

ROLE OF OREXIN 1 RECEPTOR BLOCKER SB-334867 ON CHANGES OF TRIGLYCERIDE AND CHOLESTEROL METABOLISM INDUCED BY PARADOXICAL SLEEP DEPRIVATION IN ADULT MALE RATS

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Abstract

Background: Sleep deprivation (SD) is a growing hazard through its effects on metabolism. Orexin is involved in regulation of both sleep and metabolism. Work on orexin receptors may explain the mechanisms of some hazardous effects of SD.

Aim: To test the role of orexin-1 receptor (OX1R) blocker, SB-334867 in changes of triglycerides and cholesterol metabolism induced by SD.

Method: 72 adult albino rats arranged in 4 equal groups: control, SD, SD-OX1R blocked & SD-DMSO groups. The 3 SD groups are subjected to 8 days of paradoxical SD using the modified multiple platform method. The OX1R blocked group was injected intraperitoneally daily with single dose (3 mg/kg/day) of SB-334867 dissolved in 2 ml DMSO and diluted 1:1000. The SD-DMSO group was injected by 2 ml of DMSO diluted 1:1000. Triglycerides and cholesterol levels were measured.

Results: Blood triglyceride levels dropped in all groups subjected to SD after the 1st day while the blood cholesterol level dropped in all groups subjected to SD at the 7th or 8th day. In SD-OX1R blocked group showed less drop in blood triglycerides than the other SD groups but statistically non-significant change in cholesterol level.

Conclusion: SD leads to earlier and more drop in blood triglycerides than the drop in cholesterol levels. This can be explained by high metabolism during SD with dependence on triglyceride more than cholesterol. OX1R blocker partially reduces the drop of triglyceride not cholesterol level indicating that orexin may be involved in control of triglyceride metabolism but not cholesterol.

Introduction

There is increasing studies to demonstrate that SD is associated with increased risk of cardiovascular disease including hypertension⁽³⁾. Night shift workers have a higher risk of diabetes as SD predispose for poor metabolic health by promoting excess caloric intake⁽⁴⁾. These adverse sequelae were linked to sleep disturbances, including insufficient sleep, fragmented sleep, circadian dysregulation even without altering total sleep duration⁽⁵⁾.

Rapid industrialization, new technologies as smartphones and lifestyle changes have changed the magnitude of stressors to which our current generation is exposed which if uncontrolled might lead to accelerated aging, immune suppression, heart disease, hypertension & obesity⁽¹⁾. One of these modern stressors is the sleep deprivation (SD). The average sleep duration has declined over the last few decades that represent a major public health issue⁽²⁾.

Medicine under a 12 h light/dark cycle (light on at 6:00 am). Animals were provided ad libitum & water throughout the study. Experiments were approved by the Ethical Committee of Animal Experiments, Sohag faculty of medicine and in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press).

Experimental groups

Animals were randomly divided into four equal groups (n=18):

Group I (G I-the control group):

In this group, each three rats were placed inside a cage (50 x 45 x 30cm) that contain 7 rectangular platforms specified for standing of rats and filled with sawdust to a level 1 cm below the upper surface of the 7 rectangular platforms. Rats in this group were allowed to sleep normally. This group was i.p. injected with 2 ml of saline once daily throughout the 8 days of the experiment.

Group II (GII- the sleep deprived group):

In this group, rats were subjected to paradoxical SD for 8 successive days using a modified multiple platform method⁽¹⁰⁾. Each three rats were placed inside a similar cages as G I but water was added to the bottom of the cage instead of the sawdust. The upper surfaces of the platforms were kept 1 cm above the surface of the water. Thus, the rats could move around inside the cage by jumping from one platform to another. Upon reaching the paradoxical phase of sleep, rats experience muscle atonia, which leads them to make contact with, or fall into, the water. At that point, they awaken abruptly and repeat the sleep-wake cycle. This group was injected with 2ml saline i.p. once daily for 8 successive days as GI⁽¹¹⁾.

Group III (G III - the sleep deprived-OX1R blocked group, SD-OX1R blocked group):

These effects of SD on lipid metabolism are still controversy. While some researchers find drop of triglycerides and cholesterol levels after SD⁽⁶⁾ others reported a rise in their levels⁽⁷⁾. Also the mechanisms by which SD affects lipid metabolism are still not clearly understood⁽⁵⁾.

Orexin, a newly discovered hypothalamic neurotransmitter, is involved in regulation of sleep⁽⁸⁾, the neuroendocrine system & energy homeostasis⁽⁹⁾.

Orexin has 2 types of receptors: orexin-1 receptors (OX1R) and orexin-2 receptors (OX2R). The OX1R has poor effect on sleep while OX2R has a major role in promoting wakefulness. So Blocking OX1R by SB-334867 without affecting sleep may affect lipid metabolism giving an explanation for the changes in lipid profile during periods of SD.

So, the hypothesis of this study is, orexin may play a role in the disturbances that accompany SD that will be reflected on health due to its wide roles in regulation of sleep and regulation of different parameters that affected by SD.

Aim of the work:

- To test the effect of SD on blood triglyceride and cholesterol levels.
- To evaluate the hypothesis that orexin is involved in the mechanism by which SD produces these changes.
- To test the efficacy of OX1R blocker, SB-334867 as a protective agent against changes in triglyceride and cholesterol levels.

Materials and methods

Animals:

A total of 72 adult male albino rats, aged 10-12 weeks and weighting 200-300 grams obtained from Animal Facility, Faculty of Science, Sohag University. Rats were housed in Animal Facility, Sohag Faculty of

injection of 2 ml of DMSO diluted 1:1000 in saline i.p. once daily for 8 successive days.

A blood sample was taken daily at 4:00 p.m. from the lateral tail vein of each rat using 3 ml syringe. The samples were collected in EDTA (20 µL / ml blood) containing tubes. Blood was centrifuged and plasma was separated and stored at -20°C until the time of biochemical analysis⁽¹⁰⁾.

In this group, rats were subjected to paradoxical SD as G II but with i.p. injection of SB-334867 (OX1R blocker) dissolved in 2 ml DMSO and diluted 1:1000 in saline in a dose of 3 mg/kg/day once daily for 8 successive days.

Group IV (G IV - the sleep deprived-DMSO group, SD-DMSO group):

In this group, rats were subjected to paradoxical SD as G II but with i.p.

Time	Procedure
10 a.m.	Removal of food and starting the fasting.
12 p.m.	Removal of water at the bottom of the cages of G II, G III &G IV and drying the cages allowing rats to sleep freely.
4 p.m.	Collection of blood samples for measuring of blood triglycerides and cholesterol. Adding the food after weighting it. Start of SD by filling the cages with water to a level of 1 cm below the upper surfaces of the rectangular platforms in all groups except G I. Injection of rats in G I & G II with saline, rats of G III with OX1R blocker i.p. & rats of G IV with DMSO i.p.

Table (1): Time schedule details during the 8 days of the experiment

compared with the control group. The fasting triglyceride level in G III was statistically significant higher when compared with G II beginning after the 1st day of SD (P-value < 0.0001) and continued to the 8th day (P-value = 0.0044). The difference was more obvious during the 1st 4 days.

Cholesterol levels:

In the sleep deprived group (G II) & the SD-DMSO group, there was a statistically significant lower fasting blood cholesterol level appeared at the 8th day of SD when compared with the control group (P-value = 0.0121 for G II & 0.0048 for G IV). In SD-OX1R blocked group (G III), there was a statistically significant lower fasting blood cholesterol level beginning at the 7th day of SD (P-value = 0.0199) and continued to the 8th day (P-value = 0.0021) when compared with the control group. While, by comparing fasting blood cholesterol level in G III and G II showed no statistically significant difference.

The fasting Level of triglycerides and cholesterol were measured from these blood samples using triglyceride kits & cholesterol kits respectively (S.L., Agappe diagnostics LTD., India).

Statistical analysis

In this work statistics were done by using prism program, version 7.

Data expressed at mean ± SE (standard error). Student t-test was used to determine significance between numeric data between two groups. ANOVA test was used to determine significance between numeric data of different groups. Probability value (P-value) was considered Significant if P < 0.05.

Results

Triglyceride levels:

In all groups subjected to SD (G II, G III & G IV), the fasting blood triglyceride level showed a statistically significant lower values beginning after the 1st day of SD (P-value < 0.0001 in all groups) and continued to day 8 (P-value < 0.0001 in all groups) when

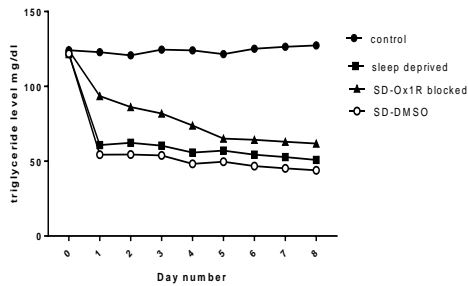


Figure (1):Time course of changes in fasting blood triglyceride level in different groups throughout the experiment.

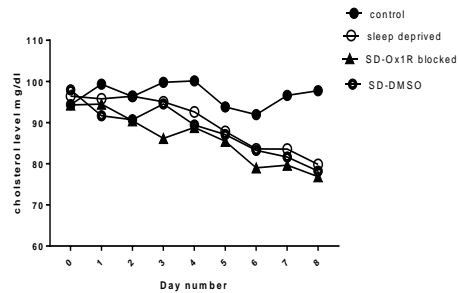


Figure (2):Time course of changes in fasting blood cholesterol level in different groups throughout the experiment.

Discussion

Our modern society suffers new kinds of stressors; one of them is the SD which has negative effects on different body functions including lipid metabolism. Nevertheless, the role of orexin in health hazards of SD is not yet evident and the mechanisms by which orexin involved in these hazards and the protective effect of orexin receptor blockers are still debated⁽¹²⁾.

To accomplish the goals, an animal model of SD was established and the experiment is formed of control group, SD group, SD-OX1R blocked group & SD-DMSO group. In this study, fasting blood triglyceride & cholesterol levels were measured and the results were recorded in all groups.

The current study showed that the triglyceride level in the sleep deprived group (G II) and SD-DMSO group (G IV) was lowered to statistically significant values after the 1st day of SD when compared with the control group. while the cholesterol level was statistically significant lower than the control group at the 8th of SD. In the SD-OX1R blocked group (G III), Triglyceride levels in G III showed statistically significant lower values after the 1st day of SD when compared with the control group but the drop in G III is less than the drop observed in G II which was statistically significant. This suggests a role of orexin in

triglyceride metabolism.

The cholesterol level showed statistically significant lower values at the 7th & 8th day of SD with no significant changes when compared with G II denoting this effect on cholesterol is orexin independent.

These results agreed with results of previous studies that reported that SD reduces the triglyceride levels in rats but doesn't affect the cholesterol level^(13, 14). Plasma concentrations of triglyceride decreased significantly after the 1st day of paradoxical SD but after 8 days of SD, the total cholesterol levels were not modified⁽¹⁰⁾. While, some studies reported a drop in both triglyceride and cholesterol levels in SD rats by lesions of VLPO nucleus⁽⁶⁾. On contrast, some studies reported higher triglyceride and cholesterol levels in rats subjected to SD⁽⁷⁾ which may be due to dietetic factor.

The drop in lipid concentration could be a result of high energy expenditure that contributes to energy deficit in sleep deprived rats⁽¹⁰⁾. This maybe explained by stimulation of stress hormones secretion specially thyroxine by orexin-B at the hypothalamic level⁽¹⁵⁾.

conclusion

SD leads to a rapid and remarkable drop in blood triglyceride levels with a

slight and delayed drop in blood cholesterol level that can be explained by the high metabolic rate during periods of SD. Blocking OX1R leads to rapid but less remarkable drop in blood triglyceride level but no significant effect on changes of blood cholesterol level.

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