Ondansetron ameliorates depression associated with obesity in high-fat diet fed experimental mice: An investigation-based on the behavioral, biochemical, and molecular approach

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Abstract:
INTRODUCTION: Obesity is an important risk factor for depression as more than half of the obese population is susceptible for depression at double rate. Our earlier studies reported the antidepressant potential of 5-HT₃ receptor antagonist, ondansetron (OND) in depression associated obesity using behavioral tasks. The present research work aimed to evaluate the effect of OND on depression associated with obesity with special emphasis on biochemical and molecular mechanisms such as hippocampal brain-derived neurotrophic factor (BDNF), cyclic adenosine monophosphate (cAMP), 5-hydroxytryptamine (5-HT), hippocampal histological examination and immunohistochemical expression of p53 proteins.

MATERIALS AND METHODS: Mice were fed with high-fat diet (HFD) for 14 weeks, followed by treatment schedule for 28 days with vehicle/OND (0.5 and 1 mg/kg, p.o.)/reference antidepressant escitalopram (10 mg/kg, p.o.). Subsequently, animals were screened in the behavioral tests of depression such as forced swim test (FST) and sucrose preference test (SPT), biochemical estimations including hippocampal cAMP, BDNF and 5-HT, and molecular assays mainly histology and p53 expression of dentate gyrus (DG).

RESULTS: HFD-fed mice showed increased immobility time in FST, reduced sucrose consumption in SPT, decreased level of signal transduction factor cAMP, neuronal growth factor BDNF and neurotransmitter 5-HT in the hippocampus, and raised and p53 expression neuronal damage in the DG region of mice fed with HFD in comparison to the mice fed with normal pellet diet. Chronic treatment with OND (0.5 and 1 mg/kg, p.o.) significantly inhibited the behavioral, biochemical and molecular modifications in HFD-fed mice.

CONCLUSION: In the preliminary study, OND attenuated depression associated with obesity in mice fed with HFD using various assays procedures, at least in part by the modulation of serotonergic transmission.

Keywords:
5-HT3 receptor antagonist, comorbid depression, serotonergic neurotransmission

Introduction

Major depression, common psychiatric disorder that affects the functioning and quality of life that ranks third in leading cause of disability which is expected to stand first by 2030, across the world.[1] Major depression is experienced by around 10%–15% of the world population and the lifetime prevalence of depression is >20%, which is one in every five individuals.[2] In India, a meta-analysis study noticed the...
raised prevalence rate over the past few decades of around 15.9% for depression. Data obtained from the primary health-care settings in India showed 21%–84% of the cases of depression. Obesity affects >300 million world population and triggers to various metabolic, cardiovascular, and psychological complications. Obesity has been reported to be associated with several neuropsychiatric disorders such as Alzheimer’s disease, cognitive decline, depression, and anxiety. Association of depression with obesity is a growing problem with children, adolescents, and adult population globally, as most of the obese people are twice prone to develop depression.

Current antidepressant suffers from limitations of lag phase, late therapeutic benefit, disturbed circadian rhythm, and weight gain issue with long-time treatment and failure at clinical trials. Hence, depression associated with obesity needs some serious attention with respect to the biochemical factors and better treatment approaches to combat such serious comorbid disorders.

The nerve growth factor brain-derived neurotrophic factor (BDNF) and second messenger in the signal transduction process cyclic adenosine monophosphate (cAMP) are very important factors involved in maintaining the neuronal survival. cAMP and BDNF in the hippocampus dentate gyrus (DG) have been reported to be severely affected in case of depression and obesity.

Serotonin (5-hydroxytryptamine [5-HT]), is an important neurotransmitter that regulates mood, sleep, and appetite. Reports have claimed the involvement of BDNF and 5-HT in regulating the neuronal survival. BDNF is co-expressed on the dorsal and median raphe serotonergic neurons and regulate the survival and differentiation in the brain.

The p53 protein-mediated neuronal damage through DNA, hypoxia, oxidative stress is well documented. p53 protein leads to the alteration of hypothalamic pituitary adrenal (HPA) axis. HPA axis hyperactivity is one of the major pathogenic factors involved in neuropsychiatric and metabolic disorders such as depression and obesity, respectively. Hence, it would be fascinating to conduct experiments and look for the role of p53 protein in such comorbid disorders.

Serotonergic type 3 receptor (5-HT3) antagonist ondansetron (OND) is reported for antidepressant potential through serotonin modulation in comorbid depression and obesity in the previous reports from our laboratory. The present study is aimed to investigate the effect of OND on the biochemical and molecular mechanisms such as hippocampal cAMP, BDNF, 5-HT level, and on hippocampal DG morphology and p53 protein expression in support to the behavioral assays such as forced swim test (FST) and sucrose preference test (SPT).

**Materials and Methods**

**Animals used in the study**

Swiss albino mice (male, 20–25 g) were procured from Choudhary Charan Singh Hisar Agricultural University, Hisar, India (Reg. No. 417/01/a/CPCSEA). Under standard housing conditions, animals were maintained with temperature 23°C ± 2°C and room humidity 60% ± 10% and light-dark cycle of 12:12 h. Filtered water and standard diet were provided ad libitum. The protocol used in the research work was approved by the Institutional Animal Ethics Committee (Protocol No. IAEC/RES/18/09).

**High-fat diet used in the study**

As per the earlier described procedure, the high-fat diet (HFD) was prepared and fed to mice for 14 weeks to induce depressive phenotypes.

**Experimental design used for the study**

The flow of the study was designed as per Figure 1. Mice were divided into 5 groups (n = 6/group). Half brain of each mice was used for various biochemical assessments and other half part of brain considered for DG morphology and p53 immunohistochemical (IHC) assays.

Escitalopram (ESC) was used as standard antidepressant. Group I–normal pellet diet (NPD) control kept on vehicle (10 ml/kg, p.o.), Group II–HFD control received vehicle (10 ml/kg, p.o.), Group III–HFD + OND (0.5 mg/kg, p.o.), Group–IV HFD + OND (1 mg/kg, p.o.), and Group V–HFD + ESC (10 mg/kg, p.o.).

**Drug solutions**

OND and ESC were freshly prepared in distilled water and administered orally once daily for 28 days.

**Note**

In our earlier study, OND showed no significant effect in the per se groups fed with normal pellet diet (NPD). Per se group was not included in the present study taking into account the guidelines by animal ethical committee for animal number.

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**Figure 1**: Time line of the study representing study protocol
Body weight measurement
During the treatment schedule with OND/ESC/vehicle for 28 days, body weight was recorded once in every 2 days.

Behavioral assays
Sucrose preference test
A method described earlier by Willner, 1997 was followed for SPT. Mice were allowed to explore both water and sucrose solution (1% v/v) for initial 5 days of treatment schedule (from day 1 to 5). To rule out the chances of side preference, each day the bottles were switched. On test day (day 29), mice were allowed to access bottles filled with both water and sucrose, 24 h later, percentage sucrose preference was calculated based on the amount of water and sucrose consumed by mice.

Spontaneous locomotor activity score
As per the earlier procedure, spontaneous locomotor activity (SLA) score was measured by using actophotometer (INCO, India). Mice were introduced in the actophotometer and lead was closed. SLA was recorded for 10 min.

Forced swim test
FST was performed according to the method mentioned elsewhere. Mice were allowed to swim a cylinder of glass (diameter: 22.5 cm and height 30 cm) filled with water (15 cm, mentioned at 23°C ± 2°C) for 6 min consisting adaption time of initial 2 min and duration of immobility was measured in remaining 4 min.

Biochemical assays
Brain hippocampal cyclic adenosine monophosphate estimation
cAMP estimation was performed as per the guidelines mentioned by the ELISA kit (Enzo Life Sciences).

Brain hippocampal brain-derived neurotrophic factor estimation
BDNF estimation was carried out according to the instructions described in the ELISA kit (Boster Biological Technology).

Brain hippocampal 5-hydroxytryptamine estimation
Hippocampus was homogenized in a mixture of 1 mM ethylenediaminetetraacetic acid, 0.4 mM Na₂S₂O₅, and 0.5M HCLO₄, and spin at 12000 rpm (Eppendorf centrifuge 5702R) at 4°C. Supernatant was used for 5-HT estimation considering of the mobile phase as a 90:10 v/v ratio of 10 mM phosphate buffer (pH 3.0) and methanol, respectively. The ionization potential of + 800 mV was applied at the electrochemical cell and 1.0 ml/min flow rate was maintained.

Molecular assays
Dentate gyrus histology study in high-fat diet-fed mice
After dissection brain was fixed in 10% formaldehyde for 2 h and then for 24 h in fresh 10% formaldehyde. Dehydration of brain was performed with 70% ethanol for 24 h, and 90% and 100% ethanol for 1 h and each. Then, brain samples were embedded in the paraffin after cleaning with xylene. Sectioning (5 μm) of DG region was done by microtone (Macro Scientific Works Pvt. Ltd., Delhi, India) and hematoxylin and eosin (H and E) staining was performed. Sections were observed under light microscope (Optika, Italy) for the morphological changes.

Dentate gyrus p53 immunohistochemical study of high-fat diet-fed mice
The brain samples were fixed in 4% paraformaldehyde overnight and then stored in 30% sucrose at 4°C. Brain samples were embedded in paraffin blocks and sections (5 μm) of the hippocampal DG region were taken. Then, sections were incubated in citrate buffer (10 mmol/l) for antigen retrieval treatment. Sections were incubated with the primary (anti-p53; mouse, 1:200 dilution; Santa Cruz Biotechnology) and secondary (antimouse Horse Radish Peroxidase conjugated) antibodies, respectively, followed by chromogenic detection of diaminobenzidine (DAB). Sections were mounted in DPX (Sigma-Aldrich) on slides after counterstaining with hematoxylin and dehydration with alcohol and xylene. Sections were observed using Optika microscope (Model no. 4083.B5, Italy) and using Image J software for calculating DAB positive area was analyzed.

Statistical analysis
Data were expressed as mean ± standard error of the mean and analyzed by using GraphPad PRISM software version 2.01 (Graph Pad Software, La Jolla, USA). Using one-way analysis of variance followed by Bonferroni post test for multiple comparisons between various groups, statistical analysis were done (P < 0.05 statistically significant).

Results
Effect of ondansetron treatment on body weight of high-fat diet-fed mice
Figure 2 showed significant higher body weight in HFD-fed mice than (P < 0.01) NPD-fed mice. No
significant effect was observed on body weight with chronic administration of OND (0.5 and 1 mg/kg, p.o.) and standard ESC (10 mg/kg, p.o.) (F(4,35) = 4473, P > 0.05) in HFD-fed mice.

**Behavioral assays**

**Effect of ondansetron on sucrose preference test in high-fat diet-fed mice**

HFD-fed mice exhibited significantly (P < 0.01) decreased sucrose consumption in comparison to NPD group. Treatment with OND (0.5 and 1 mg/kg, p.o.) and reference drug ESC (10 mg/kg, p.o.) increased the sucrose solution consumption in HFD-fed mice (F(4,25) = 6.21, P < 0.05) as shown in Table 1.

**Effect of ondansetron on spontaneous locomotor activity score in high-fat diet-fed mice**

Treatment with OND (0.5 and 1 mg/kg, p.o.) and standard ESC (10 mg/kg, p.o.) did not alter the basal locomotor activity score in HFD-fed mice (F(4,25) = 0.029, P > 0.05) [Table 1].

**Effect of ondansetron on immobility time in forced swim test in high-fat diet-fed mice**

HFD-fed mice exhibited significantly (P < 0.01) higher immobility time than NPD control group. Chronic with OND (0.5 and 1 mg/kg, p.o.) and standard ESC (10 mg/kg, p.o.) inhibited the immobility time in HFD-fed mice (F(4,25) = 13.35, P < 0.01) in comparison to HFD control group as shown in Table 1.

**Biochemical assays**

**Effect of ondansetron on brain hippocampal cyclic adenosine monophosphate, brain-derived neurotrophic factor, and 5-hydroxytryptamine level of high-fat diet-fed mice**

HFD-fed mice showed significantly (P < 0.01) reduced the level of cAMP, BDNF, and 5-HT in hippocampus than NPD-fed mice. Administration of OND (0.5 and 1 mg/kg, p.o.) and reference ESC (10 mg/kg, p.o.) significantly improved the hippocampal cAMP (F(4,25) = 37.79, P < 0.01), BDNF (F(4,25) = 63.91, P < 0.05), and 5-HT (F(4,25) = 23.18, P < 0.01) levels of HFD-fed mice in comparison to HFD control group as represented in Table 2.

**Molecular assays**

**Effect of ondansetron on DG histology of high-fat diet-fed mice**

HFD-fed mice showed remarkable (P < 0.05) higher pyknotic neurons than NPD-fed mice. Chronic administration of OND (0.5 and 1 mg/kg, p.o.) and standard ESC (10 mg/kg, p.o.) significantly (F(4,25) = 419.8, P < 0.01) attenuated the alterations in hippocampus DG region by inhibiting the pyknotic neurons in HFD fed mice [Figure 3].

**Table 1: Effect of ondansetron treatment on behavioral assays including sucrose preference test, spontaneous locomotor activity, and forced swim test in high-fat diet-fed mice**

| Groups            | Percentage sucrose preference | SLA score | Immobility time (s) FST |
|-------------------|-------------------------------|-----------|-------------------------|
| NPD control       | 83.33±4.06                    | 467.00±15.17 | 130.33±7.07             |
| HFD control       | 55.27±6.28**                  | 471.33±20.99 | 169.50±6.18**           |
| HFD + OND (0.5 mg/kg, p.o.) | 76.45±3.78*                  | 464.83±18.38 | 120.33±9.43**           |
| HFD + OND (1 mg/kg, p.o.)    | 80.50±3.61**                  | 464.67±15.56 | 88.83±8.46**            |
| HFD + ESC (10 mg/kg, p.o.)       | 76.15±3.91*                  | 464.00±16.46 | 113.83±8.69**           |

Values represent mean±SEM. **P <0.01 when compared with NPD control group, *P <0.05; **P <0.01 when compared with HFD control group, one-way analysis of variance followed by Bonferroni test, n=6/group. OND=Ondansetron, SLA=Spontaneous locomotor activity, FST=Forced swim test, HFD=High-fat diet, NPD=Normal pellet diet, ESC = Escitalopram, SEM=Standard error of the mean

**Table 2: Effect of ondansetron treatment on hippocampal cyclic adenosine monophosphate, brain-derived neurotrophic factor and serotonin level in high-fat diet-fed mice**

| Groups            | cAMP (pmol/mg of proteins) | BDNF (ng/mg of proteins) | 5-HT (ng/g) |
|-------------------|----------------------------|--------------------------|-------------|
| NPD control       | 5.61±0.12                  | 3.63±0.23                | 298.78±13.09|
| HFD control       | 2.58±0.12**                | 0.56±0.05**              | 102.01±7.37**|
| HFD + OND (0.5 mg/kg, p.o.) | 3.83±1.00**                | 1.31±0.09*               | 225.96±16.96**|
| HFD + OND (1 mg/kg, p.o.)    | 4.83±0.14**                | 2.54±0.12**              | 290.61±23.04**|
| HFD + ESC (10 mg/kg, p.o.)       | 4.06±0.32**                | 1.84±0.16*               | 275.01±19.57**|

Values represent mean±SEM. **P <0.01 when compared with NPD control group, *P <0.05; **P <0.01 when compared with HFD control group, one-way analysis of variance followed by Bonferroni test, n=6/group. BDNF=Brain-derived neurotrophic factor, cAMP=Cyclic adenosine monophosphate, HFD=High-fat diet, ESC=Escitalopram, OND=Ondansetron, NPD=Normal pellet diet, 5-HT=5-hydroxytryptamine, SEM=Standard error of the mean

**Figure 2:** Effect of ondansetron (0.5 and 1 mg/kg, p.o.) treatment on the body weight of high-fat diet-fed mice. Escitalopram (10 mg/kg, p.o.) served as reference antidepressant group in the study. Values represent mean ± standard error of the mean, **P < 0.01 as compared to normal pellet diet control group, n = 6/group.
**Effect of ondansetron on p53 immunohistochemical in dentate gyrus of high-fat diet-fed mice**

HFD-fed mice exhibited marked ($P < 0.05$) higher p53 proteins in the hippocampal DG than mice fed with NPD. Repetitive administration of OND (1 mg/kg, p.o.) significantly ($F(2,15) = 196.1, P < 0.01$) inhibited the percent p53 proteins in the DG of HFD-fed mice as shown in Figure 4.

**Discussion**

HFD feeding over a period of time heightens the risk for depressive phenotypes in mice, suggesting obesity, as one of the important causative factor that can lead to development of depression. The biochemical mechanisms linking depression and obesity are still not

![Figure 3: Effect of ondansetron on (0.5 and 1 mg/kg, p.o.) treatment on histology of brain hippocampal DG region in high-fat diet-fed mice with hemotoxylin and eosin staining at ×10 and ×40, respectively. Escitalopram (10 mg/kg, p.o.) served as standard antidepressant group. Yellow arrows show the healthy neurons whereas red arrows represent the pyknotic neurons. Values represent mean ± standard error of the mean, $^\#P < 0.01$ as compared to normal pellet diet control group, $^{**}P < 0.01$ as compared to high-fat diet control group](image)

![Figure 4: Effect of ondansetron (1 mg/kg, p.o.) treatment on immunohistochemical assay of p53 protein in the hippocampal DG region of high-fat diet fed mice at ×10 and ×40, respectively. Red arrows represent the percentage area of p53 in the hippocampal DG. Values represent mean ± standard error of the mean, $^\#P < 0.01$ as compared to normal pellet diet control group, $^{**}P < 0.01$ as compared to high-fat diet control group](image)
clearly studied. Hence, studies dealing with comorbid depression and obesity are needed to be undertaken with respect to novel therapeutic approaches against such comorbid disorders.

5-HT is an important neurotransmitter that plays a significant role in the regulation of mood, sleep, and appetite. Interestingly, in depression and obesity, the level of 5-HT is reduced. 5-HT is involved in regulation of several key pathological factors such as altered cAMP and BDNF[23] and p53 proteins expression,[22] and altered DG neuronal morphology involved in comorbid depression and obesity. Hence, the therapeutic alternative that acts by improving the serotonergic neurotransmission could be a better approach for the treatment of depression associated with obesity.

Through neuromodulation of 5-HT in the hippocampus and prefrontal cortex, 5-HT3 receptors are involved in regulation of mood disorders. Antidepressant vortioxetine has been studied for the treatment of anxiety and mood disorders through multimodal actions at various 5-HT receptor systems such as agonist at 5-HT1B and antagonists at 5-HT3, 5-HT7 receptors, respectively.[23]

Our earlier reports have reported the effect of OND on depression associated with obesity with main focus on oxidative stress, insulin, and leptin resistance in HFD-fed mice through neuromodulation of serotonin.[12,13] The current research work is focused to study the effect of OND on hippocampal neurotrophic factors cAMP, BDNF, and neurotransmitter 5-HT, and morphological changes in DG and p53 protein expression in support to preliminary high predictive validity behavioral tests of depression such as SPT and FST.

Anhedonia represents very important symptom of clinical depression in terms of loss of interest or pleasure, which is reflected in rodents by the diminished ability to consume sweet/sucrose solution.[15] FST is important preliminary behavioral assay of depression as it is highly sensitive for the neurochemical modifications, hence used for screening the antidepressant potential of known or unknown drugs/compounds.[17] As FST include the duration of immobility time as observation, to avoid the bias results SLA was performed. OND treatment in HFD-fed mice showed no alteration in the basal SLA score and improved the percent sucrose preference in SPT, inhibited the duration of immobility in behavioral despair FST.

BDNF has been found to be colocalized on the serotonergic neurons and 5-HT is involved in the regulation of hippocampal neuronal survival, neurite outgrowth, and synaptogenesis.[21] Expression of neurotrophic factors such as cAMP response element binding proteins and BDNF at least in part regulated by 5-HT.[24]

5-HT modulates BDNF which regulate the synaptic plasticity. Chronic administration of serotonergic drugs improves the expression of BDNF gene in the hippocampus DG region which is involved in neuronal survival and stability of neuron synapse.[25] In the present study, hippocampal levels of cAMP, BDNF, and 5-HT were improved with chronic administration of OND in HFD-fed mice.

Obesity is associated with pro-inflammatory cytokines and stress that leads to hyperactivity of HPA axis, oxidative stress, and altered morphology of neuron. The p53 protein plays a significant role in dysregulation of HPA axis is associated with raised oxidative stress that further results in altered neuronal morphology.[24,25] Results of the present study described that chronic treatment with OND significantly attenuated the p53 protein expression in DG and inhibited the changes in morphology of neurons in HFD-fed mice. However, this is only the preliminary study and further research with more specific strains of mice and molecular assays could enlight more details on such comorbid disorders from the mechanistic approach.

Conclusion

The present preliminary study indicates that chronic treatment with OND ameliorated depression associated with obesity by reversing the behavioral, biochemical, and molecular alterations in HFD-fed mice.

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Conflicts of interest

There are no conflicts of interest.

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