IJPSR (2010), Vol. 1, Issue 9 (Suppl.) (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 May, 2010; received in revised form 31 July, 2010; accepted 13 August, 2010

A STUDY ON THE EFFECT OF ISOBARIC HYPOXIA ON ANTIOXIDANT ENZYME ACTIVITY AND LIPID PEROXIDATION IN RAT BRAIN

Manju Antony*[1] and T. J. James [2]

Department of Life sciences, Kristu Jayanti College [1], Bangalore (Karnataka), India
Department of Zoology, Sacred Heart College [2], Thevara (Kerala), India

Keywords:

Hypoxia,

Reoxygenation,

Peroxidation,

Hippocampus,

Correspondence to Author:

Manju Antony

Department of Life sciences, Kristu Jayanti College, Bangalore (Karnataka), India

E-mail: manju_antony@hotmail.com

ABSTRACT

In the present work, an attempt was made to study the effect of isobaric hypoxic hypoxia (10%) on rat brain. Since literatures support generation of free radicals during post ischemic/hypoxic period, here hypoxia followed by reoxygenation is dealt with as the single problem. Subsequently, as brain shows regional variations in its function and metabolic activities an attempt was made to study effect of hypoxia reoxygenation in five brain regions i.e. cerebrum, cerebellum, medulla, hippocampus and hypothalamus. The changes in the antioxidant defense enzymes such as superoxide dismutase, catalase and glutathione peroxidase activity and lipid peroxidation levels in brain associated with hypoxia-reoxygenation was analyzed in this study. Antioxidant enzyme activity and lipid peroxidation levels showed a significant ($P< 0.0001$) regional variation among the five brain regions chosen for the study. Interestingly, the hippocampus region of the rat brain depicted the lowest SOD and GPx activity and highest level of lipid peroxidation. Hypoxia (10%) for 3 hours brought about significant decrease ($P<0.05$) in the activities of superoxide dismutase, catalase and glutathione peroxidase in rat brain regions. After hypoxia, hippocampus region showed a highly significant ($P<0.0001$) reduction in SOD activity and GPx activity and highest rise in lipid peroxidation levels. Thus the hippocampus region was found most vulnerable to hypoxia. Among the three enzymes, most conspicuous decrease was shown by superoxide dismutase. The present study revealed that 3 hours of hypoxia followed by reoxygenation increased lipid peroxidation levels in the rat brain.

INTRODUCTION: The pathological state of the organism caused by inadequate oxygen supply to tissues and organs or by disturbance of utilization in them has acquired in the literature the name of hypoxia. The term covers the entirely vast set of processes in the organism brought about by oxygen deficiency as the leading pathogenic factor. There are several classifications of the form of oxygen deprivation, among which most writers single out hypoxic, circulatory and histotoxic hypoxia. Being a composite part of the problem of oxygen deprivation in the oxygen deprivation in the organism as a whole, hypoxia of the brain is singularly important, since the brain is an organ most vulnerable to deficit of oxygen.

Though brain constitutes only a small fraction of total body weight (in humans about 2%) it is a highly oxidative organ and accounts for disproportionately large percentage of bodily oxygen consumption (in humans about 20%). Yet the partial pressure and concentration of oxygen in brain are low and non- uniform with major variations between closely adjacent areas. The great variability in local oxygen tension from one area of the brain to another may be further exaggerated by sudden changes in neuronal activity or under pathological conditions.

As reported by earlier works, the brain oxygen decreases very rapidly with a reduction in its supply either through a decrease in tension of the inspired gas (hypoxic hypoxia and consequent tissue hypoxia) or by a limitation of blood delivery (circulatory hypoxia/ischemia and consequent tissue hypoxia). Disturbances in cerebral circulation as well as cardiovascular and respiratory disorders subject the tissues to the condition of hypoxia. Limitation of oxygen supply to the brain below a 'critical' level reduces, and eventually blocks oxidative phosphorylation, drastically decreases cellular ATP and leads to collapse of ion gradients, which eventually leads to cessation of neuronal activity and death. In the immediate post hypoxic or post ischemic period, the reactive hyperemia temporarily augments tissue oxygen levels to values, which reach much higher than that in normal brain, at a time when neuronal activity is severally depressed. It is at this tenure the free radicals or reactive oxygen species are rapidly generated in the brain that accounts for most of the pathological conditions associated with oxidative stress.

Reactive oxygen species generated in body organs including brain comprise superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (.OH). Extremely high level of interest conferred recently on reactive oxygen species in neurobiology arises from a substantial body of information which suggest that they can be involved in post-hypoxic, post-ischemic and post-traumatic brain damage, in the ageing process and in the pathogenesis of several neurodegenerative disorders, Parkinson's disease and Alzheimer's disease [1]. The various reactive oxygen species exhibit different degrees of toxicity against their targets, which include lipids, proteins, nucleic acids and even carbohydrates.

Lipid peroxidation and formation of peroxide radical and aldehydes damages membranes, membrane-bound enzymes and receptors. Attacks on proteins may need to their unfolding, fragmentation and polymerization while damage to DNA can result in mutations or activation of certain enzymes. Antioxidant defense mechanism in the body involving defense enzymes superoxide dismutase, catalase and glutathione peroxidase; and other antioxidants like alpha-tocopherol and reduced glutathione plays an important role in keeping these deleterious radicals under check during normal conditions. An increase in the reactive oxygen species during post ischemia/hypoxia

might have altered the equilibrium between the between free radical generation and its scavenging system leading to cell injury. Hence for the same reason most of the studies pertaining to free radical mediated tissue injury involve the study of the activities of the components of defense system. So far most of works pertaining to the effect of oxygen deprivation in brain dealt with *in vitro* experiments which are routinely studied at high oxygen pressures and there is wide variation in the process *in vivo* which usually occurs normally under low ambient PO_2.

The present challenge is to explore at the level of intact organs, tissues, which have been investigated only in isolated cells. Accordingly the present work is an attempt to study the effect of isobaric hypoxic hypoxia on brain *in vivo*. Unlike works on cerebral ischemia, the present work dealt with effect of oxygen deprivation alone and did not accompany substrate deficiency in tissues. In the present work, an attempt was made to study the effect of isobaric hypoxic hypoxia (10%) on rat brain.

Since literatures support generation of free radicals during post ischemic/hypoxic period, here hypoxia followed by reoxygenation is dealt with as the single problem. Subsequently, as brain shows regional variations in its function and metabolic activities an attempt was made to study effect of hypoxia reoxygenation in five brain regions ie cerebrum, cerebellum, medulla, hippocampus and hypothalamus. The changes in the antioxidant defense enzymes such as superoxide dismutase, catalase and glutathione peroxidase activity and lipid peroxidation levels in brain associated with hypoxia-reoxygenation was analyzed in this study.

MATERIAL AND METHODS:

Materials: Experiments were performed on male wistar rats (*Ratus nonvergicus*), obtained from the inbred colony of the animal house of Department of Nutrition and Biochemistry, Central institute of Fisheries Technology (CIFT), Kochi. Male rats of 3 months old were used for the experiment. The rats weighing 150-180g were kept in polypropylene cages of size 43.5x29.0x16cm and maintained on standard pellet diet and water *ad libitum*. The cages were maintained in a well-ventilated room at room temperature of 28+/-2^{o} C.

Experimental Design: Only healthy male rats were used for the experiments. A total of 80 rats of 3 months weighing 150-180 gms were used for the study. Rats subjected to 10% isobaric hypoxia in the chamber followed by reoxygenation formed the hypoxia experimental group. After hypoxia for duration of 3 hours the rats were kept in 21% oxygen for 30 minutes. The normal control condition referred to healthy rats of same age group reared under optimum environmental conditions and fed *ad libitum*.

Experimental Hypoxia: A gadget for creating hypoxia was fabricated by modifying an inhalation chamber with an outlet and two inlets one for the entry of O_2 and the other for N_2. A gauge cum regulator, which monitors the volume and pressure of gases entering the chamber, controls the flow of gases through the inlet (**Fig. 1**). During the experiments rats were placed inside the chamber and by controlling the flow of N_2 and O_2. The amount of O_2 in the chamber is maintained to 10%.

FIG. 1: A GADGET FOR CREATING HYPOXIA, CONSISTING OF GLASS CHAMBER IN WHICH OXYGEN CONCENTRATION MAINTAINED AT 10% UNDER ISOBARIC CONDITION BY CONTROLLING THE FLOW OF N_2 AND O_2

Methods:

Biochemical Assays: After subjecting to hypoxia rats were anesthetized with ketamin (0.5 ml). The whole brain was removed by opening the cranium. The brain was washed in 0.90% cold saline and immediately weighed in a single pan electric balance. The brain is then regionalized into cerebrum, cerebellum, medulla, hippocampus and hypothalamus. Each region is weighed separately and 10% homogenate was prepared using phosphate buffer of pH 7.4. Homogenates are then centrifuged in a REMI refrigerator centrifuge. Following procedures were adopted for the biochemical analysis for:

Superoxide dismutase: Marklund and Marklund (1974) [2]

Catalase: Kar and Mishra (1976) [3]

Glutathione peroxidase: Hafeman *et al* (1974) [4]

Lipid peroxidation: Placer *et al* (1966) [5]

Statistical Analysis: The statistical package SPSS PC+ (statistical package for social science, version 4.0.1) was used for statistical analysis. Mean and standard deviation were estimated from the sample for each study group. Mean values were compared by Student's independent t-test or one - way analysis of variance appropriately. Multiple Range Test by Tukey HSD (Honestly Significant Difference) procedure was employed to identify the significant groups, if P- value in one-way ANOVA is significant. All variables were tested for normality assumption within each group by using Kolmogrove Smirnove non parametric procedure before undertaking the test of significance. In the present study, $P<0.05$ was considered as the level of significance.

RESULT:

Regional variation in antioxidant enzyme activity in the rat brain: Prior to the study of the effect of hypoxia, an attempt was made to analyze the regional variations in the superoxide dismutase, catalase, glutathione peroxidase activity and lipid peroxidation levels in the rat brain under normal control conditions. SOD, CAT, GPx activity and MDA content showed a highly significant ($P<0.0001$) regional variation in its activity among cerebrum, cerebellum, medulla, hippocampus and hypothalamus regions (**Table 1**). The highest mean SOD activity was noticed in the cerebrum and the lowest in the hippocampus in the rat brain. The highest mean CAT activity in the hippocampus region was found to be significantly higher ($P<0.05$) than that in cerebrum, cerebellum, medulla and hypothalamus. Further, the lowest CAT activity was depicted in the cerebrum. The highest mean GPx activity was noticed in the cerebellum and the lowest in the hippocampus region of the rat brain. The mean MDA content in the cerebrum and hippocampus was found to be significantly higher than mean MDA in the hypothalamus, medulla and cerebellum ($P<0.05$). Interestingly, the hippocampus region of rat brain depicted the lowest SOD and GPx activity and the highest level of lipid peroxidation.

Table 1: REGIONAL VARIATION IN ANTIOXIDANT ENZYME ACTIVITY AND LIPID PEROXIDATION IN RAT BRAIN

Brain regions	SOD activity (expressed in Units/mg protein/ml) (n=12) Mean +/-SD	Catalase activity (expressed in micromoles of H_2O_2 decomposed/mg protein /ml/minute) (n=12) Mean +/-SD	Glutathione peroxidase activity (expressed in Units/mg protein /ml) (n=12) Mean +/-SD	Malondialdehyde content (expressed in n moles/gm wet tissue) (n = 12) Mean +/-SD
Cerebrum	10.12+/-0.59	5.23+/-0.30	10.38+/-0.77	285.32+/17.60
Cerebellum	8.16+/-0.46	6.38+/-0.34	12.83+/-0.79	266.70+/-11.69
Medulla	7.21+/-0.42	7.78+/-0.38	8.89+/-0.61	255.98+/-12.21
Hippocampus	6.42+/-0.45	9.19+/-0.47	6.86+/-0.43	284.22+/-12.19
Hypothalamus	6.93+/-0.43	7.91+/-0.41	7.82+/-0.37	254.97+/-11.93

Effect of isobaric hypoxia on the enzyme activity and MDA content in the rat brain: Hypoxia for 3 hours brought about a significant (P<0.05) reduction in the brain SOD activity in the rats (**Table 2**). In the rat brain, the hippocampus depicted the highest decrease (21.3 %) in SOD activity (P<0.0001) (Table 2). The hypothalamus and the cerebrum showed 17% and 15% decrease in the SOD activity respectively. A significant decrease in the CAT activity was noticed in the hippocampus, hypothalamus and cerebrum of the rat brain (Table 2). Hippocampus depicted the maximum decrease i.e. 13.19%. The CAT activity in the cerebrum and the hypothalamus showed 9.6% and 7.7% decline respectively (Table 2). The brain glutathione peroxidase activity decreased significantly (P<0.05) in all the 5 brain regions of the rat brain (Table 2). The highest decrease was noticed in the hippocampus region (16%). The hippocampus showed a 17% increase in MDA content followed by cerebrum (15%), hypothalamus (12%), cerebellum (12%) and medulla (12%), (Table 2) after hypoxia for 3 hours.

TABLE 2: VARIATION IN ANTIOXIDANT ENZYME ACTIVITY AND LIPID PEROXIDATION IN RAT BRAIN AFTER HYPOXIA

Brain regions	SOD activity (expressed in Units/mg protein/ml) (n=12) Mean +/-SD		Catalase activity (expressed in micromoles of H_2O_2 decomposed/mg protein /ml/minute) (n=12) Mean +/-SD		Glutathione peroxidase activity (expressed in Units/mg protein /ml) (n=12) Mean +/-SD		Malondialdehyde content (expressed in n moles/gm wet tissue) (n = 12) Mean +/-SD	
	Control	**Experimental**	**Control**	**Experimental**	**Control**	**Experimental**	**Control**	**Experimental**
Cerebrum	10.11+/-0.62	8.63+/-0.56* (14.7%↓)	5.22+/-0.31	4.71+/-0.29* (9.7%↓)	10.38+/-0.81	9.30+/-0.62* (10.4%↓)	285.51+/-12.36	329.28+/-16.27** (15.3%↑)
Cerebellum	8.16+/-0.48	7.38+/-0.46* (9.6%↓)	6.39+/-0.36	6.05+/-0.35(NS) (5.2%↓)	12.84+/-0.76	11.59+/-0.64* (9.8%↓)	266.63+/-12.19	298.86+/-12.86* (12.1%↑)
Medulla	7.20+/-0.45	6.53+/-0.43* (9.4%↓)	7.78+/-0.41	7.46+/-0.39(NS) (4.1%↓)	8.90+/-0.60	7.95+/-0.50* (10.7%↓)	255.74+/-13.06	286.54+/-11.49* (12%↑)
Hippocampus	6.42+/-0.47	5.06+/- 0.46** (21.3%↓)	9.20+/-0.49	7.99+/-0.46* (13.2%↓)	6.87+/-0.44	5.76+/-0.33* (16.1%↓)	283.94+/-12.95	331.88+/-17.83** (16.9%↑)
Hypothalamus	6.93+/-0.44	5.73+/-0.44* (17.2%↓)	7.92+/-0.44	7.31+/-0.33(NS) (7.7%↓)	7.88+/-0.37	6.80+/-0.57* (13.9%↓)	254.97+/-12.51	285.98+/-12.10* (12.2%↑)

Control young Vs Experimental young: ***** denotes P<0.05 (significant) and ****** denotes P<0.0001 (highly significant); Figures in parentheses show the percentage increase (↑) in the experimental group as compared to respective regions in the control

DISCUSSION: In the recent years, free radical pathology as it pertains to hypoxic/ischemic conditions in tissues has been the subject of renewed interest in several investigative laboratories. Since the brain is an organ most vulnerable to deficit of oxygen [6], the investigator focused on the effect of hypoxia in this organ. Though much work has been done on the effects of cerebral ischemia, the effect of hypoxic hypoxia on the brain were less explored. So far the studies on the effect of hypoxic hypoxia on neurons were confined to *in vitro* experiments.

The present work is a fresh attempt made to study the effect of hypoxic hypoxia *in vivo*. In this work hypoxia (isobaric) of 10% was chosen as it was found that, at oxygen concentration below 10% the rats were less viable and an oxygen concentration above this level had no effect on any of the parameters analyzed in rat brain. The study of effects of hypoxic hypoxia in brain attains relevance, as this situation is associated with respiratory and cardiovascular disorders and most importantly during childbirth. Superoxide dismutase, catalase and glutathione peroxidase, which forms the main defense against free radicals, has been the focus of study in most of the experiments pertaining to the analysis of hypoxic/ischemic injury.

Their results suggests major fluctuations in the activity of these enzymes which in turn is found to be responsible for the rise in lipid peroxidation levels and other pathological conditions associated with oxidative injury. And hence for these reasons investigator chose to study the fluctuations in these enzyme levels in rat brain under hypoxia. Lipid peroxidation levels in rat brain were also analyzed as it form the basis of most of the pathological changes associated with hypoxic/ischemic oxidative injury. Prior to the study of effect of hypoxia on rat brain, an attempt was made to study the regional variations in the activity of these enzymes in rat brain. Several workers [7, 8, 9, 10] has reported a differential pattern in the expression of antioxidant defense enzymes between and within organs, which they presumed correlated with metabolic profile, function and disease vulnerability of the different cell types. In the present investigation, a highly significant regional variation was observed in the activities of superoxide dismutase, catalase and glutathione peroxidase among cerebrum, cerebellum, medulla, hippocampus and hypothalamus regions of the rat brain.

However, these enzymes did not show any uniformity in the pattern of regional variation. When hippocampus showed lowest SOD activity, a highest catalase activity was recorded in this region. Cerebrum and cerebellum showed high SOD activity, whereas lowest catalase activity was depicted in the cerebrum. Glutathione peroxidase showed maximum activity in cerebrum and lowest in hypothalamus. It is interesting to note that all the defense enzymes showed a similar pattern of regional variation in the rat brain. Whether the regional variation in the activities of defense enzymes in rat brain correlate with metabolic activities of that particular region is open for further analysis.

However, studies [11, 12] also proved absence of any correlation between SOD expression in different brain regions and its function or disease vulnerability. But they suggested a correlation between the lowest superoxide dismutase activity in hippocampus region (Sommer's sector) and its vulnerability to hypoxic/ischemic injury. In accordance with their observation the present study also found hippocampus region more susceptible to hypoxia, which in turn can be due to its low SOD activity. Hypoxic hypoxia dealt with in the present study

must be completely demarcated from widely studied cerebral ischemia. Though both conditions subject the brain tissue to a condition of oxygen deficiency, in the cerebral ischemia oxygen deprivation is supplemented by substrate deprivation. However cerebral pathology associated with ischemia and hypoxia is attributed to the free radicals induced oxidative damage. Most reports [13, 14, 15] support generation of free radical during the reoxygenation phase following hypoxia/ischemia rather than hypoxia alone. But there are also observations supporting the generation of free radicals even during hypoxic phase [16].

In the present work, hypoxia followed by reoxygenation is dealt with as single problem. Hypoxia for 3 hours brought about significant decrease in the activities of superoxide dismutase, catalase and glutathione peroxidase in rat brain regions. Among the three enzymes, most conspicuous decrease was shown by superoxide dismutase. Though, glutathione peroxidase also showed significant decrease in its activity in all regions, the decrease in brain catalase activity following hypoxia was less pronounced. A feeble response of catalase to hypoxia might due to the comparatively smaller role played this enzyme in brain[17]. In brain, glutathione peroxidase that performs the identical function (removal of H_2O_2) to that of catalase is reported to be more prominent than its counterpart [18].

In agreement with the present reports most of the works on the effect of ischemia/reperfusion also report a decrease in the activities of antioxidant defense enzymes in various organs [19]. It seems that these enzymes are related in such a way that the decrease in the activity of a particular enzyme will also decrease the functioning efficiency of the others. It has been reported that O_2^- has an inhibitory effect on the catalase and glutathione peroxidase, which can be removed by superoxide dismutase [20]. Thus decrease in SOD activity will affect glutathione peroxidase and catalase and impaired functioning of either glutathione peroxidase or catalase will in turn affect SOD activity. The present work revealed accelerated lipid peroxidation levels in rat brain following hypoxia-reoxygenation. So our results on the effect of hypoxia on antioxidant defense system suggests a down regulation of antioxidant defense enzymes during hypoxia/reoxygenation which is not sufficient to meet the increased demand to overcome the free radical onslaught.

Role of free radicals in initiating lipid peroxidation is a widely accepted phenomenon. Lipid peroxides could be detected in brain as early as 30 minutes after 10 minutes of ischemia and degree of lipid peroxidation increased with time [21]. Brain with its high lipid content, comprising phospholipids as the main component forms an easy target to lipid peroxidation. The present study revealed that 3 hours of hypoxia followed by reoxygenation increased lipid peroxidation levels in the rat brain. It has been reported that during hypoxic/ischemic condition in tissues, activation of phospholipases especially phospholipase A_2 possibly triggered by Ca^{2+} will lead to the accumulation of free fatty acids [22].

These fatty acids especially unsaturated fatty acids form an easy target for lipid peroxidation [23]. Following rapid influx of oxygen during reoxygenation, free radicals will be generated which in turn initiate lipid peroxidation. However, in normal aerobic tissue, initiation and propagation of the lipid peroxidation is kept under check by antioxidant defense system [24]. Since the present work reported a decrease in antioxidant defense enzyme activities accompanied by GSH depletion during hypoxia-reoxygenation, the defense

system become insufficient to curb the rapid influx of free radicals in tissues during early reoxygenation phase. The un-proportionate increase in free radicals can initiate lipid peroxidation in rat brain, which in turn leads to a cascade of pathological changes, most important being changes in cell membrane structure and function. This altered cell function can inversely affect antioxidant enzyme activities. Thus this cycle of events continue until the equilibrium between enzymes and free radical damage is restored. This might be a possible explanation for increased lipid peroxidation in tissues following hypoxia-reoxygenation**.**

Based on the fact that regional variation exists in defense enzyme activities in rat brain one may speculate that certain neuronal populations may be more vulnerable to oxidative stress as a result of lower antioxidant protection. As observed in the present study, hippocampus region in the rat brain showed maximum decrease in SOD, catalase and glutathione peroxidase activity following hypoxia-reoxygenation. It correlated with the present observations that hippocampus region depicted lowest SOD and glutathione peroxidase activity even under normal conditions.

Various studies have reported a lowest SOD activity in hippocampus (CA1 sector) region of human brain and they correlated it to selective vulnerability of hippocampus to hypoxia/ischemia [11, 12]. In the present work it is also demonstrated that along with the decrease in superoxide dismutase, glutathione peroxidase and catalase activity in hippocampus region of rat brain, this region showed highest increase in lipid peroxidation levels after 3 hours of hypoxia/reoxygenation. This selective vulnerability of the hippocampus to damage by lipid peroxidation must be aggravated by low amount of glutathione peroxidase, an enzyme that detoxifies hydrogen peroxides and lipid hydroperoxides. Thus finding of the present work gives an explanation for the selective vulnerability of hippocampus to hypoxia-reoxygenation as due to the presence of low amount of antioxidant enzymes /low activity of antioxidant enzymes in this region.

CONCLUSION: The present clearly indicated regional variation in the defense enzyme activities in the rat brain. Interestingly, comparatively low antioxidant enzyme activities (superoxide dismutase and glutathione peroxidase) noticed in the hippocampus region of the rat brain correlated with its selective vulnerability to hypoxia. After 3 hours of hypoxia, significant decrease in the antioxidant defense enzymes (superoxide dismutase and glutathione peroxidase) was noticed along with a corresponding increase in peroxidation levels, which in turn pointing towards a pathological situation in tissues. In the present work it is also demonstrated that along with the decrease in superoxide dismutase, glutathione peroxidase and catalase activity in hippocampus region of rat brain, and this region showed highest increase in lipid peroxidation levels. Thus the hippocampus region of the rat brain is most vulnerable to hypoxia. It can, thus, be concluded that long durations of hypoxia (3 hours) can bring about deleterious biochemical changes in the rat brain leading to oxidative damage.

REFERENCES:

1. Facchinetti F, Dawson VL, Dawson TM: Free radicals as mediators of neuronal injury. Cell. Mol. Neurosci .1998; 18: 667-682.
2. Marklund S, Marklund G: Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 1974; 47:468-473.
3. Kar HI, Mishra BK: quoted from Vohra BPS, Sharma SP Kansal VK Maharishi Amrit Kalash rejuvenates ageing central nervous system's antioxidant defence system: an in vivo study. Pharmacol. Res. 1999; 40(6): 497-502.

4. Hafeman DC, Sundae RA, Hoekstra WG: Effect of Dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J .Nutr. 1974; 104: 580-587.
5. Placer ZA, Cusmann LL, Johnson BC: Estimation of product of lipid peroxidation, malodialdehyde, in biological systems. Analyt. Biochem.1996; 16: 359-361.
6. Astrup J, Siesjo BK, Symon L: Thresholds in cerebral ischemia. The ischemic penumbra. Stroke 1981; 12:723-725.
7. Muse KE, Oberley TD, Sempf JM, Obrley LW: Immuno-localization of antioxidant enzymes in adult hamster kidney. Histochem. J. 1994; 26:734-753.
8. Moreno S, Mugnaini E, Ceru MP: Immunocytochemical localization of catalase in the central nervous system of the rat. J. Histochem. Cytochem. 1995; 43(12):1253-1267.
9. Brannan TS, Maker HS, Weiss C, Cohen G: Regional distribution of glutathione peroxidase in the adult rat brain. J. Neurochem. 1980; 35:1013-1014.
10. Zhang P, Damier P and Nicol A: Preferential expression of superoxide dismutase messenger RNA in melanized neurons in human mesencephalon. Neuroscience. 1993; 55(1): 167-175.
11. Bergeron C, Petrunka C and Weyer L: Copper/Zinc superoxide dismutase expression in the human central nervous system: Correlation with selective vulnerability. Am. J. Pathol 1996; 148:273-279.
12. Ceballos I, Javoy-Agid F, Agid Y: Neuronal localization of copper-Zinc superoxide dismutase protein and mRNA within the human hippocampus from control and Alzheimer's disease brains. Free. Radic. Res .Commun 1991; 12-13pt2:571-80.
13. Hess ML, Manson NH: Molecular oxygen: friend and foe. The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischemia/reperfusion injury. J .Mol. cell. cardiol. 1984; 16(11): 969-85.
14. Hori O, Matsumoto M, Kinoshita T, Kamada T: metabolic and biosynthetic alterations in cultured astocytes exposed to hypoxia/reoxygenation. J. Neurochem. 1994; 62(4): 1489-1495.
15. Lievre V, Bianchi A, Dauca M, Daval JL: Free radical production and and changes in superoxide dismutases associated with hypoxia/reoxygenation-induced apoptosis of embryonic rat fore brain neurons in culture. Free. Radic. Biol. Med 2000; 29(12):1291-1301.
16. Rosenbaum DM, Kalberg J, and Kessler JA: Superoxide dismutase ameliorates neuronal death from hypoxia in culture. Stroke. 1994; 25:857-863.
17. Cohen G: Oxygen radicals and Parkinson's disease, in Halliwell B,ed. Oxygen radicals and tissue injury. Bethesda, Maryland, FASEB, 1988; pp.130-135.
18. Jain A, Martensson J, Stole E, Miester A: Glutathione deficiency leads to mitochondrial damage in brain. Proc. Natl. Acad. USA.1991; 88:1913-1917.
19. Dobashi K, Ghosh B, Orak JK, Singh I, and Singh AK: Kidney ischemia-reperfusion: modulation of antioxidant defenses. Mol. cell. Biochem. 2000; 205 (1-2): 1-11.
20. KonoY, Fridovich I: Superoxide radical inhibits catalase. J. Biol. Chem. 1982; 257: 5751-5754.
21. Gardner M, Nilssn B, Rehncrona S, Siesjo BK: Free fatty acids in the rat brain in moderate and severe hypoxia. J. neurochem. 1981; 36: 1500-1505.
22. Bazan NG: Free arachidonic acid and other lipids in the nervous system during early ischemia and after electroshock. Adv. Exp. Med. Biol. 1976; 72:317-335.
23. Yoshida S, Inoh S, Asano T, Ueta N: Effect of transient ischemia on free fatty acids and phospholipids in gerbil brain. J. Neurosurg. 1980; 53: 323-331.
24. Fridovich I: Biological effects of the superoxide radical. Arch. Biochem. Biophys. 1986; 247:1-11.

****