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Research Article

Sleep deprivation alters prostaglandin and anti-oxidant enzymes in rats induced with neuropathic pain

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Abstract

Human studies of sleep and pain are particularly challenging and must confront potential confounds such as co-existing disease and poly pharmacy. As an example of co-existing disorders, more than 40% of individuals with symptoms of insomnia report at least one chronic painful physical condition. The effects of 72 hours Rapid eye movement sleep deprivation (REMSD) was investigated on serum levels of nociceptive mediator (Prostagladin E_2-PGE_2) and oxidative stress markers (Malondialdehyde-MDA & Super oxide dismutase-SOD) in rats with neuropathic pain. Twenty male Wistar rats were divided into 4 groups (un-ligated control, ligated control, sham control and test), each containing 5 rats. All the animals were ligated by chronic constriction injury (CCI) of the sciatic nerve except those in sham and un-ligated control groups. This was followed by 72 hours of REMSD using the multiple platform method, after which the serum level of PGE_2, SOD and MDA were assessed. The induction of 72 hours REMSD led to a significant ($P<0.05$) decrease in serum PGE_2 in test group compared with ligated control. This study also showed that REMSD reduced production of reactive oxygen species as shown by a significant ($P<0.05$) decrease in serum MDA and an insignificant decrease in serum SOD compared with ligated control group. In conclusion, 72 hours REMSD showed a decrease in serum levels of PGE_2, MDA, and insignificant decrease in SOD in sciatic nerve-ligated Wistar rats. It can be deduced from this study that 72 hours REMSD has hypoalgesic effect on rats with neuropathic pain.

Keywords: *Sleep deprivation, Neuropathic pain, PGE_2, Anti-oxidant enzymes*

INTRODUCTION

It is well known that sleep loss makes an individual more susceptible to disease and, conversely, that sleep is important for recovery from illness. Specific immunological active peptides or neuro-endocrine hormones influence the sleeping-waking brain, and sleep disturbances may affect inflammatory components. Cellular (macrophages, neutrophils, eosinophils, basophils, natural killer, and T and B lymphocytes) and molecular (pro-inflammatory cytokines and acute phase proteins) inflammatory components that act as mediators of the acute phase response in inflammatory diseases, additionally, play a role as modulators of metabolic functions that involve the central nervous system, including sleep (Irwing *et al.,* 2001).

Activation of inflammatory components is a central feature in various types of painful conditions, a finding frequently reported under conditions of experimental sleep loss. Partial and acute sleep deprivation in healthy volunteers have been shown to increase pro-inflammatory cytokines such as interleukin (IL)-6, Tumour necrosis factor (TNF)-alpha, its soluble receptor p55, and C-reactive protein (Haack *et al.,* 2007). Little is known about the effects of sleep loss on the prostaglandin system, though it plays a significant role in sleep-wake regulation in animals, and is well-studied for its involvement in the origin of inflammation (Huang *et al.,* 2007).

Free radicals have been hypothesized to accumulate during prolonged waking as a result of enhanced metabolic activity, and may be responsible for some of the effects of sleep deprivation (Reimund 1994). Free radicals are difficult to detect and quantify directly due to their extreme reactivity. The production of free radicals can be inferred from measurement of antioxidant responses and/or oxidative stress-induced products (e.g. Malondialdehyde). Antioxidant responses include changes in the activities of anti-oxidative enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) and in the levels of the endogenous antioxidant, glutathione (GSH). If antioxidant responses are unable to successfully scavenge the free radicals, this will lead to oxidative stress resulting in damage to lipids, proteins and/ or nucleic acids (Giordano 2005).

Oxidative stress has been implicated in the activation of p38MAPK pathway (Xu *et al.,* 2007). After nerve injury, p38MAPK pathway increases the production of various inflammatory mediators such as TNF-α, ILs, COX-2 which are important mediators for the progression of neuropathic pain (Raghavendra *et al.,* 2003).

Sleep disturbances have been shown to precipitate some forms of chronic painful conditions and alterations of some blood parameters. Human studies of sleep and pain are particularly challenging and must confront potential confounds such as coexisting disease and polypharmacy. As an example of co-existing disorders, more than 40% of individuals with symptoms of insomnia report at least one chronic painful physical condition.

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There is paucity of information on serum mediators of pain, inflammation and oxidative stress in relation to sleep deprivation. As a result, this study aims at investigating the effect of sleep deprivation on serum mediators of pain and inflammation (PGE_2) and oxidative stress in Sciatic nerve lesion induced neuropathic pain in Wistar rats. This will further provide experimental explanation for the relationship that exists between sleep deprivation and neuropathic pain.

MATERIALS AND METHODS

Experimental animals and grouping

A total of twenty male Wistar rats with average weight of 160.5±15.4g were employed in this study. They were housed under standard environmental conditions of temperature (23±2ºc) and relative humidity of 30-50% at 12:12h light-dark cycles. Animals were kept in wire mesh cages at the Animal House of the Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria. All animals had free access to water and standard pelletized laboratory animal diet *ad libitum* and were acclimatized to laboratory conditions for two weeks before the experiment. The rats were randomly divided into 4 groups (A-D), each containing 5 rats as shown in Table 1.

Table 1:
Animal grouping

GROUP (n)	SLEEP DEPRIVATION (SD)	CCI
A- Normal (unligated) control	Negative	Negative
B-Sham control	Negative	Negative
C-Ligated control	Negative	Positive
D- Test (ligated+Sleep-deprived)	Positive	Positive

n=5 animals in each group, CCI= chronic constriction injury, Under CCI, +ve Animals were ligated, −ve Animals were not ligated. In sleep deprivation (SD), +ve animals were sleep deprived while –ve were not sleep deprived

Induction of REM sleep deprivation

The animals were deprived of REM sleep based on the method described by Jouvet *et al.,* (1964) which was modified by Van-Hulzen and Coenen (1981). Briefly, a large cylindrical metal cage that could conveniently contain four rats was placed inside a relatively large bowl of water. The rats were then gently placed inside the cage. The wire gauge of the cage provided a dry platform for the rats to stay in order to prevent them from staying inside water for too long.

Once the rats enter the stage of paradoxical sleep, their muscles get relaxed and as a result, they fall into water. To prevent this, they must hold the wire gauge for support and thus keeping them alert. The rats were kept in this sleepless state for 72 hours but were allowed free access to food and water (Hajali *et al.,* 2012). This method of sleep deprivation is called multiple platform method, a modified version of the flower pot method. Only the rats in the test (ligated sleep deprived) group went through this procedure of sleep deprivation in this experiment as shown in Table 1.

Induction of neuropathic pain by sciatic nerve ligation

Chronic constriction injury (CCI), a method described by Bennette and Xie, (1988). CCI was used to induce neuropathic pain. CCI was used because it produces sustained tactile allodynia which mimics conditions observed in patients with neuropathic pain (Bennett and Xie, 1988).

Following anaesthesia with 50mg/kg ketamine (Kaur *et al.,* 2012), a cut was made through the Bicep femoris muscle in order to expose the sciatic nerve of the left thigh. A suture silk 4.0 was used to ligate the nerve at two sites with 1mm gap in between. This ligation was carefully done to avoid puncturing any blood vessel. After tying the ligature, the overlying muscle and skin layers were sutured and a topical antibiotic was applied. The rats were closely monitored to regain their consciousness before being placed in their cages. Only rats in the test (ligated sleep deprived) and ligated control groups went through this procedure. Rats in the sham group had their sciatic nerve exposed but not ligated.

Experimental procedures

In this study, rats in ligated control and test (ligated sleep deprived) groups underwent sciatic nerve ligation, after which only those in the test group were sleep-deprived for 72 hours. Those in normal and sham control groups were neither ligated nor sleep deprived. On the fourth day, the rats were sacrificed and blood was obtained via cardiac puncture. Serum samples were collected from the blood which were then analysed for Prostaglandin E_2, Malondialdehyde (MDA) and Super oxide dismutase (SOD).

Assessment of Nociceptive mediator

Determination of serum PGE_2 concentration: This test principle is based on competitive ELISA method. The microtiter plate provided in PGE_2 kit has been pre-coated with PGE_2. During the reaction, PGE_2 in the sample or standard competes with a fixed amount of PGE_2 on the solid phase supporter for site on the Biotinlylated Detection Ab specific to PGE_2. Excess conjugate and unbound sample or standard were washed off the plate, and Avidin conjugated to Horseradish peroxidise (HRP) was added to each microplate well and incubated. Then, a Tetramethylbenzidine TMB substrate solution was added to each well. The enzyme substrate reaction was terminated by the addition of sulphuric acid solution and the colour change was measured spectrophotometrically at a wavelength of 450nm ± 2nm. The concentration of PGE_2 in the sample was then determined by comparing the optical density of the sample to the standard curve.

Assessment of Oxidative Stress

Determination of superoxide dismutase (SOD) activity: A method originally described by Misra and Fridovich (1972) was employed. This method involves inhibition of epinephrine auto-oxidation, in an alkaline medium at 480 nm in an ultraviolet spectrum. The enzyme SOD inhibits the auto-oxidation of adrenaline by catalyzing the breakdown of superoxide anions. The degree of inhibition is thus a reflection of the activity SOD and is determined at one unit of the enzyme activity.

Determination of serum malondialdehyde (MDA) level:

The assay method of Hunter *et al.,* (1963), modified by Gutteridge and Wilkins (1982) was adopted. MDA, a product of lipid peroxidation, when heated with 2-thiobarbituric acid (TBA) under acid conditions forms a pink colouration product which has a maximum absorbance at 532nm. MDA kit comprises trichloroacetic acid (TCA), TBA, and hydrochloric acid (HCl). Stock solution of TCA-TBA-HCl composed of 15g of TCA, 0.375g of TBA and 0.25N of HCl was prepared

RESULTS

Estimation of serum PGE_2 level

As indicated by Fig.1, Chronic Constriction Injury (CCI) caused a significant ($p<0.05$) increase in serum level of PGE_2 from 45.20±5.70pg/ml in normal unligated control to 378.00±38.03pg/ml in ligated control. There was also a significant increase in PGE_2 in the Sham control 200.00±24.75pg/ml compared to the normal unligated control 45.20±5.70pg/ml and this increase was lower to that observed in normal control 45.20±5.70pg/ml compared with ligated control 378.00±38.03pg/ml. However, the effect of sleep deprivation on the test group produced a significant ($p<0.05$) decrease in the serum level of PGE_2 from 378.00±38.03pg/ml in ligated control to 167.60±19.85pg/ml in the test (ligated sleep deprived) group.

Estimation of serum MDA level

As indicated in Fig. 2, the mean serum level of MDA increased significantly ($p<0.05$) in ligated control 24.92±0.75nm/ml compared with normal control 22.15±0.12nm/ml and also increased insignificantly from 22.15±0.12nm/ml in normal control to 23.09±0.51nm/ml in sham control. CCI resulted in a significant ($p<0.05$) increase in serum MDA level. However, induction of sleep deprivation caused a significant ($p<0.05$) decrease in mean MDA level in the test (ligated sleep deprived) group 22.30±0.31nm/ml compared with ligated control 24.92±0.75nm/ml.

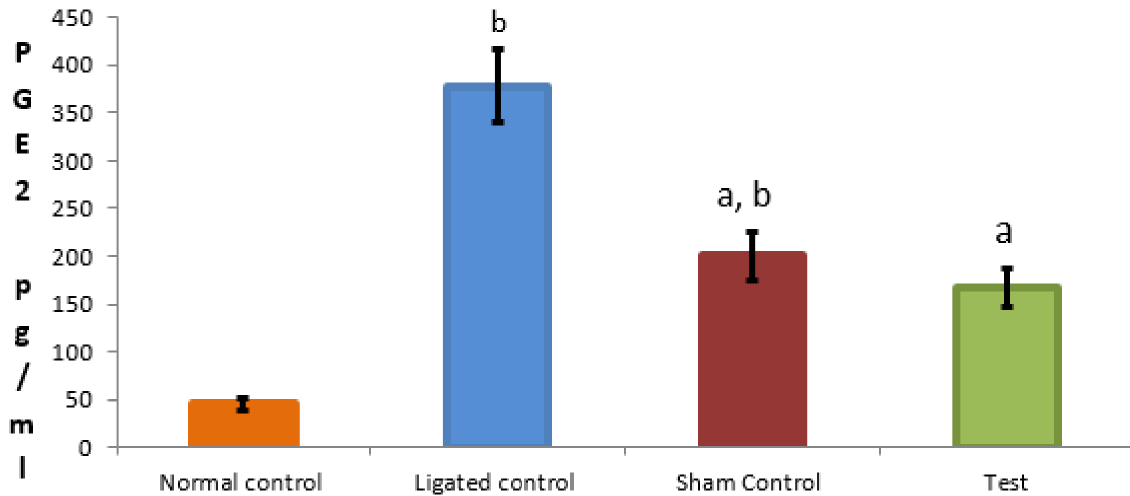

Figure 1:
Effect of REM sleep deprivation on serum PGE_2 level.
[a]$p<0.05$ is significant compared with ligated control group. [b]$p<0.05$ is significant compared with normal control.

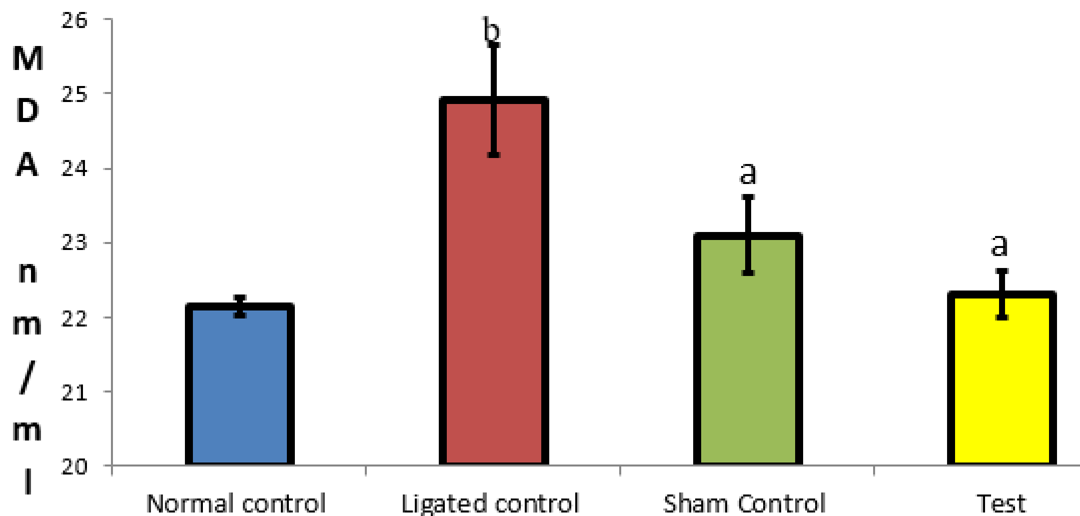

Figure 2:
Effect of REM sleep deprivation on serum Malondialdehyde (MDA) level.
[a]$p<0.05$ is significant compared with ligated control group. [b]$p<0.05$ is significant compared with normal control group.

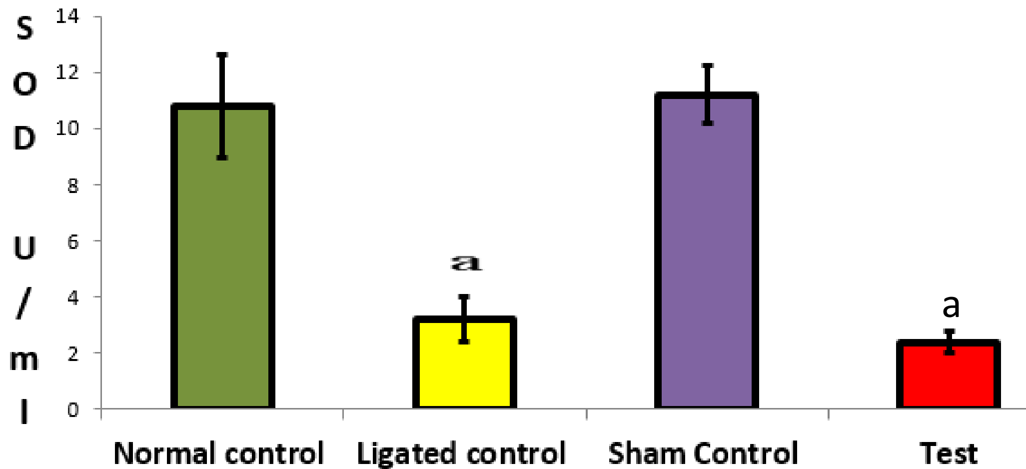

Figure 3:
Effect of REM sleep deprivation on serum super oxide dismutase (SOD) level.
[a]$p<0.05$ is significant compared with normal control.

Estimation of serum SOD level

Fig.3 shows that there was a significant ($p<0.05$) decrease in mean serum level of SOD from 10.80±1.85u/ml in normal control to 3.20±0.80u/ml in ligated control. CCI resulted in significant decrease ($p<0.05$) in serum SOD level when normal unligated control was compared with ligated control but sleep deprivation of the test (ligated sleep deprived) group produced a statistically insignificant decrease in mean serum SOD level from 2.40±0.40u/ml in test group to 3.20±0.80u/ml in ligated group.

DISCUSSION

Given that the prostaglandin system is critically involved in nociceptive processing and sleep-wake regulation, this study attempted to look at the serum level of PGE_2 following sleep deprivation. The hyperalgesia caused by chronic constriction injury was accompanied by increased serum PGE_2 in the ligated group. This can be explained by the fact that PGE_2 is one of the principal mediator of inflammation and thus, promote the development of inflammatory sign including pain (Haack *et al.,* 2009). PGE_2 is able to sensitize the nociceptive system through binding to Enzyme-Prostaglandin receptors located on peripheral terminals of primary sensory neurons resulting in an increased sensitivity to noxious stimuli and also changes neuronal excitability and synaptic dis-inhibition in the spinal cord which manifest in hyperalgesia and this has been suggested to play a role in the development of spontaneous pain (Vanegas and Schaible, 2001).

Our previous finding of anti-nociceptive effect of REM sleep deprivation on neuropathic pain (Under review) was further elucidated by this present study which showed a corresponding decrease in the serum level of PGE_2 in the test (ligated sleep-deprived) group. This may be said to be the basis for reduction in pain perception observed in the test group since PGE_2 is a nociceptive mediator. Though, the molecular mechanism responsible for this decrease in PGE_2 was not part of the scope of this study, future studies can look into this. Also, very few reports have shown the relationship between sleep deprivation, PGE_2 and pain perception, and this result is contrary to the study of Haack *et al.,* (2009) which reported an increase in urinary level of PGE_2 following sleep deprivation in which they opined that the loss of inhibitory pain control mediated by PGE_2 probably accounted for most of the chronic pain symptoms, including the occurrence of spontaneous pain. Based on our search, we could not access any report on the link between REM sleep-deprivation, serum level of PGE_2 and neuropathic pain except for the report of Haack *et al.,* (2009) which is contrary to our finding. We propose that the probable reason for the difference may be attached to medium of assessing the PGE_2 i.e., urinary versus serum.

Studies have linked sciatic nerve ligation which is a stressor to an imbalance in reactive oxygen species (ROS) and anti-oxidant enzymes (Senoglu *et al.,* 2009). Free radicals have been found to induce tissue injury and pain in neuropathic pain models (Muthuraman *et al.,* 2008). This was also confirmed by the present study in which CCI of the sciatic nerve caused oxidative stress (generation of free radicals) which was expressed by an increase serum in MDA and a reduction in serum SOD of the ligated group. Following the sciatic nerve ligation of the test group, one would have expected that sleep deprivation which has been confirmed a stressor, to cause a further increase in oxidative stress with a corresponding increase in products of tissue peroxidation and decrease in anti-oxidant enzymes. In contrast, sleep deprivation produced a decrease in serum level of MDA (a product of tissue peroxidation) with an insignificant reduction in serum SOD level (an antioxidant enzyme) meaning that there was no release of free radicals and thus, the increase in pain threshold (decreased pain perception).

Many studies have reported existing relationship between mediators of inflammatory disorder and markers of oxidative stress as a complex one. The inflammatory process is accompanied by oxidative stress. Pro-inflammatory cytokines and growth factors stimulate release of reactive oxygen species (ROS) which act as signalling mediators for a variety of signal transduction pathways and gene expression. The transcription factors that have been implicated in many inflammatory responses are the nuclear factor-kappa B (NF-kB) and activator protein-1 (AP-1). Both NF-kB and AP-1 are sensitive to many different oxidative stress stimuli and some findings suggest that they may mediate cytokine and adhesion molecules expression (Vlahopoulos *et al.,* 1999). NF-kB when activated induces the expression of cytokines and adhesion molecules in a positive feedback loop. Moreover, this pathway

is also redox-dependent and is activated by oxidative stress. Therefore, it can be postulated that antioxidant defence capacity may affect inflammatory response. For example, increased antioxidants in blood could, by inhibiting NF-kB, lead to a decrease in intercellular adhesion molecule-1 (ICAM-1), and interleukin-8 (IL-8) which are inflammation markers. For instance, in chronic obstructive pulmonary disease (COPD) which is accompanied by inflammation (both airway and systemic) and by oxidative stress, the pathogenic mechanisms explaining this association are multi-factorial and involve an intricate interplay between inflammatory and oxidative processes. On one hand, ROS activates NF-kB and other redox-sensitive transcription factors such as AP-1, which cause an increased gene expression of both pro-inflammatory cytokines and protective enzymes. On the other hand, these cytokines play an important role as activators of neutrophils and as chemo attractants, which will, in their turn, determine the inflammatory mediators released from neutrophils (Mac-Nee 2000; Sadowskaa *et al.,* 2005).

Another probable factor that may explain the observed decrease in MDA level could be that the multiple platform method used in sleep depriving these animals reduced stress compared to the single platform method. The multiple platform method has been reported to reduce stress as it allows for movement of animals and interaction with cohorts thus reducing stress that might have resulted from social isolation and restricted movement (Machado *et al.,* 2004).

In conclusion, Induction of neuropathic pain by chronic constriction injury of the sciatic nerve followed by REM sleep deprivation caused a decrease in serum levels of PGE_2, Malondialdehyde, and no significant effect on Super oxide dismutase. We therefore propose that REM sleep deprivation alters prostaglandin and anti-oxidant enzymes in rats induced with neuropathic pain.

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