SEASONAL VARIATIONS IN MARKERS OF STRESS AND OXIDATIVE STRESS IN RATS

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ABSTRACT

High ambient temperature has been reported to increase oxidative stress by increasing lipid peroxidation and decreasing antioxidant defence in transition dairy cows. It is also known to cause an increase in plasma cortisol levels in goats, European hedgehog and human volunteers. High levels of glucocorticoids have been reported to decrease blood glutathione and erythrocyte superoxide dismutase activity in rats. Although institutional animal houses in research laboratories of developed countries maintain animals in air-conditioned rooms at constant temperature, the same is not true of animal houses in the developing countries especially those belonging to smaller institutions and this could affect the results of the experiments being conducted on these animals. The present research study was done to assess the effects of seasonal variations on the status of erythrocyte oxidative damage, antioxidant defence and plasma cortisol levels in adult female Wistar rats. Rats were kept in their home cages and were left in non-air-conditioned procedure rooms in two different seasons, Hot season (March-May) and Cool season (June to September). Erythrocyte Thiobarbituric acid reactive substances and plasma cortisol levels were significantly increased in rats exposed to high ambient temperature and humidity of the hot season as compared to the rats of the cool season. Erythrocyte reduced glutathione levels, erythrocyte glutathione peroxidase and superoxide dismutase activities were significantly decreased in the hot season group of rats. The results of our experiments showed that exposure of adult female Wistar rats to high ambient temperature and humidity of the hot season increases neuroendocrine stress, oxidative stress and decreases antioxidant defence in them.

KEY WORDS

Cortisol, Temperature, Oxidative stress, Season, Antioxidants

INTRODUCTION

Reactive oxygen species (ROS) produced during aerobic metabolism are known to cause oxidative damage to macromolecules (1). Malondialdehyde (MDA) is produced during the ROS mediated peroxidation of polyunsaturated fatty acids and is a widely used marker of oxidative stress. Reduced glutathione, glutathione peroxidase and superoxide dismutase form a part of the antioxidant defence systems produced by the body to protect the cellular constituents from the damages

caused by ROS (2). A disturbance in the prooxidant/antioxidant balance in favour of the prooxidants leading to potential damage is known as oxidative stress (3). Neuroendocrine stress is known to increase oxidative stress. Emotional stress is known to increase the plasma levels of MDA, which has been reported to directly correlate with the severity of emotional stress in human beings (4). Stress is also known to cause an increase in glucocorticoid levels. High levels of glucocorticoids have been reported to decrease blood glutathione and erythrocyte superoxide dismutase activity in rats (5).

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While institutional animal houses in research laboratories of developed countries are well established with animals being maintained in air-conditioned rooms at a constant temperature of 25°C, many of the animal houses in the developing countries especially those belonging to smaller institutions lack air-conditioning facilities. As a result, experimental animals are exposed to varying ambient temperatures during different

seasons of the year. This could affect the results of the experiments being conducted on these animals. Bernabucci et al have reported higher erythrocyte thiobarbituric acid reactive substances (TBARS) in transition dairy cows during summer (6). Hao et al, in their studies on pilots have found that heat environments induce increased lipid peroxidation and decreased antioxidant ability in erythrocytes (7). Seasonal and ambient temperature dependent variations have also been shown in cortisol levels in the plasma of goats (8), European hedgehog (9), healthy women (10) and male volunteers (11). A few reports are also available suggesting ambient-temperature dependent changes in markers of stress and oxidative stress in rats. Shido et al (12) have found that plasma cortisol level was higher in heat acclimated rats exposed to an ambient temperature of 33°C for 5 hours for 16 consecutive days. Miura et al (13) have reported that lipid peroxidation occurred temperature dependently between 27°C and 42°C in rat brain homogenates. However, to our knowledge, not much work has been done to study the variations in neuroendocrine stress, oxidative stress and antioxidant profile in different seasons of the year in rats, although rats are the most commonly used laboratory animals in research.

The objectives of the present study were to assess the effect of different seasons on the status of (i) Neuroendocrine stress as reflected by plasma cortisol levels, (ii) Membrane lipid peroxidation in erythrocytes measured as TBARS and (iii) Antioxidant defence in erythrocytes using the levels of reduced glutathione and activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) as markers in adult female Wistar rats.

MATERIALS AND METHODS

Female Wistar albino rats, *Rattus norvegicus*, of 4-5 months age, weighing 150-225 g were used in this study. Rats were maintained in their home cages left in procedure rooms without air-conditioning facilities. Standard pelleted food and water was provided *ad libitum* to all the animals. Proper ventilation was provided. Standard hygienic conditions were maintained in the animal house and the rats were exposed to proper light and dark cycle (12 hours each of light and darkness). The experimental protocol was approved by the Institutional Animal Ethics Committee.

The experiment was carried out from the beginning of March till the end of September in Manipal, a coastal town in the southern part of India (74°53' East, 12°55' North). Manipal is recorded to have a hot and humid climate between March and May with average temperatures ranging from 33-35°C

and average humidity ranging from 60-70%. Wet and cool climate is recorded between June and October with average temperatures ranging from 26-29°C and average humidity ranging from 80-90%. There is no clear-cut winter in this region but southwest monsoons are heavy during the months of June to September with an average rainfall of 260-300 inches/year. The first group of rats was sampled for estimation of stress parameters between the months of March and May and were categorized as the Hot Season group, (HS; n=12). The average temperature of the well ventilated room in which the animals were kept, matched with the atmospheric temperature and ranged from 33-35°C. Likewise, the average humidity of the room matched with that of the atmosphere and was found to range from 60-70%. The second group of rats sampled between the months of June and September belonged to the Cool Season group, (CS; n=19). The average temperature of the room matched with the atmospheric temperature and ranged from 26-29°C and the average humidity of the room matched with that of the atmosphere and was found to range from 80-90%. Rats of both groups were left in the non-air-conditioned procedure room for 14 days during the respective seasons at the end of which they were sampled. Blood was obtained from both the groups of rats in oxalate coated tubes between 9.00 a.m. and 10.00 a.m. on the 15th day. The blood obtained was centrifuged to separate the plasma and cells. Packed red cells were washed three times in ice-cold phosphate buffered saline (PBS-phosphate buffer 0.01M, pH 7.4, containing 0.15 M NaCl) and used for the estimation of TBARS and reduced glutathione levels. An equal volume suspension of erythrocytes in PBS was prepared and used for assay of glutathione peroxidase activity and superoxide dismutase activity. Plasma was used for the estimation of cortisol.

Packed red cells (0.2 ml) were used for the estimation of MDA as TBARS by the method of Jain et al (14). TBARS was expressed as nanomoles/gram haemoglobin. Haemoglobin concentration was measured by cyanmethaemoglobin method of Drabkin (15). Reduced glutathione (GSH) level in rat erythrocytes was measured by the method of Beutler et al (16) and expressed as mg/g Hb. The erythrocyte suspension in PBS was used for the assay of GSHPx by the method of Paglia et al (17). Values were expressed as units of enzyme activity/gram haemoglobin.

Inhibition of the reduction of nitro blue tetrazolium (NBT) by superoxide radicals, generated by the illumination of riboflavin in the presence of oxygen and the electron donor methionine was used as the basis for the assay of SOD (18). Values were expressed as units of enzyme activity/gram haemoglobin.

Plasma cortisol was estimated by the fluorimetric method of Gowenlock et al (19) and was expressed as nanomoles/litre of plasma

All statistical analysis were done using the two tailed unpaired t-test, linear regression and correlation in the Graph Pad Instat (GPIS) package.

RESULTS

The results for the various measurements are shown in Table 1. High levels of erythrocyte TBARS ($p<0.01$) and plasma cortisol ($p<0.05$) were observed in HS group of rats as compared to the CS group. All parameters of antioxidant defence were lower in the HS group as compared to the CS group (erythrocyte GSH, $p<0.01$; erythrocyte GSH-Px activity, $p<0.01$ and erythrocyte SOD activity, $p<0.001$). There was a significant positive correlation of erythrocyte GSH levels with erythrocyte activity of GSH-Px ($r=0.85$, $p<0.001$) and erythrocyte GSH-Px activity with that of erythrocyte SOD ($r=0.59$, $p<0.05$) in HS group.

Table 1 : Parameters of neuroendocrine stress, oxidative stress and antioxidant defence in rats in the hot (HS) and cool (CS) seasons (values are Mean $\pm$ SD)

	HS (n=12)	CS (n=19)	p value
Plasma Cortisol (nmol/l)	4473.96 $\pm$ 1060.03	3663.53 $\pm$ 986.21	<0.05
Erythrocyte TBARS (nmol/g Hb)	7.19 $\pm$ 1.18	6.09 $\pm$ 0.9	<0.05
Erythrocyte GSH (mg/g Hb)	1.84 $\pm$ 0.43	2.4 $\pm$ 0.5	<0.01
Erythrocyte GSH-Px (U/g Hb)	70.94 $\pm$ 10.79	80.12 $\pm$ 8.13	<0.01
Erythrocyte SOD (U/g Hb)	2145.47 $\pm$ 635.38	3172.02 $\pm$ 863.2	<0.001

DISCUSSION

The results of the present study confirm occurrence of increased stress and oxidative stress in the hot season. Although other seasonal factors may affect the oxidative stress status of hot season rats and cool season rats, previous studies on other species have indicated that seasonal alterations of the oxidative stress depend more on weather conditions such as temperature (20). Lamba et al (21) have reported circannual variations in plasma level of cortisol and have correlated the steroid rhythms with environmental factors such as photoperiod, temperature and rainfall. In this study, the role of

photoperiod as an influencing factor is ruled out since both the groups of rats were exposed to similar durations of light/dark cycle. It seems possible that temperature and humidity have been the main factors in influencing the stress status. Calamari et al (22) found an increase of plasma TBARS and a decrease in plasma lipid soluble antioxidants in moderately heat stressed, mid-lactating cows during summer. Bernabucci et al have reported higher erythrocyte TBARS in transition dairy cows exposed to moderate heat stress due to summer temperature humidity index (6). Harmon et al (23) reported the reduction of antioxidant activity of plasma in midlactating cows exposed to a chamber temperature of 29.5°C. The variations in erythrocyte antioxidant levels and plasma cortisol levels in our study agrees well with those reported by Harmon et al and Shido et al (12). Miura et al (13) have reported that the temperature dependent lipid peroxidation observed by them is caused by endogenous iron which may bind to cell components. In the light of this and previous report (24), it can be said that the high polyunsaturated fatty acid content of erythrocyte membrane and the high iron content of haemoglobin in association with the high ambient temperature may have resulted in increased lipid peroxidation in HS group of rats as reflected by the increase in erythrocyte TBARS in these rats. We have found a lowered antioxidant defence status in erythrocytes of HS group of rats as indicated by lower levels of GSH and decreased activities of GSH-Px and SOD. One of the most important non-enzymatic protection against ROS-induced lipid peroxidation involves the glutathione pathway. Constant use of GSH as a result of lipid peroxidation results in a decrease in its levels. This is very well reflected in the HS group of rats that show higher erythrocyte TBARS and lower erythrocyte glutathione levels. The lowering of GSH in erythrocytes has resulted in the associated decrease of GSH-Px activity as reflected by the significant positive correlation between erythrocyte GSH and GSH-Px activity. High levels of glucocorticoids have been reported to decrease blood glutathione and erythrocyte superoxide dismutase activity in rats (5). The increase in plasma cortisol in response to high ambient temperature in hot season could be an additional factor responsible for increasing the oxidative stress in the HS group of rats as reflected by the decreased GSH level and SOD activity in their erythrocytes.

In conclusion, it can be said that the high atmospheric temperature and humidity of summer increases neuroendocrine stress and lipid peroxidation which in turn contributes to the reduced erythrocyte antioxidant response. These findings are important as they suggest that any study of antioxidants and oxidative stress in laboratory rats must take into consideration seasonal variations in their levels for

accurate assessment of changes due to pathological conditions.

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