Circadian Rhythms of Oxidant-Antioxidant Agents, HPA Axis and Protein Carbonyl Content in Serum, Brain and Adrenal Gland Tissues.

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Abstract

The aim of this experiment is to study the circadian rhythm of oxidant-antioxidant components in the body. A total of 20 adult male rabbits were divided into 2 groups (G1 and G2) and kept in 2 different rooms. Serum was isolated after blood collection at 12 A.M or 12 P.M respectively from G1 and G2. The results reveal a significant elevation in serum GSH, MDA and SOD during night hours. The B-endorphin showed a value of 84.36±1.36 in rabbits serum during night compared with 53.23±1.12 during day hours. Similarly, ACTH and cortisone concentration during night hours expressed a value of 71.03±0.16 and 24.46±0.38 compared to 52.80±0.37 and 11.80±0.57 during day respectively. Protein carbonyl contents shows a regular variation between serum and tissues during day and night hours. It was concluded that the oxidant-antioxidant imbalance lead to such variation between day and night hours due to activities and metabolism. Extensive research is needed to minimize and overlap such stress.

Keywords: Oxidant-antioxidant, B-endorphin, Protein carbonyl contents.

The objective of this study was to examine the circadian rhythm of oxidant-antioxidant components in the body. A total of 20 adult male rabbits were divided into two groups (G1 and G2) and kept in two different rooms. Serum was isolated after blood collection at 12 A.M or 12 P.M respectively from G1 and G2. The results revealed a significant elevation in serum GSH, MDA and SOD during night hours. The B-endorphin showed a value of 84.36±1.36 in rabbit serum during night compared with 53.23±1.12 during day hours. Similarly, ACTH and cortisone concentration during night hours expressed a value of 71.03±0.16 and 24.46±0.38 compared to 52.80±0.37 and 11.80±0.57 during day respectively. Protein carbonyl contents showed a regular variation between serum and tissues during day and night hours. It was concluded that the oxidant-antioxidant imbalance lead to such variation between day and night hours due to activities and metabolism. Extensive research is needed to minimize and overlap such stress.

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الابعاد اليومي لعوامل الأكسدة ومضاداتها ومحور HPA ومحتجز البروتين الكربوني في المصل وأنسجة الدماغ والغدة الكظرية

الخلاصة

الهدف من هذه التجربة هو دراسة الإيقاع اليومي لتراكيز عوامل الأكسدة ومضاداتها في الجسم ، حيث تم تقسيم عشرون من ذكور الأرانب البالغة إلى مجموعتين (G1 و G2) ووضعوا في غرفتين مختلفتين ، وتم عزل المصل بعد جمع الدم في 12 صبيحا أو 12 ليلا.

B-endorphin مستوي 84.36±1.36 في مصل الأرانب أثناء الليل مقارنة بـ 53.23±1.12 أثناء النهار. كما ظهر مستوى ACTH و cortisone خلال ساعات الليل مستوى 71.03±0.16 و 24.46±0.38 مقارنة بـ 52.80±0.37 و 11.80±0.57 في المصل خلال ساعات النهار. استنتج أن عدم توازن مضادات الأكسدة يؤدي إلى مثل هذا الاختلاف بين ساعات النهار والليل بسبب الأنشطة والتمثيل الغذائي، وهكذا حاجة إلى إجراء أبحاث مكثفة لتقليل وتجاهل هذا الإجهاد.
Introduction

In recent years, the circadian rhythms of reactive oxygen species and antioxidants have been of great concern because of their critical role in the development of many diseases, including malignant tumors (1). Disruption of the circadian cycle is closely correlated with metabolic imbalance, as seen by working night individuals or rotating shifts (2). Thus, the aim of this research is the knowledge of a systematic study of circadian variations of oxidant-antioxidant components in serum and some tissues in the body.

It is understood that the biological clock that regulates the endogenous circadian rhythms of most aspects of mammalian physiological and biochemical processes resides in the suprachiasmatic nucleus of the hypothalamus and the primary external signal that trains the biological clock as the light-dark cycle (3).

Little is known about the role of oxidant-antioxidant agents during day and night hours. The imbalance between the production of reactive oxygen species (ROS), reactive nitrogen species (RNS) and their removal is characterized by oxidative stress (4). Protein carbonyl content (PCC), which is the most frequently used marker of oxidative protein modification (5). While ACTH activates the adrenal gland to initiate a peripheral stress response, β-endorphin attenuates the stress response, at least in part, by inhibiting CRH secretion (6). Beta-endorphin is a morphine-like active ingredient secreted by the pituitary and hypothalamic glands (7). Thus, the object of this experiment to investigated variations of oxidant-antioxidant markers and the hypothalamic–pituitary–adrenal axis (HPA axis) during day and night hours.

Material and methods

This experiment used twenty adult male New Zealand rabbits weighing 950-1200g and aged between 2.5-3.5 months. They were divided into 2 groups (G1 and G2) and they were rearing in cages in two air conditioned rooms (22-23 C°) in the animal house of the college of veterinary medicine at the university of Baghdad. The rooms were provided with an automatic electric timer to control day-night cycle (12 hours each). The animals were left for two weeks for acclimatization to the experimental conditions and were given anticoccidial drug (amprolium) via drinking water (1g/liter) for three days. Blood samples were collected by cardiac puncture technique from each rabbit at 12 A.M. and 12 P.M. Respectively for G1 and G2. Serum was isolated to test GSH (8), MDA (9), SOD (10). Serum β-endorphin (pg / ml), ACTH and cortisone concentrations were determined using a commercially available ELISA kit for each (Elabscience, China) according to the manufacturer’s instructions (11). Protein carbonyl content (PCC) in serum was determined as described by (12). After anesthetizing the animals, brains and adrenals were isolated and hemogenized for the test of PCC using colorimetric assay Kit (Elabscience. china). All the data were subjected to statistical analysis.
using T-test (SPSS program version 24) to express differences between the two groups. The values were represented as means ± SE at P≤0.05 (13).

**Results and Discussion**

Serum concentration of GSH, MDA, SOD, B-endorphin, ACTH, Cortisone and PCC concentration in serum, brain and adrenal of normal adult male rabbits during day and night hours of the day.

Table (1) represents the differences in concentration of many parameters between day and night hours of the day. Serum GSH concentration shows a significant (P<0.05) increase during night hours(2.33±0.033) compare to that during day hours (1.56±0.057). At the same time, serum MDA levels is significantly (P<0.05) increased at night in comparism to that at day 0.66±0.03 compared to 0.14±0.005 respectively. The same rabbits show a significant increases (P<0.05) in serum SOD activity during night (21.18±0.338) compared to the level during day (19.62±0.288). Moreover, these animals have a significant increases in serum B-endorphin, ACTH and Cortisone concentrations between night and day hours of the day. On the other hand, nonsignificant found in PCC in serum, brain and adrenal tissues between night and day hours of the day.

Table 1. Serum concentration of GSH, MDA, SOD, B-endorphin, ACTH, Cortisone and PCC concentration in serum, brain and adrenal of normal adult male rabbits during day and night hours of the day.

<table>
<thead>
<tr>
<th>Time Parameter</th>
<th>G1(Day hours) (12 A.M)</th>
<th>G2(Night hours) (12 P.M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (µmol/l)</td>
<td>1.56±0.057 B</td>
<td>2.33±0.033 A</td>
</tr>
<tr>
<td>MDA (µmol/l)</td>
<td>0.14±0.005 B</td>
<td>0.66±0.03 A</td>
</tr>
<tr>
<td>SOD Activity(U/ml)</td>
<td>19.62±0.288 B</td>
<td>21.18±0.338 A</td>
</tr>
<tr>
<td>B-endorphin (pg/ml)</td>
<td>53.23±1.12 B</td>
<td>84.36±1.36 A</td>
</tr>
<tr>
<td>ACTH (pg/ml)</td>
<td>52.80±0.37 B</td>
<td>71.03±0.16 A</td>
</tr>
<tr>
<td>Cortisone (nmol/L)</td>
<td>11.80±0.57 B</td>
<td>24.46±0.38 A</td>
</tr>
<tr>
<td>Serum PCC (nmol/mg/prot)</td>
<td>0.52±0.023 A</td>
<td>0.48±0.065 A</td>
</tr>
<tr>
<td>Brain PCC (nmol/mg/prot)</td>
<td>0.40±0.057 A</td>
<td>0.50±0.057 A</td>
</tr>
<tr>
<td>Adrenal PCC (nmol/mg/prot)</td>
<td>0.23±0.033 A</td>
<td>0.26±0.033 A</td>
</tr>
</tbody>
</table>

-Values are presented as Means ± SE (n = 5 rabbits /group).
- The different capital letters refer significant differences between groups within one row at (P≤0.05).

This study clearly demonstrates that there is diurnal changes in serum oxidative-antioxidant
components in rabbits, as a result, a significant increase in GSH and SOD activities at night compared to daytime. Moreover, we found a significant increase in MDA and there were no significant differences in PCC.

As by-products of many endogenous and exogenous sources, including UV light, radiation, neutrophil activity, metabolism and oxygen free radicals are produced (14). In the present study, GSH and SOD activity were highest during night time than during day time. Although, the literature lacks a direct discussion for such changes but we could explain that depending on fact mentioned before that there is more ROS production during day light hours than night due to UV light, radiation, so that antioxidants may be lowered to maintain the balance between oxidant and antioxidant production.

Daily rhythmicity in SOD activity was first stated by (15) that SOD activity peaks in the dark phase in the rat cerebral cortex, coinciding with the peak level of malondialdehyde, a lipid peroxidation product. Furthermore, (16) mentioned that the development peaks of glutathione peroxidase in rodent brain and liver in a circadian way and clarified that this event is powered by melatonin and is most likely temporarily coordinated to explicitly scavenge reactive oxygen species (ROS) as the abundance peaks of ROS just before the production of glutathione peroxidase reaches its highest.

On the other hand, at night hours, we observed an increase in MDA. Circadian rhythmicity could be seen in many physiological processes, such as body temperature, exercise, sleep, metabolism, heart rate, blood pressure, secretion of hormones and neurotransmitters (17). Interestingly, many antioxidant enzymes oscillate in a circadian fashion and with a profile that reflects the metabolic requirements of a specific tissue. For example, the oxidative metabolism in the brain of rodents was found to be highest during the dark phase (18), this is associated with the high serum lipid peroxides in this study. Moreover the genes associated with metabolism also showed differences in circadian expression (19). In this way, the cellular metabolism is greatly affected by mitochondrial protein rhythmic development and behavior, which explains why the rise in MDA in our experiment may be triggered by the accumulation of oxidative agents.

In the current research, the diurnal circadian rhythm of serum ACTH, cortisone and β-endorphin is in accordance with (20) that rabbits housed in a regulated laboratory colony secrete both corticosterone and cortisol in an acircadian rhythm that peaks in the afternoon and reaches a nadir at 0600 h. This period is around 12 hours out of time with the circadian rhythm of human glucocorticoid.

Rovirosa et al. (2005) recorded that the peak levels of cortisone occur at the time of feeding, and there was an anticipatory elevation of cortisone several hours prior to the initiation of feeding (21).

The release of the opioid peptide β-endorphin was also increased significantly at night hours as compared to light hours in rabbit
However, the available literatures are not enough to explain this result at the moment.

Dent et al. in 1981 found variation of diurnal rhythm of plasma β-endorphin in 10 studied healthy men were kept in a sleep laboratory setting for a 24-h period. They found a clear diurnal variation of β-E with lowest levels at night between 2200 and 2400 h and highest levels at day between 0400 and 1000 h. (22).

In addition, Esel et al. (2001) explained in their study an increase in plasma levels of β-endorphin, which was synchronous with an increase in ACTH and cortisol during early and late alcohol withdrawal, since β-endorphin and adrenocorticotropic hormone (ACTH) are derived from the same precursor molecule (proopiomelanocortin), so changes can be expected (23). Our observation has shown that an increase in the amount of β-endorphin in rabbit serum at night is correlated with an increase in ACTH relative to its concentrations during daytime hours.

The data obtained in the table reveals a non significant variation of protein oxidation marker involved protein carbonyl content (PCC) in serum and tissues include brain and adrenal gland between night and day time. It is obvious that during night hours, there is a decrease in serum PCC coincided with an increase in tissues which is exactly opposite to their concentration during day hours.

The ROS has caused oxidative damage to the carbonyl derivatives of amino acid residues such as lysine, proline, threonine and arginine (24). Thus, the formation of PCC due to high production of ROS means imbalance between oxidants and antioxidant. Therefore, we concluded that the PCC level does not undergo significant changes between periods (night and day) may be attributed to the existing balance between oxidizing agents and antioxidants in serum and tissues (brain and adrenal gland). However, an extensive research is needed to explain these points.

**Conclusion**

This study concluded that the oxidant-antioxidant imbalance contributes to such variance between day and night hours due to activities and metabolism and the normal values of β-endorphin, ACTH and cortisone in male rabbits during night and day hours. To reduce and overlap such stress, extensive research is needed.

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