Methylphenidate-induced hepatotoxicity in rats and its reduction by buspirone

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Abstract: Methylphenidate is commonly use for the treatment of attention deficit hyperactivity disorder (ADHD), but its long term use was found to produce hepatic necrosis in mice. Purpose of this study was to investigate that co-administration of buspirone (drug which attenuates methylphenidate induced sensitization) may attenuate methylphenidate-induced hepatotoxic effects and to determine the effect of challenge dose of haloperidol (D2 antagonist that blocks the effects of methylphenidate in case of intoxication) on SGPT and SGOT levels in methylphenidate treated rats. Estimation of SGPT and SGOT were performed using kit method. Prolong oral administration of methylphenidate at a dose of 2.0 mg/kg/day, buspirone at a dose of 10 mg/kg/day, their co-administration and challenge dose of haloperidol (1 mg/kg i.p.) in rats increased SGPT concentration and decreased SGOT concentration, effect is more pronounced in methylphenidate treated rats and potentiate with administration of haloperidol challenge dose. In conclusion our analysis showed that methylphenidate and challenge dose of haloperidol is associated with elevation of SGPT in rats, which is attenuate in co-administration of methylphenidate buspirone treated rats. To quantify the risk of methylphenidate-induced hepatic injury and role of buspirone to reduce the injury further pharmacoepidemiological investigations are required.

Keywords: Methylphenidate, SGPT, SGOT, buspirone, haloperidol.

INTRODUCTION

Drugs are important cause of liver injury. Worldwide database of adverse drug reactions in children contained 9036 hepatic injury cases, among the top 40 causes were 2 agents used for attention deficit disorder; atomoxetine [14th, 64 cases] and methylphenidate [11th, 96 cases] (Ferrajolo *et al*., 2010). There are increasing incidences of drug-induced liver disease thus, monitoring of liver enzymes is considered necessary, especially in case of those drugs that are reported to overt injury (Lewis, 2000).

Methylphenidate is commonly use for the treatment of Attention Deficit Hyperactivity Disorder (Pliszka, 2007; Greenhill *et al*., 2002) and has been found to be a safe and effective drug after rigorous studies (Hoover-Stevens and Kovacevic-Ristanovic, 2000). However in some cases it can cause intolerable side effects and was found to produce hepatic necrosis in male ICR mice (Roberts *et al*., 1994). *It has been mention in review of hepatotoxicity of antidepressants that methylphenidate has been implicated in hepatocellular injury* (Larrey and Ripault, 2013; Zimmerman, 1999). Sponsor received reports of enzyme elevations after it was marketed (Lewis *et al*., 2007). Methylphenidate is extensively metabolized in the liver (Leonard *et al*., 2004) but the mechanism of liver injury by methylphenidate is not known, however direct toxicity to hepatic tissues may occur after intravenous administration of drug (Molleston *et al*., 2011).

Previously it has been shown that buspirone which is antagonist at certain postsynaptic $5HT_{1A}$ receptor and partial agonist at $5\text{-}HT_{1A}$ autoreceptors (ZifaandFillion, 1992) it can also blocks the presynaptic rather than the postsynaptic (McMillen and McDonald, 1983**)** D_2 dopamine receptors, thus can attenuate methylphenidate-induce adverse effects related to behavior and growth and haloperidol, a D_2 receptor antagonist could block the effects of methylphenidate (Levy and Hobbes, 1996) so beneficial in cases of intoxication.

The study was designed to analyze hepatotoxicity following repeated administration of methylphenidate. It was hypothesized that buspirone could attenuate to methylphenidate-induced hepatotoxicity. Effect of challenge dose of haloperidol was also monitored on treated groups of rats to investigate its hepatotoxic effects and to determine whether its exposure increases or decreases the risk of hepatic myopathy.

MATERIALS AND METHOD

Animals

Locally bred forty eight male rats weighting 180-200gms were used for experiment. Seven days before the start of experiment the rats were caged in pair in a quiet room,

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under a 12 hr cycle of light/dark and room temperature was controlled at 24+2°C with cubes of standard rodent diet with free tap water access so that rats could become familiar to the environment. They were acclimatized to various handling procedures in order to nullify stress effects. All the performed experiments were according to the approved protocols and were in accordance to the rules and regulation given by local animal care committee.

Drugs

Methylphenidate HCl purchased from Sigma, St. Louis, USA and prepared in saline NaCl 0.9% and buspirone (Research Biochemicals Incorporated) prepared in distilled water. Administered the drugs by per oral route twice a day individually and also co-administered to the 3rd group (methylphenidate at the dose of 2mg/kg/day (Wargin *et al*., 1983; Aoyama *et al*., 1990), buspirone at the dose of 10 mg/kg/day (Vis *et al*., 2001; Naito *et al*., 2003) and co-administered methylphenidate at the dose of 2 mg/kg/day and buspirone at the dose of 10 mg/kg/day according to the weight of the rats) of treated animals where as control rats were treated with (0.9%) saline per oral twice a day. Haloperidol available in 5mg/ml ampoules (Searle) was used in the present study.

Dose and drug administration

The protocol of experiment was designed to administer methylphenidate and saline to 1st group of treated rats, buspirone and saline to 2nd group of treated rats, methylphenidate and buspirone to 3rd group of treated rats and saline to control rats orally two times daily (8.00 AM and 8.00 PM) for six weeks. Among the three groups of treated rats 1st group was given methylphenidate in the dose of 2 mg/kg/day (0.18-0.2 ml of methylphenidate suspension 2 times daily), 2nd group was given buspirone at the dose of 10 mg/kg/day (0.9-1 ml of buspirone suspension 2 times daily) and 3rd group was given methylphenidate at the dose of 2 mg/kg/day (0.18-0.2 ml of methylphenidate suspension 2 times daily), and buspirone at the dose of 10 mg/kg/day (0.9-1 ml of buspirone suspension 2 times daily) according to the weight of the rats. The tablets were powdered and 10 mg methylphenidate tablet was added in 10 ml 0.9% NaCl and 5mg buspirone tablet was added in 5 ml distilled water to make the suspension i.e. 1mg/ml, then calculated amount of suspension was administered to each rat with the feeding tubes. Control rats were given Saline 2.0 ml/kg/day i.e. 0.18-0.2ml 2 times daily according to the weight of the rats. Before decapitation challenge dose of haloperidol 1mg/kg i.p. was given to all treated and control rats to monitor the effect of drug on behavioral parameters 30 minutes post injection and on blood tests.

Decapitation and blood sample collection

To avoid the order effect the experiment was performed in such a way that control and drug treated rats were killed by using guillotine alternately in a balanced design. Immediately after decapitation blood samples were collected in different heparinized tubes for the estimation of SGPT and SGOT. Blood tests were performed using kit method.

STATISTICAL ANALYSIS

The results are represented as mean ±S.D. Statistical analysis was performed by using SPSS software (version 16.0). Statistical analysis of effect of haloperidol on SGPT and SGOT in treated and control animals were performed using three-way ANOVA analysis of variance. Newman-Keul test was performed for Post hoc comparison of groups. The level of significance was considered significant if the values lie between $p<0.05$ and $p<0.01$.

RESULTS

Effect of haloperidol on SGPT in methylphenidate treated, buspirone treated and co -administration of methylphenidate-buspirone treated rats.

Fig. 1 shows the effect of haloperidol on SGPT in repeated methylphenidate, buspirone and co-administration of methylphenidate-buspirone treated animals. Data analyzed by three-way ANOVA showed significant effects of methylphenidate ($F=98.18$, $df=1,24$, $p<0.01$), buspirone ($F=52.95$, $df=1,24$, $p<0.01$) haloperidol ($F=84.45$, $df=1,24$, $p<0.01$) and significant interactions between haloperidol*buspirone ($F=16.33$, $df=1,24$, $p<0.01$) and buspirone*methylphenidate ($F=149.78$, $df=1,24$, $p<0.01$). Interactions between haloperidol*methylphenidate ($F=1.133$, $df=1, 24$, $p>0.05$) and haloperidol*buspirone*methylphenidate ($F=2.237$, $df=1, 24$, $p>0.05$) are not significant.

Post hoc by Newman-Keuls test demonstrated that haloperidol ($p<0.01$) increased SGPT levels in saline treated, methylphenidate treated and co-administration of methylphenidate-buspirone treated rats than similarly treated saline injected rats. SGPT levels increased significantly in ($p<0.01$) saline and haloperidol injected groups of methylphenidate, saline injected buspirone treated rats and in ($p<0.05$) saline injected co-administration of methylphenidate-buspirone treated rats from similarly injected saline treated rats. SGPT levels decreased in both groups of co-administration treated rats from ($p<0.01$) similarly injected methylphenidate treated rats and ($p<0.05$) in saline injected group from similarly injected buspirone treated rats.

Effect of haloperidol on SGOT in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats.

Fig. 2 shows the effect of haloperidol on SGOT in repeated methylphenidate, buspirone and co-administration of methylphenidate-buspirone treated animals. Data analyzed by three-way ANOVA showed significant effects of methylphenidate ($F=11.63$, $df=1,24$, $p<0.05$), buspirone ($F=54.01$, $df=1,24$, $p<0.01$)

haloperidol (F=57, df=1,24, p<0.01) and significant interactions between haloperidol* methylphenidate (F=17.37, df=1,24, p<0.01), buspirone*methylphenidate (F=348.2, df=1,24, p<0.01) and haloperidol* buspirone* methylphenidate (F=115.04, df=1,24, p<0.01) but non-significant interaction between haloperidol*buspirone (F=1.79, df=1,24, p>0.05).

Effect of haloperidol in methylphenidate, buspirone and their co-administration treated rats on SGPT

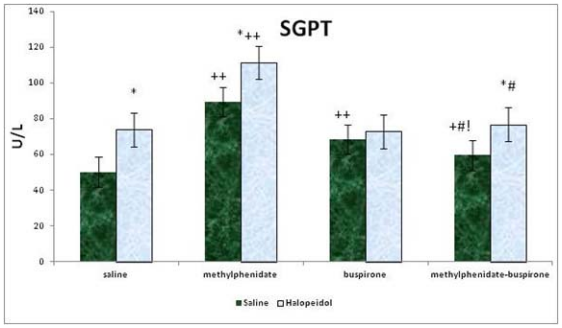

Fig. 1: Effect of Haloperidol 1mg/kg I.P. on levels of SGPT in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Newman–Keuls test:*p<0.01 from similarly treated saline injected rats +p<0.05,++p<0.01 from similarly injected saline treated rats. #p<0.01 from similarly injected methylphenidate treated rats. !p<0.05 from similarly injected methylphenidate treated rats following three-way ANOVA.

Post hoc analysis by Newman–Keuls test demonstrated that haloperidol (p<0.01) decreased SGOT levels in saline treated and co-administration of methylphenidate-buspirone treated rats and increased (p<0.01) significantly in methylphenidate treated rats than similarly treated saline injected rats. SGOT levels decreased (p<0.01) significantly in saline and haloperidol injected groups of methylphenidate and in saline injected buspirone treated rats and increased (P<0.05) significantly in haloperidol injected co-administration of methylphenidate-buspirone treated rats from similarly injected saline treated rats. SGOT levels increased in saline and haloperidol injected groups of co-administration treated rats from (p<0.01) similarly injected methylphenidate treated rats and (p<0.05) from similarly injected buspirone treated rats.

DISCUSSION

Methylphenidate is a medication of choice for persons, in specifically young children, who are suffering from attention-deficit/hyperactivity disorder (Sharma and Couture, 2014; Habibzadeh *et al*., 2011; Dopheide and Pliszka, 2009). Prolong oral administration of methylphenidate, buspirone, their co-administration and challenge dose of haloperidol in rats increased SGPT concentration and decreased SGOT concentration, effect is more pronounced in methylphenidate treated rats and potentiate with administration of haloperidol challenge dose. It has been reported that methylphenidate administration produce hepatic necrosis in mice (Roberts *et al*., 1995) which can be attenuated by buspirone co-administration.

Effect of haloperidol in methylphenidate, buspirone and their co-administration treated rats on SGOT.

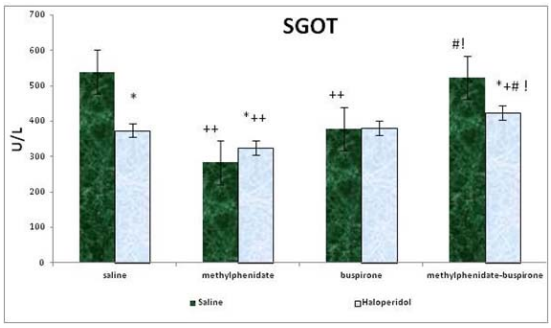

Fig. 2: Effect of Haloperidol 1m/kg I.P. on levels of SGOT in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Newman–Keuls test:*p<0.01 from similarly treated saline injected rats. +p<0.05,++p<0.01 from similarly injected saline treated rats. #p<0.01 from similarly injected methylphenidate treated rats. !p<0.05 from similarly injected methylphenidate treated rats following three-way ANOVA.

Methylphenidate metabolized extensively in the liver (Leonard *et al.*, 2004) and has many drug-drug interactions. In both therapeutic and abuse scenarios it inhibits the CYP450 system. This effect was dependent on both the duration of methylphenidate administration and the isoform of CYP450 (Le Nedelec and Rosengren, 2002). The injury of liver due to methylphenidate oral administration resolves rapidity and is usually self-limited. There have been no reports of vanishing bile duct syndrome or chronic liver injury associated with either intravenous or oral methylphenidate administration. However, it has been demonstrated that there is recurrence of injury with re-exposure (Stecy *et al.*, 1985; Mehta *et al*., 1984).

Previously it has been shown that buspirone a partial agonist at 5-HT_{1A} autoreceptors and an antagonist at certain postsynaptic $5HT_{1A}$ receptor site (Zifa and Fillion, 1992), also preferentially blocks the presynaptic rather than the postsynaptic (McMillen and McDonald, 1983) D_2 dopamine receptors, can attenuate methylphenidate-induce adverse effects related to behavior and growth. Liver or kidney damage has not been reported as a direct result of taking buspirone and adverse events reports on organ toxicity are rare for buspirone (Dykens *et al.*, 2008). Present study shows that buspirone co-administration can reduce adverse effects of methylphenidate on liver.

Effect of haloperidol in methylphenidate, buspirone and their co-administration treated rats on sgpt.

Table 1: Effect of Haloperidol 1m/kg I.P. on levels of SGPT in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Newman–Keuls test:*p<0.01 from similarly treated saline injected rats .+p<0.05,++p<0.01 from similarly injected saline treated rats. #p<0.01 from similarly injected methylphenidate treated rats. !p<0.05 from similarly injected methylphenidate treated rats following three-way ANOVA.

	SGPT														
Parameters	Saline				Haloperidol				Halo. Effect	Bus. Effect	MPH effect	Halo *bus	Halo *MPH	Bus. *MPH	Halo *busp *MPH
	Saline		Buspirone		Saline		Buspirone		df=1,24	df=1,24	df=1,24	df=1,24	df=1,24	df=1,24	df=1,24
	Saline	MPH	Saline	MPH	Saline	MPH	Saline	MPH	F=84.4	F=52.9	F=98.1	F=16.33	F=1.133	F=149.7	F=2.237
Mean	50.25	++ 89.5	++ 68.5	+#! 59.75	*74	*++ 111.7	73	*# 77	P<0.01	P<0.01	P<0.01	P<0.01	P>0.05	P<0.01	P>0.05
S.d	3.4034	4.654	4.795	1.70	3.559	3.40	3.741	2.58							

Effect of haloperidol in methylphenidate, buspirone and their co-administration treated rats on sgot

Table 2: Effect of Haloperidol 1m/kg I.P. on levels of SGOT in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Newman–Keuls test:*p<0.01 from similarly treated saline injected rats. +p<0.05,++p<0.01 from similarly injected saline treated rats. #p<0.01 from similarly injected methylphenidate treated rats. !p<0.05 from similarly injected methylphenidate treated rats following three-way ANOVA.

	SGOT														
Parameters	Saline				Haloperidol				Halo. Effect	Bus. Effect	MPH effect	Halo *bus	Halo *MPH	Bus. *MPH	Halo *busp *MPH
	Saline		Buspirone		Saline		Buspirone		df=1,24	df=1,24	df=1,24	df=1,24	df=1,24	df=1,24	df=1,24
	Saline	MPH	Saline	MPH	Saline	MPH	Saline	MPH	F=57	F=54.0	F=11.6	F=1.788	F=17.37	F=348.1	F=115.038
Mean	539.5	++ 283.2	++ 377.5	#! 523	* 374.5	*++ 324.7	379.75	*+#!4 23.5	P<0.01	P<0.01	P<0.05	P>0.05	P<0.01	P<0.01	P<0.01
S.d	4.795	12.44	17.521	11.97	5.321	19.51	4.7871	6.78							

Haloperidol, a D_2 receptor antagonist could block the effects of methylphenidate (Levy and Hobbes, 1996) so beneficial in cases of methylphenidate intoxication. It has been reported that increase of liver enzymes is slightly more frequent with haloperidol and is responsible for hepatic disorders (Manceaux *et al*., 2011), which is consistent with our findings.

In conclusion our analysis showed that methylphenidate and challenge dose of haloperidol is associated with elevation of SGPT in rats, which is attenuate in co-administration of methylphenidate buspirone treated rats. Further pharmacoepidemiological investigations are needed to quantify the risk of drug-induced hepatic injury and role of buspirone to reduce the injury.

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