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Research Article

Role of σ1R antagonist on Serotonin Syndrome like Effect Treated by Different Combination of Antidepressant Drug on Mice.

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ABSTRACT

Serotonin is one of three monoamine neurotransmitters, majorly associated with clinical depression. The serotonin syndrome is the clinical manifestation of serotonin toxicity in patients taking one or more serotonergic agents. Swiss Albino mice of either sex weighing between (20-30gm) for the Purpose of Control and Supervision of Experimental Animal (CPCSEA).Artificial cerebrospinal fluid (aCSF) was used as control (composition: 125mMNaC1, 10mM glucose, 1.25mM NaH_2PO_4, 2.5mM $CaCl_2$, 1.5mM $MgSO_4$, 26 mM$NaHCO_3$, adjust pH to 7.4 with 0.1 M NaOH), and sterile saline solution.

Fluoxetine (selective serotonin reuptake inhibitor)
Tranylcypromine (mono amino oxidase inhibitor)
Quetiapinefumearte (5-HT_{2A} and D_2 and receptor antagonist)
BD-1063 (Selective σ 1 R antagonist)

We found that pre-administration of σ1 antagonist BD-1063 significantly attenuated serotonin syndrome induced by combination of tranylcypromine and fluoxetine. σ1R in serotonin syndrome like effect of tranylcypromine and fluoxetine in mice as confirmed by antagonism of σ1R. Hence it must be further studied for underlying mechanism to be projected as a therapeutic target for the treatment of serotonin syndrome caused due to antidepressant therapy.

Keyword:- Serotonin, Neurotransmitter, Fluoxetine, Tranylcypromine, Quetiapinenfumearte, Antidepressant.

INTRODUCTION

Serotonin is one of three monoamine neurotransmitters, majorly associatedwith clinical depression. Physiologically serotonin regulates sleep, body temperature, appetite, mood, blood pressure and theperception of pain.Serotonin Syndromeis characterized by featuresof neuromuscular hyperactivity, autonomicinstability and alteration of mental status. Itisexcess stimulation of the 5-HT1Areceptors.

The syndromeis manifested by-

- Cognitive effect-headache, agitation, hypomania, mental confusion, hallucination, and coma.
- Autonomic effect- shivering, sweating, hyperthermia, tachycardia,nausea,diarrhea.
- Somatic effect- myoclonus (muscle twitching), hyperreflexia (manifested by clonus), tremor (Mills, 1997).

Sigma receptors(R)discovered in 1976in the central nervous system wasoriginally referred to as sigma/opioid receptors (Martin et al., 1976). At present, it is generally accepted that sigmareceptors are not subclass of the opioid, dopaminergic, and NMDA(N-methyl-D-aspartate)receptors (Quirion et al., 1992).

Signal transduction system involved inσRshave also been linkedto the modulation or production ofintracellular second messengers, such ascGMP(Mamiya et al. 2000), inositol phosphates (Novakova et al., 1998),protein kinases (Derbez et al., 2002),and calcium (Brent et al., 1997).
σRagonist modulation of classical neurotransmitter system;-Glutamatergic modulation accumulating evidence indicates that NMDA receptor function, and glutamatergicsignaling in general, are compromised in depression, and that modulation of glutamatergic neurotransmission contributes to the therapeuticeffect of antidepressant drugs (Skolnick, 2002; Paul and Skolnick, 2003).It is therefore significant that glutamatergicresponses that are mediated through NMDA receptors can be modulated by σR ligands, where activation of σRsresult in enhanced NMDA neurotransmission (Iyengar et al., 1990; Monnet et al., 1990; Bergeron et al., 1993). This enhanced NMDA neurotransmission may facilitate compensatory glutamatergic signaling in system compromised by depressive pathology.
Serotonergic modulation - The therapeutic effect of chronic administration of antidepressant drugs are associated with an enhancement of serotonin neurotransmission (Blier and Bouchard, 1994). Acute administration of antidepressant drugs, such as SSRIs, causes a reduction in the firing of serotonergic neurons, which recover with long term administration (Chaput et al., 1986;Beiqueet al., 2000). This recoveryin firing of serotonin neurons is thought to develop after desensitization of somatodendritic 5-HT 1Aautoreceptors, activated in response to serotonin released from axon collaterals. This phenomenon has been proposed as a mechanism that explains the three to four week delay in clinical efficacy of antidepressant drugs (Chaput et al., 1986; Blier and de Montigny, 1994).
Fluoxetine hydrochloride is the first agent of the class of antidepressants known as selective serotonin – reuptake inhibitors (SSRIs). Despite distinct structural differences between compounds in this class, SSRIs possess similar pharmacological activity. SSRIsare potentinhibitors of neuronal serotonin reuptake. They have little to noeffect on norepinephrine or dopamine reuptake and do not antagonizeα- or β-adrenergic, dopamine D2 or histamine H1receptors.
Fluoxetine in serotonin syndrome:-Co-administration of SSRIs withother serotonergic drugs (e.g. tramadol, triptans, Tranylcypromine) or with dopaminergic drugs (e.g. selegiline) may also increase the risk of serotonin syndrome (Stockley, 2002).
Tranylcypromine hydrochloride:-Tranylcypromine irreversibly and nonselectivelyinhibits monoamine oxidase (MAO).Within neurons, MAO appears to regulate the levels of monoamines released upon synaptic firing. Since depression isassociated with low levels of monoamines, the inhibition of MAO serves to ease depressive symptoms, as this result in an increase in the concentration of theseamines within the CNS.
Tranylcypromine inserotonin syndrome:- Tranylcypromineand SSRIs like Fluoxetine, Paroxetine, etc.interact. In certain individuals, the interaction between approved doses may produce serotonin syndrome without life-threatening effects.Combining Tranylcypromineand SSRIsmay be pleasurable butdisruptive to normal activities, sleep, and medication scheduling.
Quetiapine:-Quetiapinemarketed by AstraZeneca as Seroquel and by Orion Pharma as Ketipinor, is an atypical antipsychotic approved for thetreatment of schizophrenia, acute episodes of bipolar disorder (manic, mixed or depressive), and as an augmenter for the maintenance treatment of depression and bipolar disorder. Quetiapineenhances central serotonergic neurotransmissionthrough its high affinity for serotonergic receptors (i.e., 5-HT_2A receptor antagonism). In particular, 5-HT 1Areceptor modulation (partial agon-ism)may also be salient to quetiapine'san atypical antipsychotic withhigh affinity for H1 and moderate affinity for sigmaα1,

$5HT_2$,α2and D2receptor (Alexandro et al., 2004). Recent double-blind, placebo-controlled, pharmacotherapyefficacystudies in research samples bipolar disorder patients with minimal co-morbidity have provided encouraging data for acutemonotherapy(Tohen et al., 1999).

MATERIALS AND METHODS

Experimental animals

Swiss Albino mice of either sex weighing between (20-30gm) were housed under controlled environmental condition at 24±1˚C under 12:12 h light/dark cycle (light on 07:00-19:00 h) with free access to food and water were used. All experimental procedures were carried out under strict compliance with Institutional Animal Ethical Committee according to guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animal (CPCSEA) , Ministry of Environment and Forests; Government of India ; New Delhi. Every possible effort was made to reduce the suffering of animals in all the experimental design.

Drugs and administration

Artificial cerebrospinal fluid (aCSF) was used as control (composition: 125mMNaC1, 10mM glucose, 1.25mM NaH_2PO_4, 2.5mM $CaCl_2$, 1.5mM $MgSO_4$, 26 $mMNaHCO_3$, adjust pH to 7.4 with 0.1 M NaOH), and sterile saline saline solution.

- Fluoxetine (selective serotonin reuptake inhibitor)
- Tranylcypromine (mono amino oxidase inhibitor)
- Quetiapinefumearte (5-HT_{2A} and D_2 and receptor antagonist)
- BD-1063 (Selective σ 1 R antagonist)

Fluoxetine, Tranylcypromine were obtained from VAMA pharmaceuticals Nagpur, Quetiapinefumearte, were obtained from Cipla pharmaceuticals Mumbai, BD-1063 were obtained from Tocris Bioscience, London, UK. All drugs were dissolved in aCSF just before the experiments and infused by intracerebroventricularinjections in volume of 1 µ1/side bilaterally while, for ip administration drugs were dissolved in sterile saline.

Intracerebroventricular cannula implantation

The mice were anaesthetized with ketamine (75 mg/kg, ip)andxylazine)5 mg/kg ip) and skull was shaved with hair remover and iodine solution was applied as an antiseptic to the exposed skin. Animal was fixed in stereotaxic (David kopf, USA) by placing the ear bars into the ear canals and tightened into frame. An anterior/posterior incision was made on the scalp with a sterile scalpel extending from the lambda to just in-between the eyes of the animal. The guide cannula (C_{315} GH/4/SPC, Plastic one Virginia, USA) was positioned to the correct icv coordinates AP= -0.7 mm ; = ±0.2 mm; DV = -2mm relative to bregma) (Paxinos and Franklin, 1997) by adding or subtracting from bregma. Pencil mark was made with a sterile pencil at this location on the skull. Carefully, drill was holed at the pencil mark until it drills through the width of the skull. Next, using a hand drill, four holes were made for skull screws: two anterior and two posterior to the cannula hole. Screws were tightly anchored on to the skull. The guide cannula was cleaned with ethanol and saline, mounted, and lowered it slowly to the proper ventral coordinate. After the insertion guide cannula was fixed by the dental cement. A 28-gauge stainless steel dummy cannula (C_{315} 1H/4/SPC, Plastic one Virginia, USA) was inserted to occlude the guide cannula when not in use.

After surgery, the animals were placed individually in cages and allowed to recover for not less than 7 days during which they were handled to condition for future experimental procedure. They were treated with oxytetracyclin(25 mg/kg, im) and Neosporin ointment to avoid infection.

The intracerebroventricular injection were given by 33-guage internal cannula (internal diameter 0.180 mm and outer diameter 0..20mm), which was attached to a Hamilton microliter syringe (10 µ1) via polyethylene tubing (PE-10) (internal diameter, 0.28 mm; outer diameter 0.61 mm),that extended 0.5 mm beyond the guide cannula.The internal cannula was held in position for another 1 min before being slowly withdrawn to prevent backflow and promote diffusion of drug (Geiger et al., 2008).

EVALUATION PARAMETER

Forced swim test

The Procedure was quite similar to that described by Porsolt et al., (1977) except that mice were subjected to a "pretest session" to maintain consistency in the basal immobility time between different groups. Briefly, mice were placed individually in plexiglass cylinders (21 cm height- 12 cm internal diameter) containing fresh water upto a height of 9 cm at ± 1°C and forced to swim for 15 min. Twenty four hours later, the animal were randomly divided into different group (6-8 animal/group) and treated with either a drug (test group) or vehicle (control group). Each mouse was again forced to swim in a similar environmental for the period of 5 min in a "test session" and immobility time was measured by the trained observer blind to the treatment. A mouse was judged to be immobile when it remained floating motionless in the water, making only necessary movements to keep its head above water. Each mouse was used only once in "test session". Reduction in the duration of immobility was considered asantidepressant like effect of the drug. Forced swimming tests were conducted 30 min after the administration of drugs.

Measurement of rectal temperature

The rectal temperature of the mice was measured every 30 min after the drug injections. The rate were gently restrained while their body temperature was measured. Athermocouple probe connected to a digital thermometer (Appolo Pharmacy) was inserted up to 4 cm into the rectum and steady temperature read out was obtained within 10 s of the probe insertion.

Open field test

The open field test was used to measure locomotoractivity (Walsh and Cummins, 1997). The open field apparatus consisted of a wooden chamber (55×40×50 cm^3) with a dark gray floor subdivided into 12 fields. The box was laced in a quiteroom , with the same illumination utilized in elevated plus maze room. Animals were observed individually for 3 min (Barros et al., 2001).

Rotarod apparatus

The rotarod is a device which tests motor coordination. The apparatus consisted of a horizontal metal rod with 3 cm diameter attached to a motor with the speed adjusted to 20 revolutions per minute. The rod is 50 cm in length and is divided into 5 sections by plastic discs, thereby allowing the simultaneous testing of 5 mice. The rod is in a height of about 25 cm above the table top in order to discourage the animals from jumping off the roller if it jumps then inactivation of timer occur.

PROCEDURE

Motor coordination was assessed using a standard rotarod treadmill for mice set at a fixed speed of 20 r.p.m. All experimental were performed between 8:00 and 11:00 am. Prior to drug administration. All animals were pre-screened toensured the normal motor coordination (walking on rotarod for 180 s). therefore, each animal served as its own control. Animals that were unable to walk 180 s. during screening were excluded from the experiment. Motor impairment was

evaluated after 15 min. each mouse were allowed three attempts at evaluation time and the highest time (seconds) an animal walked on the rotarod were recorded and used for data analysis.

Social Interaction Test (SIT)

The social interaction test arena is a plastic cage 12 in long ×17 cm × 12 cm high walls . A pair of mice belonging to different home cages will be placed in the arena, and the social interaction behavior between mice will be observedfor 5 min.In all tests mice the same age and gender are use as a standard opponent. Standard opponent is used up to one time per day. The experiment will be started with the animal being placed in the test cage, after hich a standard opponment will be carefully put in the opposite corner of the test cage. observations start when the experimental animal sniff the partner for the first time and last 5 min if no aggressive attacks take place. The experiment will be terminated 2 min after the first attack of the experimental animal. During the period, the cumulative time spent in social interaction behaviors including sniffing, following and grooming of partner; genital investigation, tail licking, facing, neck licking, trunk sniffing, aggressive attacks were scored (Scearce - Levie et al., 2007).

EXPERIMENTAL DESIGN

Exeriment-1:Dose dependent effect of fluoxetine (ip) in mice

Separate groups of mice were either treated with saline (1 ml/kg, ip) and fluoxetine (10,20 mg/kg, ip) administration . 30 minutes after ip administration, animal were subjected to forced swim test , open field test, rotarod apparatus, social interaction test and measurement of rectal temperature.

Experiment-2: Dose dependent effect of quetiapine (ip) in mice

Separate group of mice were either treated with saline (1 ml/ kg, ip) and quetiapine (50,100 mg/kg, ip)administration. 30 minutes after ip administration, animal were subjected to forced swim test, open field test, rotarod apparatus, social interaction test and measurement of rectal temperature.

Experiment-3: Effect of tranylcypromine (ip) in mice

Separate groups of mice were either treated with saline (1 ml/kg, ip) and tranylcypromine (0.5 mg/kg, ip) administration. 30 minutes after ip administration, animalwere subjected too forced swim test, open field test, rotarod apparatus, social interaction test and measurement of rectal temperature.

Experiment-4: Induction of serotonin syndrome by tranylcypromine (ip)and fluoxetine (ip)

Separate groups of mice were either treated with the combination of tranylcypromine (0.5mg/kg,ip) and fluoxetine (10-20mg/kg,ip) or saline (1 ml/kg, ip). Animal were subjected to forced swim test , open field test, rotarod apparatus, social interaction test and measurement of rectal temperature.

Experiment-5:Induction of serotonin syndrome by tranylcypromine(ip) and quetiapine (ip)

Separate groups of mice were either treated with the combination of tranylcypromine (0.5 mg/kg, ip) and quetiapine (50-100 mg/kg, ip) or saline (1 ml/kg, ip). Animal were subjected to forced swim test, open field test, rotarod apparatus, social interaction test and measurement of rectal temperature.

Experiment-6: Dose dependent effect of σ 1 R antagonist BD-1063 (icv) on serotonin syndrome

Separate groups of mice were either treated with different doses of BD1063 (5-10 µg/mice, icv) or aCSF (2µ1/mice, icv) animal were subjected to forced swim test, open field test, rotarod apparatus, social interaction test and measurement of rectal temperature.

Experiment-7 : Effect of σ 1 R antagonist BD1063 (icv) on serotonin syndrome induced by tranylcypromine(ip) and fluoxetine (ip)

A separate groups of mice were treated with BD1063 (10µg/mice, icv) prior to the administration of tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) and animal were subjected to forced swim test, open field test, rotarod apparatus, social interaction test and measurement of rectal temperature.

Experiment-8: Effect of BD-1063 (icv) on serotonin syndrome induced tranylcypromine(ip) and quetiapine (ip)

A separate group of mice were treated with BD-1063(10 µg/mice, icv) prior to the administration of tranylcypromine (0.5 mg/kg, ip) and quetiapine (100 mg/kg, ip) and animals were subjected to forced swim test, open field test, rotarod apparatus, social interaction test and measurement of reactal temperature.

RESULT

Effect of tranylcypromine and fluoxetine on immobility time in mouse FST

Combination of subeffective doses of tranylcypromine (0.5 mg/kg, ip) fluoxetine (20 mg/kg,ip)significantly decreased the immobility time as compared with saline treated animals (One –way ANOVA-F (5, 29) =12.56, P<0.001]. Post hoc analysis by Newman-Keuls multiple comparison test reduction in the immobility time by fluoxetine (10 mg/kg, ip) alone or in combination with tranylcypromine failed to influence the immobility time in FST as compared to saline treated mice.

Effect of σ 1R antagonist , BD -1063 on the immobility time in mouse FST

BD-1063 (5-10 µg/mice, icv) failed to influence the immobility time as compared to vehicle treated group [F(2, 14)=2.291, P=0.15]. The mean immobility time in BD-1063 injected mice was not significantly different from the control animals.

σ 1R antagonist, BD-1063 attenuates the serotonin syndrome like effect tranylcypromine and fluoxetine in mice.

Tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) in animals pre-treated with BD-1063 (10 µg/mice, icv) failed to show significant serotonin syndrome-like effect as compared with vehicle treated group.Significant decrease in immobility time with tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) compared to aCSF treated mice. Pre-treatment of BD1063 significantly attenuated the serotonin syndrome-like effect of tranylcypromine and fluoxetine.

Effect of tranylcypromine and fluoxetine on rectal temperature in mouse

Combination of subeffective doses of tranylcypromine(0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) significantly increased the rectal temperature as compared with saline treated animals. Tranylcypromine pre-treatment significantly potentiated the increase in rectal temperature by fluoxetine while lower dose of fluoxetine (10 mg/kg, ip) alone or in combination with tranylcypromine failed to influence the rectal temperature as compared to saline treated mice.

Effect of tranylcypromineand fluoxetine on ambulation in mouse in open field test

Combination of subeffective dosesof tranylcypromine (0.5 mg/kg, i) and fluoxetine (20 mg/kg, ip) significantly increased the ambulation as compared with saline treated.Tranylcypromine

pretreatment significantly potentiated the increase in ambulation by fluoxetine while lower dose of fluoxetine (10 mg/kg, ip) alone or in combination with tranylcypromine failed to influence the ambulation in open field test as compared to saline treated mice.

Effect of tranylcypromine and fluoxetine on grooming in open field test

Combination of subeffectivedoses of tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg, i) significantly increased the grooming as compared with saline treated animals.Tranylcypromine pretreatment significantly potentiated the increase in grooming by fluoxetine (20 mg/kg, ip) while lower dose of fluoxetine (10 mg/kg, ipp) alone or in combination with tranylcypromine failed to influence the grooming in open field test as compared to saline treated mice.

Effect tranylcypromine and fluoxetine on rearing in mouse in open field test

Combination of subeffective doses of tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg ip) significantly increased the rearing as compared with saline treated animals.Tranylcypromine pretreatment significantly potentiated the increase in rearing by fluoxetine while lower dose of fluoxetine (10 mg/kg, ip) alone or in combination with tranylcypromine failed to influence the rearing in open field test as compared to saline treated mice.

Effect of tranylcypromine and fluoxetine in fall latency on rotarod apparatus in mouse

Combination of subeffective doses of tranylcypromine (0.5 mg/kg ,ip) and fluoxetine (20 mg/kg, ip) significantly decreased the fall latency as compared with saline treated animals .Tranylcypromine pre-treatment significantly potentiated the reduction in fall latency by fluoxetine while lower dose of fluoxetine (10 mg/kg, ip) alone or in combination with tranylcypromine failed to influence the fall latency as compared to saline treated mice.

Effect of tranylcypromine and fluoxetine on sniffing in social interaction test in mouse

Combination of subeffective doses of (tranylcypromine(0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) did not show any significant effect in sniffing as compared with saline treated animals.Tranylcypromine pretreatment significantly not potentiated the increase in sniffing by fluoxetine while lower dose of fluoxetine (10 mg/kg, ip) alone or in combination with tranylcypromine failed to influence the sniffing in open field test.

Effect of tranylcypromineand fluoxetine on facing in social interaction test in mouse

Combination of subeffective doses of tranylcypromine(0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) significantly increased the facing as compared with saline treated animals.Tranylcyprominepre-treatment significantly potentiated the increase in facing by fluoxetine while its lower dose of fluoxetine (10 mg/kg, ip) alone or in combination with tranylcypromine failed to influence the facing in social interaction test as compared to saline treated mice.

Effect of tranylcypromine and fluoxetine on the genital investigation in social interaction test in mouse

Combination of subeffective doses tranylcypromine (0.5 mg/kg, ip) and fluoxetine (10 mg/kg, ip) significantly increased the genital investigation as compared with saline treated animals .Tranylcyprominepretreatment significantly potentiated the increase in grooming by fluoxetine

while higher dose of fluoxetine (20 mg/kg, ip) alone or in combination with tranylcypromine failed to influence the genital investigation in social interaction test as compared to saline treated mice.

Effect of σ 1R antagonist BD- 1063 on rectal temperature in mouse

BD-1063 (5-10 µg/mice, icv) failed to influence the rectal temperature as compared to vehicle treated group. The mean the rectal temperature in BD-1063 injected mice was not significantly different from the control animals.

Effect of σ1R antagonist , BD- 1063 on ambulation in open field test in mouse

BD-1063 (5-10 µg/mice ,icv) failed to influence the ambulation compared to vehicle treated group.The mean ambulation in BD – 1063 injected mice not significantly different from the control animals.

Effect of σ1R antagonist, BD-1063 on grooming in open field test in mouse

BD- 1063 (5-10 µg/mice, icv) failed to influence the grooming as compared to vehicle treated group. The mean grooming in BD-1063 injected mice was not significantly different from the control animals.

Effect of σ1R antagonist, BD-1063 on rearing in open field test in mouse

BD-1063 (5-10 µg/mice, icv) failed to influence the rearing as compared to vehicle treated group. The mean rearing in BD-1063 injected mice was not significantly different from the control animals.

Effect of σ1R antagonist, BD-1063 on fall latency in mouse

BD-1063 (5-10 µg/mice, icv) failed to influence the fall latency as compared to vehicle treated group. The mean fall latency in BD-1063 injected mice was not significantly different from the control animals.

Effect of σ1R antagonist , BD-1063 on sniffing in social interaction test in mouse

BD-1063 (5-10µg/mice, icv) failed to influence the sniffing as compared to vehicle treated group. The mean sniffing in BD-1063 injected mice was not significantly different from the control animals.

Effect of σ1R antagonist, BD-1063 on facing in social interaction test in mouse

BD-1063 (5-10 µg/mice, icv) failed to influence the facing as compared to vehicle treated group. The mean facing in BD-1063 injected mice was not significantly different from the control animals.

Effect of σ1R antagonist , BD-1063 on genital investigation in social interaction test in mouse

BD-1063 (5-10 µg/mice, icv) failed to influence the genital investigation as compared to vehicle treated group.The genital investigation in BD-1063 injected mice was not significantly different from the control animals.

σ1R antagonist, BD-1063 attenuate the serotonin syndrome-like effect of tranylcypromine and fluoxetine in mice

Tranylcypromine(0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) in animals pre-treated with BD-1063 (10 µg/mice, icv)failed to show any significant serotonin syndrome-like effect as compared with vehicle treated groups.Increase in rectal temperature with tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg//kg, ip) compared to aCSF treated mice. Pre-treatment of BD1063 significantly attenuated the serotonin syndrome-like effect of tranylcypromine and fluoxetine.

σ 1R antagonist, BD-1063 attenuate the serotonin syndrome-like effect tranylcypromine and fluoxetine in mice

Tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) in animal pre-treated with BD-1063 (10 µg/mice, icv) failed to show any significant serotonin syndrome-like effect as compared with vehicle treated groups.Significant increase in ambulation with tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) compared to aCSFtreated mice. Pre-treatment of BD-1063significantly attenuated the serotonin syndrome-like effect of tranylcypromine and fluoxetine.

σ 1R antagonist, BD-1063 attenuate the serotonin syndrome-like effect tranylcypromine and fluoxetine in mice

Tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg ip) in animals pre-treated with BD-1063 (10 µg/mice, icv) failed to show any significant serotonin syndrome-like effect as compared with vehicle treated groups significant increase in grooming with tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) compared to aCSF treated mice. Pre- treatment of BD-1063 significantly attenuated the serotonin syndrome-like effect significantly and fluoxetine.

σ 1R antagonist, BD-1063 attenuate the serotonin syndrome –like effect of tranylcypromine and fluoxetine in mice

Tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) in animals pre-treated with BD-1063 (10 µg/mice, icv) failed to show any significant serotonin syndrome-like effect as compared with vehicle treated groups significant increase in rearing with tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg , ip) compared to aCSF treated mice. Pre-treatment of BD-1063 significantly attenuated the serotonin syndrome-like effect of tranylcypromine and fluoxetine.

σ1R antagonist, BD-1063 attenuate the serotonin syndrome-like effect tranylcypromineand fluoxetine in mice

Tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg, kg, ip) in animals pre-treated with BD-1063 (10 µg/mice, icv) failed to show any significant serotonin syndrome-like effect as compared with vehicle treated groups.

σ 1R antagonist, BD-1063 attenuate the serotonin syndrome-like effect of tranylcypromine and fluoxetine in mice

Tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) in animals pre-treated with BD-1063 (10 µg/mice, icv) failed to show any significant serotonin syndrome-like effect as compared with vehicle treated groups.No any significant increase in sniffing with tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) compared to aCSF treated mice.pre-treatment of BD1063 shows no any significant effect with tranylcypromine and fluoxetine.

σ 1Rantagonist, BD-1063 attenuate the serotonin syndrome-like effect of tranylcypromineand fluoxetine in mice

Tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) in animals pre-treated with BD-1063 (10 µg/mice, icv) failed to show any significant serotonin syndrome-like effect as compared with vehicles treated groups. Pre-treatment of BD1063 ($P < 0.05$) significantly attenuated the serotonin syndrome-like effect of tranylcypromine and fluoxetine.

σ 1R antagonist, BD-1063 attenuate the serotonin syndrome-like effect of tranylcypromine and fluoxetine in mice

Tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) in animals pre-treated with BD-1063 (10 µg/mice, icv) failed to show any significant serotonin syndrome-like effect as compared with vehicle treated groups.No any significant increase in genital investigation with tranylcypromine (0.5 mg/kg, ip) and fluoxetine (20 mg/kg, ip) compared to aCSF treated mice. Pre-treatment of BD-1063 shows no any significant effect with tranylcypromine and fluoxetine.

- **Effect of tranylcypromine and quetiapine on the immobility time in mouse FST**

  Combination of subeffective doses of tranylcypromine (05 mg/kg, ip) and quetiapine (100 mg/kg, ip) significantly decreased the immobility time as compared with saline treated animals.Tranylcypromine pre-treatment significantly potentiated the reduction in the immobility time by quetiapine while lower doses of quetiapine (50 mg/kg, ip) alone or in combination with tranylcypromine failed to influence the immobility time in FST as compared to saline treated mice.

- **Effect of tranylcypromine and quetiapine on rectal temperature in mouse**

  Combination of subeffective doses of tranylcypromine (0.5 mg/kg, ip) and quetiapine (100 mg/kg, ip) significantly increased the rectal temperature as compared with saline treated animals. Tranylcypromine pre-treatment significantly potentiated the increase in rectal temperature by quetiapine while lower dose of quetiapine (50 mg/kg, ip) alone or in combination with tranylcypromine failed to influence the rectal temperature as compared to saline treated mice.

- **Effect Of tranylcypromine and quetiapine on ambulation in mouse in open field test**

  Combination of subeffective doses of tranylcypromine(0.5 mg/kg, ip) and quetiapine (100 mg/kg, ip) significantly decreased the ambulation as comared with saline treated animals.Ambulation by quetiapine while lower doses of quetiapine(50 mg/kg, ip) alone or in

combination with tranylcypromine failed to influence the ambulation in open field test as compared to saline treated mice.

- **Effect Of tranylcypromine and quetiapine on gromming in mouse in open field test**

Combination of subeffective doses of tranylcypromine (0.5 mg/kg, ip) and quetiapine(50 mg/kg, ip) significantly increased the grooming as compared with saline treated animals.Tranylcypromine pretreatment significantly potentiated the increase in grooming by quetiapine whilehigher dose of quetiapine(100 mg/kg, ip) alone or in combination with tranylcypromine failed to influence the grooming in oen field test as compared to saline treated mice.

- **Effect of tranylcypromine and quetiapine on rearing in mouse in open field test**

Combination of subeffective doses of tranylcypromine (0.5 mg/kg ip) and quetiapine (50 mg/kg, ip) significantly increased the rearing as compared with saline treated animals.Tranylcypromine pre-treatment significantly potentiated the increase in rearing by while higher doses of quetiapine (100 mg/kg, ip) alone or in combination with tranylcypromine failed to influence rearing in open field test as compared to saline treated mice.

- **Effect of tranylcypromine and quetiapine in fall latency on rotarod apparatus in mouse**

Combination of subeffective doses of tranylcypromine (0.5 mg/kg, ip) and quetiaine (100 mg/kg, ip) significantly decreased the fall latency as compared with saline treated animalstranylcypromine pre-treatment significantly potentiated the reduction in fall latency by quetiapine while lower dose of quetiapine (50 mg/kg, ip) alone or in combination with tranylcypromine failed to influence the fall latency as compared to saline treated mice.

- **Effect of tranylcypromine and quetiapine on sniffing in social interaction test in mouse**

Combination of subeffective doses of tranylcypromine (0.5 mg/kg, ip) and quetiapine (100 mg/kg, ip) significantly decreased the sniffing as compared with saline treated animals.Tranylcypromine pretreatment significantly potentiated the reduction in sniffing by quetiapinewhile lower dose of quetiapine(50 mg/kg, ip) alone or in combination with tranylcypromine failed to influence sniffing in open field test.

- **Effect of tranylcypromine and Quetiapineon facing in social interaction test in mouse**

Combination of subeffective doses of tranylcypromine (0.5 mg/kg, ip) significantly increased the facing as compared with saline treated animals .Tranylcypromine pre-treatment significantly potentiated the increase in facing by quetiapine while higher dose of quetiapine (100 mg/kg, ip) alone or in combination with tranylcypromine failed to influence the facing in social interaction test as compared to saline treated mice.

- **Effect of tranylcypromine and Quetiapine on genital investigation in social interaction test in mouse**

Combination of effective doses of tranylcypromine (0.5 mg/kg, ip)quetiapine (50 mg/kg, ip) significantly increased the genital investigation as compared with saline treated animals tranylcypromine pre-treatment significantly potentiated the increase in grooming by quetiapine

while higher dose of quetiapine(100 mg/kg, ip) alone or in combination with tranylcypromine failed to influence the genital investigation in social interaction test as compared to saline treated mice.

DISCUSSION

Major depression is very common neuroendocrine disorder affecting million of people worldwide. The present pharmacotherapy of depression mainly involves selective serotonin reuptake inhibitors, tricyclic antidepressants, monoamino oxidase inhibitor and atypical antidepressants. However, in chronic used resistance develops to monotherapy and treatmentrequired combination of two or more antidepressants of differentpharmacological classes. This combination therapy of resistant depression commonly associated with some typical adverse effect profile like mania, mental confusion, hallucination, shivering, sweating, hyperthermia, hypertension, hyperactivity, tachycardia, nausea, diarrhea, myoclonus (muscle twitching), hyperreflexia and tremor (Mills, 1997) collectively referred as serotonin syndrome. The present study revealed that combined administration of MAO inhibitors, tranylcypromine and SSRI, fluoxetine as well as tranylcypromine and atypical antidepressant, quetiapine significantly induced serotonin syndrome in mice as evidenced by decreased fall latency in rotarod test, immobility in FST, increased ambulatory behavior in open field and social interaction test in addition there was marked elevation in rectal temperature of animal. Serotonin syndrome is a potentially life-threatening reaction resulting from drug interactional overdose involving drugs acting on central and peripheral serotonergic receptors (Hilton et al., 1997). Therapeutic approaches targeting serotonergic antagonism (5-HT_2 antagonist cyproheptadine) and some antipsychotics (bupropine, olanzapine) are clinically used to alleviate symptoms of serotonin syndrome (Graudins et al., 1998; Boddy et al., 2004; Duggal et al., 2002). However antipsychotics themselves have potential to precipitate serotonin syndrome when administered concomitantly with serotonergic medication (Frank, 2008). Clinical studies only suggested use of 5HT antagonist in severe cases as it is associated with severe side effects. This data strongly suggests the need to explore the new therapeutic agents for management of serotonin syndrome.

In the present study , we found that pre-administration of σ1 antagonist BD-1063 significantly attenuated serotonin syndrome induced by combination of tranylcypromine and fluoxetine. MAOIs, SSRIs and newer generations of antidepressant drugs like neurosteroids has significant affinity for σR, particularly the σ1 subtype (Su et al., 1988; Su et al., 1990; Maurice, 2004). Apart from classical monoamines, σRshave emerged as potential and compelling targets for antidepressant drug development. Numerous in vivo studies indicate that σRagonists produce antidepressant like effects in animals and humans (Matsuno et al., 1996; Ukai et al., 1998; Skuza&Rogoz, 2002; Skuza, 2003; Wang et al., 2007). This activity was also shown to be inhibited by the selective σ1R antagonist NE-100 (Akunne et al., 2001; Matsuno et al., 1996; Ukai et al., 1998; Wang et al., 2007). These findings clearly demonstrated the role of σR in antidepressant activity. However it is not clear whether it also contribute to side effect profile of clinically used antidepressants. Although $5HT_{2A}$receptor is considered as treatment option for serotonin syndrome, it is not very clear whether serotonin syndrome is primarily mediated by stimulation of $5HT_{1A}$ or $5HT_{2A}$ receptor. No. of preclinical studies have shown direct correlation between σRactivation and serotonergic neurotransmission in brain. σRs represent unique binding sites in brain and exert potent influence on a number of neurotransmitter systems, signaling pathways and brain regions involved in depression.

In previous study σ1R agonist and $5HT_{1A}$ ligand OPC and σRagonist pentazocine, increase in firing rate of 5-HT neurons in dorsal raphe nucleus (Bermack et al., 2004; Bermack and Debonnel, 2001). This increase was prevented by the co-administration of σ1R antagonist NE-100 suggests an effect mediated via a subtype of σ1 receptors (Bermack and Debonnel, 2001). In this line, earlier data from our laboratory also showed that σ1 ligand pentazocine increases antidepressant activity of quetiapine and pre-treatment with the σR antagonists BD-1063 abolished the antidepressant-like actions of this combination. Interestingly quetiapine and fluoxetine used in the present study also possess moderate affinity towards $5HT_{1A}$ receptors.

In view of these evidences, we proposed the σR antagonist, BD-1063 might affect serotonin syndrome via σR and indirect stimulation of $5HT_{1A}$ receptor. Further it should be verified whether BD-1063 have any affinity toward $5HT_{2A}$ receptors.

In conclusion, the present study demonstrated serotonin syndrome by combined administration of tranylcypromine + fluoxetine and tranylcypromine + quetiapine as evidenced by decreased fall latency in rotarod test, immobility in FST, increased ambulatory behavior in open field and social interaction test in addition there was marked elevation in rectal temperature of animal. Importantly this effect was completely antagonized by selective antagonist of σR, BD-1063. We assume that direct antagonist of σ1R and indirect stimulation of $5HT_{1A}$ receptor could be responsible for this beneficial effect of BD-1063. We proposed that BD-1063 should be considered for this beneficial effect BD-1063. We proposed that BD-1063 should be considered as treatment option for serotonin syndrome or in combination therapy with clinically used antidepressant.

SUMMARY AND CONCLUSION

In the present study we have observed that combination effect of ipadministration of tranylcypromine and fluoxetine as well as tranylcypromine and quetiapine produced serotonin syndrome as evidenced by decreased fall latency in rotarod test, immobility in FST, increased ambulatory behavior in open field and social interaction test, and significant elevation in rectal temperature of animal. Current available therapies for management of syndrome vis. $5\text{-}HT_{1a}$ antagonist and antipsychotics possess uncertainly for serotonergic modulations and thereby limited itself.σRhave been potentially emerged as target for psychiatric disorders and are significantly involved in antidepressant like effect of most of the marketed preparations. Hence to investigate the involvement of σR system in serotonin syndrome. It was found to attenuate expression of serotonin syndrome lie effect of tranylcypromine and fluoxetine in mice as compared to control animals.

The present study thus suggests crucial role of σ1R in serotonin syndrome like effect of tranylcypromine and fluoxetine in mice as confirmed by antagonism of σ1R. Hence it must be further studied for underlying mechanism to be projected as a therapeutictarget for the treatment of serotonin syndrome caused due to antidepressant therapy.

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