RESEARCH ARTICLE

Study the Effects of some Neuroleptic Drugs on the Haloperidol-induced Tardive Dyskinesia in Male Mice

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ABSTRACT

Tardive dyskinesia (TD) is a neurological iatrogenic disorder and is one of the neurodegenerative movement syndromes affecting mainly orofacial region which includes vacuous chewing movements (VCMs), tongue protrusion (TP), and facial jerking (FJ), resulting from chronic neuroleptic treatment of schizophrenia. The aims of this paper are to evaluate the effects of some a typical neuroleptic drug (risperidone, olanzapine, and aripiprazole) to reduce the TD in mice and study the effects of these drugs on dopamine levels in the brain, see Scheme 1.

Adult, albino mice were enrolled in this experiment. The animals were randomly divided into five groups ,each group has 10 mices. Each mouse of group1 received normal saline in equal volume to the haloperidol dose intraperitoneally (IP) for 21 days. Each mouse of group 2,3,4 and 5 received haloperidol 2 mg/kg IP for 21 days. mices of group 3,4 and 5 received risperidone 1 mg/kg, olanzapine 2.5 mg/kg, aripiprazole 3 mg/kg orally by gastric tube respectively for 3 days. On the 25th day, each mouse was placed in the glass box for 10 minutes, and VCM was recorded by video camera. Then each mouse of the whole groups was decapitated and the brain were removed from the skull for measurement of dopamine(DA).

Dopamine level decreased significantly in group 2 as compared with group1 ($p < 0.05$), there were significant increase in the DA levels between group 3 and group 4 as compared with group 2 ($p > 0.05$), but there were no significant differences between group 5 as compared with group 2 ($p > 0.05$). VCM increased significantly in group 2 as compared with group1 ($p < 0.05$). VCM decreased significantly in group 3 and 4 as compared with group 2 ($p < 0.05$), but there were no significant differences between group 5 as compared with group 2 ($p > 0.05$).

Keywords: Tardive dyskinesia, Vacuous chewing movements, Neuroleptic drugs.

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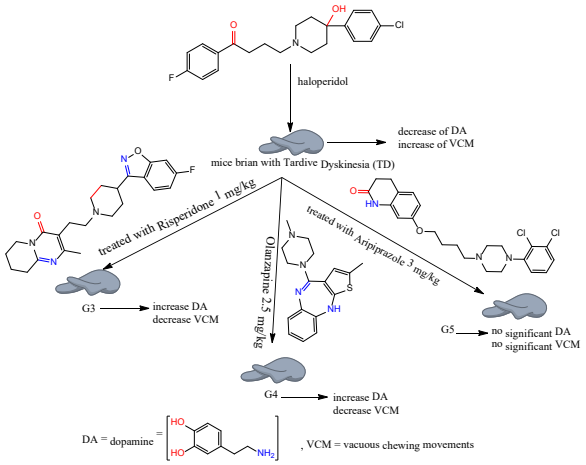
INTRODUCTION

The term dyskinesia is defined as involuntary muscle movements ranging from slight tremor to abnormal and uncontrollable movement of the entire body. Tardive Dyskinesia (TD) can be explained as slow-or tardive-onset of involuntary movements of the lips, face, tongue, trunk, and extremities.[1,2]

The first generation ‘‘typical’’ neuroleptics with high dopamine D_2 receptor occupancy have been found with higher risk of causing TD than next generations medications, often referred to as ‘‘atypical’’ antipsychotics, with low D_2 receptor occupancy, such as clozapine and quetiapine. TD is also associated with variety of other medications.[1,2]

The pathophysiology of TD still requires a universally accepted theory and mechanism. Various hypotheses have been proposed including prolonged blockade of postsynaptic



Scheme 1: Schematic diagram about the Effect of neuroleptic drug on provoked Taridive Dyskinesi in mice

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DA receptors leading to DA receptor super sensitivity, gamma aminobutyric acid (GABA) depletion, cholinergic deficiency, oxidative stress, and neurotoxicity.[3,4]

The hypothesis behind oxidative stress state that antidepressants block DA receptors and increase DA synthesis and metabolism resulting in increased production of free radicals The basal ganglia are subcortical nuclei comprised of several brain regions including the striatum and substantiating and they get highly innervated by DA neurons and are therefore remain at risk for oxidative stress and the occurrence of TD.[3,4] The aim of this paper was to evaluate the effect of some neuroleptic drugs on TD, dopamine and dopamine receptor levels (DRL) in brain mice.

EXPERIMENTAL WORK

Animals

Fifty male, adult, albino mice were enrolled in this experiment. Their weights were 20–44 g. The mice were housed in animal House of the College of Medicine, Babylon University, and kept on 25°C and 12 hours light and 10 hours light-dark cycles with water and food ad libitum. After two weeks of adaptation, the animals were randomly divided into 10 mice in each group.

Procedure

Each mice of group1 received normal saline in equal volume to the haloperidol dose IP for 21 days. Each mice of group 2,3,4 and 5 received haloperidol 2 mg/kg for 21 days. Then mice of group 3, 4 and 5 received risperidone 1 mg/kg, olanzapine 2.5 mg/kg, aripiprazole 3 mg/kg orally by gastric tube respectively for 3 days. On the 25th day, each mice was placed in the glass box for 10 minutes, then in the open field box for 5 minutes, then on the narrow beam for 5 minutes and all behaviors were recorded by video camera. Then each mice of the whole groups was decapitated and the brain were removed from the skull for chemical examination.

Brain Dissection

On the 25th days of treatment, the animals were sacrificed and the brains were removed after dissection of skull from foramen magnum posteriorly. Olfactory pulps and cerebellum were removed and the brain removed gently from the skull and the mid and forebrain were taken and dissected out and rinsed in isotonic saline and weighted.

Steps of Preparation of Sample

Tissue homogenates: residual blood removed by washing tissue with pre-cooling PBS buffer (0.01 M, pH = 7.4). After weighing it, tissues get homogenized in PBS (Normally, 9mL PBS are appropriate to 0.5 gram tissue pieces). Further to that, protease inhibitors were added into the PBS with a glass homogenizer on ice (adding at 1: 100 (v/v) dilution to 1 solution samples before assaying). For further breaking the cells, sonication have been done with an ultrasonic cell disrupter or subjection the suspension to freeze-thaw cycles. Then homogenates are centrifuged for 5 minutes at 5000×g to get the supernatant.

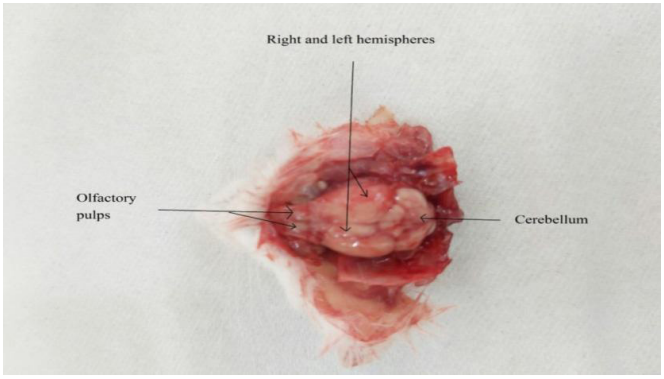
Assessment of Dopamine using ELISA kit

DA was measured by enzyme linked immunosorbent assay (ELISA), for more information see the reference.[19]

RESULTS

A. Dopamine Level

DA level decreased significantly in group 2 (haloperidol 2 mg/kg) ,group 3 (risperidone 1 mg/kg), group 4 (olanzapine 2.5 mg/kg) and group 5 (aripiprazole 3 mg/kg) as compared with group1 (normal saline) ($p < 0.05$) (Table 1 and Figure 2).



Picture 1: Skull dissection of the mouse.

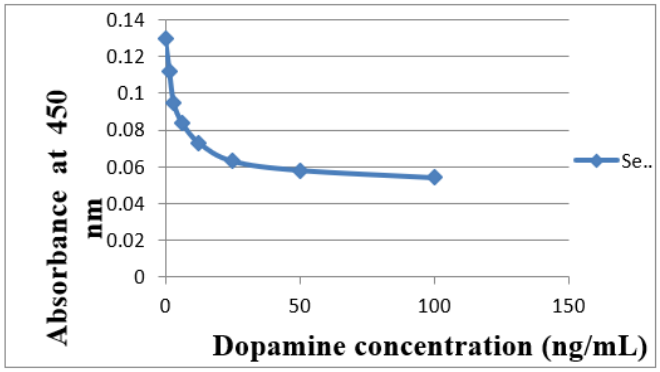


Figure 1: Standard curve of dopamine.

Table 1: A comparison of the mean differences of DA level between different groups (group 1:control group; group 2: haloperidol 2 mg/kg; group 3: risperidone 1 mg/kg; and group 4: olanzapine 2.5 mg/kg; group 5: aripiprazole 3 mg/kg).

Dopamine level Mean	*Control 1*	*Haloperidol 2*	*Risperidone 3*	*Olanzapine 4*	*Airpiprzole 5*
Control (1)	X	4.8*	2.8*	2.7*	6*
Group (2)	-4.8*	X	-1.9*	-2.1*	1.1
Group (3)	-2.8*	1.9*	X	-0.1	3.1*
Group (4)	-2.7*	2.1*	0.1	X	3.2*
Group (5)	-6*	-1.1	-3.1*	-3.2*	X

*$p < 0.05$ significant

Table 2: A comparison of the mean differences of the No. of vacuous chewing movements between different groups (group 1:control group; group 2: haloperidol 2 mg/kg; group 3: risperidone 1 mg/kg; and group 4: olanzapine 2.5 mg/kg; group 5: aripiprazole 3 mg/kg).

Mean of VCM	*Control 1*	*Haloperidol 2*	*Risperidone 3*	*Olanzapine 4*	*Airpiperizole 5*
Group 1	X	-1*	-0.3	-0.1	-0.5
Group 2	1*	X	0.7*	0.9*	0.5
Group 3	0.3	-0.7*	X	0.2	-0.2
Group 4	0.1	-0.9*	-0.2	X	-0.4
Group 5	0.5	-0.5	0.2	0.4	X

*p <0.05 significant

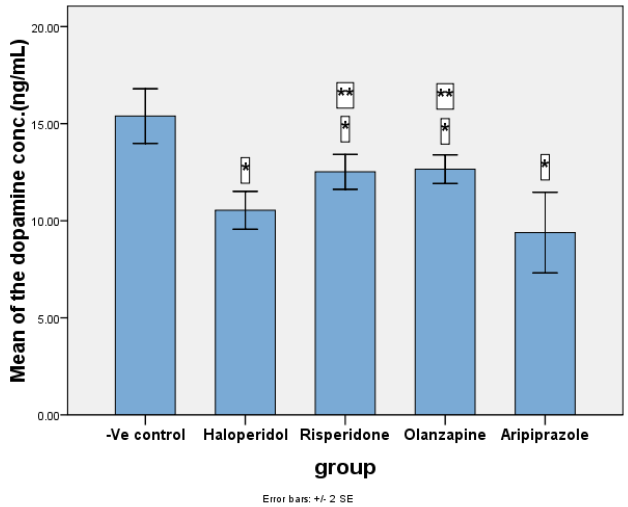


Figure 2: Means of the DA concentration of all groups; group 1 (control group), group 2 (haloperidol 2 mg/kg), group 3 (risperidone 1 mg/kg), group 4 (olanzapine 2.5 mg/kg) and group 5 (aripiprazole 3 mg/kg), (No. of animal = 10 for each group).
*: Significant differences (P <0.05) between 2,3,4,5 groups as compared with group 1
**: Significant differences (P <0.05) between 3, 4, 5 groups as compared with group 2

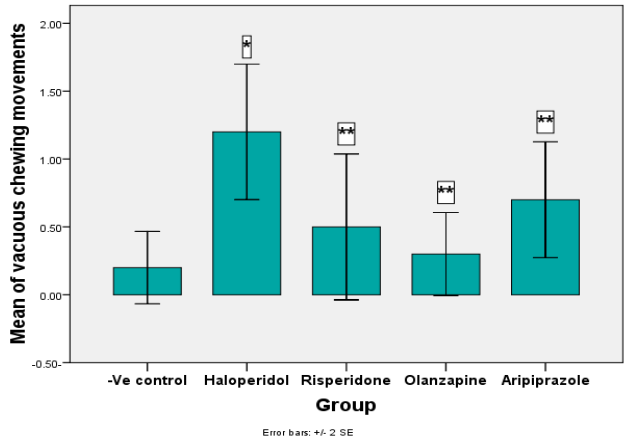


Figure 3: Means of the No. of vacuous chewing movements of all groups; group 1 (control group), group 2 (haloperidol 2 mg/kg), group 3 (risperidone 1 mg/kg), group 4 (olanzapine 2.5 mg/kg) and group 5 (aripiprazole 3 mg/kg), (No. of animal = 10 for each group).
*: Significant differences (P <0.05) between 2, 3, 4, 5 groups as compared with group 1
**: Significant differences (P <0.05) between 3, 4, 5 groups as compared with group 2

There were significant differences in the DA levels between group 3 (risperidone 1 mg/kg) and group 4 (olanzapine 2.5 mg/kg) as compared with group 2 (haloperidol 2 mg/kg) (p >0.05), but there were no significant differences between group 5 (aripiprazole 3 mg/kg) as compared with group 2 (haloperidol 2 mg/kg) (p >0.05) (Table 1 and Figure 2).

Vacuous Chewing Movement

No. of VCM increased significantly in group 2 (haloperidol 2 mg/kg) as compared with group1 (normal saline) (p <0.05). While there were no significant differences between group 3 (risperidone 1 mg/kg), group 4 (olanzapine 2.5 mg/kg) and group 5 (aripiprazole 3 mg/kg) as compared with group1 (p >0.05) (Table 2 and Figure 3).

No. of VCM decreased significantly in group 3 (risperidone 1 mg/kg) and group 4 (olanzapine 2.5 mg/kg) as compared with group 2 (haloperidol 2 mg/kg) (p <0.05), but there were no significant differences between group 5 (aripiprazole 3 mg/kg) as compared with group 2 (p >0.05) (Table 2 and Figure 3).

DISCUSSION

Dopamine Level

According to the Data of the present results demonstrated that administration of haloperidol decreases the brain dopamine level in the brain of the mice. This finding agrees with those reported by.[12] The molecular mechanisms by which haloperidol decreased DA are that it acts by chronic blockade of dopamine D_2 receptor in nigrostriatal neurons of the brain lead to increase in DA turnover in basal ganglia and classical neuroleptics such as haloperidol remains bound to dopamine D_2 receptors and increased uptake of DA, particularly after withdrawal of antipsychotics resulting in TD.[12]

Also the administration of risperidone decreases the brain DA level in the brain of the mice. This finding disagrees with those reported by.[6] But risperidone showed significant increase in DA level in the mice as compared with haloperidol. Also there was convincing evidence about interaction between serotonergic and dopaminergic neurons in the basal ganglia region. Specifically, serotonin inhibits dopamine release through an interaction with receptors in the axon terminals of the dopaminergic neurons.[7] Hence, we can consider the hypothesis that atypical neuroleptic drugs (serotonergic/dopaminergic antagonists) such as risperidone could cause lesser extrapyramidal effects by blocking serotonergic receptors in the axon terminals of the dopaminergic neurons, enhancing dopamine release in the nigrostriatal system

and regressing the effects of D_2 blockade and developing haloperidol-induced oral dyskinesia.

The olanzapine and aripiprazole decrease the brain DA level in the mice. Such significant decreases in the DA synthesis was spotted following treatment with olanzapine in comparison with the control group, specifically in the medial prefrontal cortex (PFC) and ventral tegmental area (VTA) brain regions of the DA neurotransmitters system. But olanzapine showed significant increase in DA level in the mice as compared with haloperidol.[8] The acute administration of olanzapine significantly reversed haloperidol-induced suppression of striatal nitric oxide synthase (NOS) activity, a well-recognized effect of nitric oxide which is synthesized by NOS is to promote striatal DA release by promoting DA efflux or by inhibiting DA reuptake.[9]

On the other hand the administration of aripiprazole decreases the brain DA level in the mice. Also, these findings agree with those reported by.[11] DA synthesis mRNA expression in VTA was decreased after aripiprazole administration. Tyrosine hydroxylase, being a rate-limiting enzyme for DA synthesis indicates reduction of DA synthesis in this brain region. The particular effects of aripiprazole on reducing DA production may provide a mechanism about its long-term efficacy. D_2 auto receptors may mediate reduction in DA synthesi.[10] Aripiprazole's potent agonist activities at DA auto receptors have been found in various in-vivo studies.[11] D_2 auto receptor synthesis in the VTA may be increased in answer to the decrease of DA synthesis and release caused by aripiprazole treatment as a compensatory mechanism,. Along with this, an increase in D_2 receptor mRNA expression was observed.[11]

Model of Tardive Dyskinesia in Mice

During experiment , the haloperidol increases VCM in the mice.[12,13] Chronic administration of typical neuroleptics haloperidol led to significant increase in VCMs which is associated with significant decrease in serotonin, DA and norepinephrine levels where as atypical antipsychotics showed less prevalence of extrapyramidal side effects.[14] *Yasmin et al* (1996) observed that it appears that VCMs may also develop rapidly, particularly following intraperitoneal or subcutaneous (SC) injection. and acute VCMs may develop with treatment that rapidly blocks D_2 receptors, such as SC and IP injections.[13] *Bishnoi M et al (2007)* has mentioned that chronic administration of neuroleptics are associated with proliferation of D_2 receptors in caudate putamen and NAc. Also chronic blockade of dopamine D_2 inhibitory receptor, located in the glutamatergic terminals in the striatum, leads to continuously enhanced release of glutamate which damages the striatal output neurons resulting in increased orofacial movements and oxidative damage.

It is possible typical and atypical antipsychotic differently affects neuronal survival and death and that these effects considerably contribute to the differences in the development of TD as reported by Bishnoi et al.[16]

The haloperidol is metabolized by an oxidase which generates more toxic metabolites inducing oxidative stress. Chronic blockade of dopamine D_2 receptors by neuroleptics in nigrostriatal neurons leads to an increase in DA turnover in basal ganglia and further to overproducing free radicals**.** The DA super sensitivity hypothesis states about effect of antipsychotic drug treatment resulting D2 receptors hypersensitization through increased density in every dopaminergic pathway.[12] This alters DA levels in brain regions effecting in motor dysfunction. Neuroleptics similar to haloperidol remain bound to dopamine D_2 receptors and aggregate in brain tissue. It enhances the density of dopamine D_2 receptors and further, uptake of DA. It happens particularly post withdrawal of antipsychotics resulting in TD.[11] It has been on served that the outcomes further show that haloperidol may produce EPS by D_2 receptor blockade and in addition to that, prolonged free DA reuptake in caudate putamen.

This study has revealed that administration of risperidone inhibits the VCMs which are induced by haloperidol in the mice. This effect may be due to blocking effect of risperidone on 5-HT_2 in the CNS.[7] [13]reported that risperidone treatment resulted in insignificant increase in VCM as compared to control.[7] an interaction exists between serotonergic and dopaminergic neurons in the basal ganglia region and recorded by *Carvalho R (2003)* .It has been well noted that serotonin system inhibits dopaminergic function. Therefore, risperidone could cause less extrapyramidal effects by blocking 5-HT_2 receptor.[13] It has been observed that short-term (<45 days) treatment studies in rats have been found with increased oxidative stress and oxidative neural cell injury with typical antipsychotics e.g. haloperidol. It doesn't have such effects with atypical antipsychotics like risperidone. This drug displays different alterations in different brain areas.

Also showed that administration of olanzapine inhibits the VCMs which are induced by haloperidol in the mice. This effect may be due to blocking effect of olanzapine on 5-HT_2 in the CNS**.** This finding agrees with those reported by[17] Mccullumsmith et al (2003).[17] Mccullumsmith et al reported that haloperidol-induced VCM measured after olanzapine were reduced compared to prior to olanzapine treatment. It reveals that additional treatment with olanzapine reflected in spontaneous recovery from chronic VCM.[9] Nel A and Harvey B. H (2002) have observed that the limbic-selective actions, low D_2 receptor occupancy, antagonism of 5-HT2 receptors, or antimuscarinic results of olanzapine have been engaged to explain its enhanced motor side-effect profile. It is[9] suggested that while treating TD, its efficacy may involve another lesser observed site of action which is basically, relevant to the underlying neurobiology of TD.[13] It has been reported that short-term (<45 days) treatment in rats have enhanced oxidative stress and oxidative (i.e., oxygen free radical-mediated) neural cell injury with typical antipsychotics such as haloperidol, but not with the a typical such as risperidone. This drug displays different alterations in different brain areas.

While the administration of aripiprazole shows no significant difference in VCM in the mice. about our knowldgement, there is no previous data about the effects of aripiprazole on VCM.[18]it has been observed that aripiprazole's

partial DA agonistic activity may neutralize D_2 receptor upregulation which tends to the TD remission.[18]

CONCLUSION

Risperidone and olanzapine decreased VCM of TD in male mice. Aripiprazole has no effect on model of TD in mice. Risperidone and olanzapine decreased DA level in brain of mice (but not aripiprazole). see Scheme 1.

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