F=14.69$ $df=4,80$ $p<0.01$) and haloperidol*IMI*weeks ($F=13.98$ $df=4,80$ $p<0.01$) were also significant. Post-hoc analysis showed that administration of haloperidol elicited VCMs in saline injected animals after the second week of treatment. Saline injected animals exhibited an increase in the intensity of haloperidol-induced VCMs after the 5th week of treatment. IMI injected animals exhibited a significant increase in VCMs after 4th and 5th weeks. An attenuation of haloperidol-induced VCMs in IMI injected animals were observed following 2nd to 5th weeks.

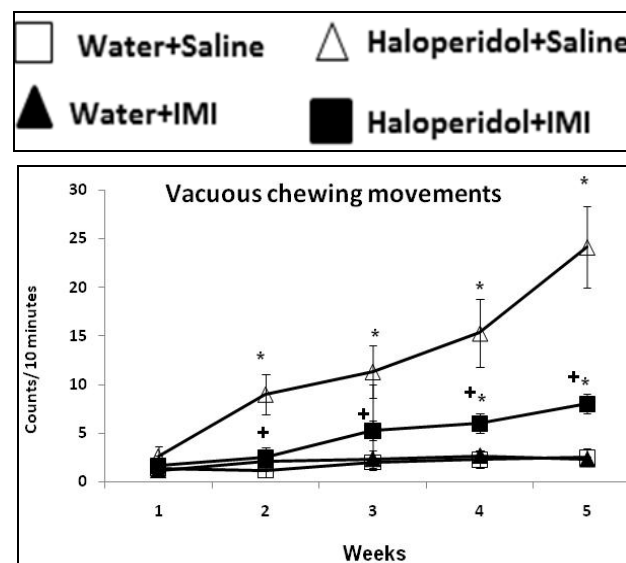


Fig. 4: Intensity of haloperidol-induced VCMs in animals treated with saline and IMI. Values are means \pm S.D. ($n=6$). Significant differences by Newman-Keuls test: * $p<0.01$ from water+saline and water+IMI treated animals, + $p<0.01$ from haloperidol +saline treated animals following three-way ANOVA.

DISCUSSION

Studies showed that haloperidol (at a dose of 1.5 mg/kg/day for 3 weeks induced facial twitching could be overturned by the co-administration of 8-OH-DPAT (Kulkarni and Naidu, 2001) and sarizoton (Brocco *et al.*, 2002). Haloperidol-induced orofacial dyskinesia (Haleem *et al.*, 2007a, b) as well as reserpine-induced orofacial dyskinesia (Quiroz *et al.*, 1999) were also overturned by the co-administration of buspirone. The aim of the present study is to evaluate the effect of haloperidol-induced TD by the co-administration of IMI (an antidepressant and 5-HT reuptake inhibitor). Tokonova *et al.* (2006) have shown that IMI could prevent the severity of catalepsy. The present study shows that 2 weeks treatment with haloperidol at a dose of 0.2mg/rat/day induced VCMs that increased in a time dependent manner as the treatment

sustained for 5 weeks. Co-administration of IMI at a dose of 5mg/ml/kg/day attenuated and completely overturned the induction in a time dependant manner (fig. 4).

Other authors have proposed a role of somatodendritic 5-HT-1A receptors in the onset of orofacial dyskinesia (Haleem and Khan, 2003, Samad *et al.*, 2007) because treatment with haloperidol for 2 weeks educed VCMs (Kulkerni and Naidu, 2001; Haleem *et al.*, 2007 a,b) and increased the sensitivity of somatodendritic 5-HT-1A receptors in rats (Haleem *et al.*, 2007 a,b). The present study reveals an important finding that reversal of haloperidol-induced VCMs in rats co-treated with IMI was associated with the reversal of haloperidol-induced increase in the responsiveness of somatodendritic 5-HT-1A receptors that have an important role in the precipitation and alleviation of haloperidol-induced VCMs. The present study also shows long term administration of IMI did not affect exploratory activity and motor coordination (fig. 1, 2, 3) while an impairment of motor coordination by haloperidol was completely overturned by the co-administration of IMI.

IMI is an antidepressant drug that preferentially inhibits 5-HT reuptake (Mokoena *et al.*, 2010; de Borotoli *et al.*, 2008). It is previously suggested that antidepressant effects of IMI are mediated by some of 5-HT receptor subtypes. The drug has been to reduce the sensitivity of catalepsy and functional activity of 5-HT-1A receptors (Tokonova *et al.*, 2006). Mochizuki *et al.* (2002) have reported that chronic administration of IMI may have resulted in desensitization of somatodendritic 5-HT-1A receptors. Furthermore, Shen *et al.* (2002) have shown that chronic treatment with IMI or fluoxetine enhanced postsynaptic 5-HT-1A receptors sensitivity in the hippocampus and decreased it in the dorsal raphe. In the present study IMI was injected at a dose of 5 mg/kg, the mechanism by which IMI could attenuate haloperidol induced increase in the effectiveness of somatodendritic 5-HT-1A receptors (fig. 3 and 4) might involved in desensitization of somatodendritic 5-HT-1A receptors by repeated administration of the drug (Mochizuki *et al.*, 2002).

In conclusion, present study shows that 5 weeks administration of haloperidol (0.2mg/rat/day) induced TD and impaired motor coordination in a time dependent manner by stimulation of somatodendritic 5-HT-1A receptors. On the other hand co-administration of IMI (5 mg/ml/kg/day) attenuated and completely reversed impairment of motor coordination and induction of TD by desensitization of somatodendritic 5-HT-1A receptors. It is suggested that drugs that affect somatodendritic 5-HT-1A receptors may be use in alleviating EPS.

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