HEPATOPROTECTIVE INFLUENCES OF MELATONIN ON THE LEVELS OF ANTIOXIDANTS AND LIPID PEROXIDATION IN HYPERAMMONEMIC WISTAR RATS

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 ABSTRACT

The antioxidant potential of melatonin (MLT) on hyperammonemia induced by ammonium acetate treatment was studied in rats. In liver tissue, the levels of thiobarbituric acid reactive substances and lipid profile variables was observed to be increased significantly in ammonium acetate treated rats and decreased significantly in rats treated with melatonin and ammonium acetate. Further, enzymatic, superoxide dismutase, catalase and glutathione peroxidase and non-enzymatic, reduced glutathione antioxidants in liver tissues decreased significantly in ammonium acetate treated rats and increased significantly in rats treated with melatonin and ammonium acetate. These biochemical alterations could be due to the ability of melatonin to (i) scavenge a variety of radicals and reactive oxygen species (ii) induce antioxidative enzymes which reduce steady state levels of reactive oxygen species and (iii) stabilize cell membranes which assist them in reducing oxidative damage and thus could prevent oxidative stress in rats.

1. Introduction:

Ammonia is a catabolic product of protein and nitrogenous compounds that is formed in mammals and humans. At high levels, ammonia is neurotoxic; it affects the functions of the central nervous system, and leads to coma and death (Plum et al. 1976). Hyperammonemia, caused by insufficient removal of ammonia in the liver (Meijer et al.1990) or portacaval shunting (Butterworth et el. 1987), which is responsible for the development of hepatic encephalopathy (Butterworth et al. 1995). Ammonia intoxication impairs mitochondrial function (Kosenko et el. 1997) which could lead to decreased ATP synthesis and increased formation of free radicals (Kosenko et al. 2000). The major toxic effects of ammonia likely involve changes in cellular pH and the depletion of certain citric acid cycle intermediates, in particular α-keto-glutarate. Sustained hyperammonemia in mice leads to increased lipid peroxidation in liver and brain, reflecting an oxidative stress condition (Connor and Costell 1990; Dakshayani et al. 2002). Melatonin (*N*-acetyl-5-methoxy-tryptamine) is the main secretory product of the pineal gland. It is present in virtually all organisms ranging from bacteria (Manchester et al. 1995) to mammals (Poeggeler et al. 1991). Further, Melatonin is an endogenous free radical scavenger (Tan et al. 1993) and a broad spectrum antioxidant (Reiter et al.1993). It detoxifies a variety of free radicals and reactive oxygen intermediates including the hydroxyl radical, peroxynitrite anion, singlet oxygen and nitric oxide (Reiter et al.1999). Melatonin, which shows extreme diffusibilty through membranes, is important for its scavenging action, since it could enter all cells and every subcellular compartment.

Systematic investigations of the levels of lipid peroxidation products and the levels of enzymic and non-enzymatic antioxidants under the conditions of hyperammonemia are lacking. The present study deals with the levels of thiobarbituric acid reactive substances (TBARS-the products of lipid peroxidation) and the levels of catalase, superoxide dismutase and glutathione peroxidase (enzymatic antioxidants) and reduced glutathione (non-enzymatic antioxidant) in the tissue under the conditions levels of hyperammonemia and during melatonin treatment in rats. Furthermore, the levels of lipid profile variables (free fatty acids, triglycerides, phospholipids and cholesterol) in all the groups were investigated.

Materials and Methods

Adult male Wistar rats (180-220 g), obtained from National Centre for Laboratory Animal Sciences, Hyderabad, were maintained in polypropylene cages in a controlled environment (22-240 C) under 12:12h light dark cycles. Standard pellet diet (Kamadhenu Agencies, Bangalore, India) and water were provided *ad libitum*. All studies were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (National Institute Guide, 1985). Melatonin was purchased from Sisco Research Laboratories Private Limted, Mumbai, India. Ammonium acetate and all other chemicals used in this study were of analytical grade.

The animals were divided into four groups of six rats each and all were fed with the standard pellet diet. Group I animals served as controls. Group II animals were administered with ammonium acetate intraperitoneally (100 mg/kg) every day for 45days (Hilgier et al. 1990). Group III animals were treated with ammonium acetate as group II animals along with melatonin (5mg/kg) intraperitoneally (Liu and Ng 2000). Group IV animals received melatonin (5 mg/kg) intraperitoneally throughout the experiment.

The experiment was terminated after 45 days and all animals were killed by cervical decapitation. Blood samples were collected from each group of rats. Biochemical determinations were done by the methods mentioned in Table 1

The data were analysed using an analysis of variance (ANOVA) and the group means were compared by Least Significant Difference (LSD) test. The results were considered statistically significant if the p-value was 0.05 or less.

Table1**: Biochemical determinations and methods**

|  |  |  |
| --- | --- | --- |
| Parameter | Studies carried out in Tissue | Method |
| Thiobarbituric acid reactive substances | liver | Nichans and Samuelson (1951) |
| Reduced glutathione | liver | Ellman (1959) |
| Superoxide dismutase | liver | Kakkar et al. (1984) |
| Catalase | liver | Sinha (1972) |
| Glutathione peroxidase | liver | Rotruck et al. (1973) |
| Free fatty acids | liver | Falholt et al (1973) |
| Phospholipids | liver | Zilversmit et al. (1950) |
| Triglicerides | liver | Foster and Dunn (1973) |
| Cholestrol | liver | Zlatkis et al. (1953) |

 Results and Discussion

 Ammonia is removed either in the form of urea in periportal hepataocytes and/or as glutamine in perivenous hepatocytes (Nelson and Cox 2000) Elevated levels of ammonia, in ammonium acetate treated rats may be due to the tissue damage caused by ammonia induced free radical generation, leading to oxidative stress and tissue damage (Kosenko et al. 2000; Dakshayani et al. 2002; Vidya et al.2003). Melatonin is an effective free radical scavenger (Reiter et al. 1999), which by its antioxidant potential decreases the ammonia levels of, Under hyperammonemic conditions, elevated levels of ammonia result in the production of free radicals such as hydroxyl radicals, superoxide radicals, peroxyl radicals, alkoxyl radicals and reactive nitrogen species.

TABLE: 2

Changes in the levels of TBARS and antioxidants in liver

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Group I | Group II | Group III | GroupIV |
| TBARS(nmoles /100g tissue) | 1.77 ± 0.08 | 3.73 ± 0.32xxx | 2.42 ± 0.16xxxa | 1.73 ± 0.10ns |
| GSH(mg/g tissue) | 23.62 ± 1.97 | 11.30 ± 0.91xxx | 18.23 ± 0.79xxx,a | 23.14 ± 1.61ns |
| SOD(50% inhibition of NBT redn /min/mg/protein) | 4.94 ± 0.49 | 2.22 ± 0.09xxx | 4.40 ± 0.49xxx,a | 5.03 ±0.51ns  |
| CAT μ moles of H 2 o 2Consumed/min/mg/protein) | 77.13 ± 7.43 | 34.28 ± 2.35xxx | 68.62 ± 3.32xxxa | 74.80 ± 6.06ns |
| GPx μg of GPx consumed/ min/mg/protein | 12.03 ± 1.24 | 4.71 ± 0.25xxx | 8.78 ± 0.64xxx,a | 12.00 ± 1.16ns |

Statistical significance was evaluated using ANOVA followed by Least Significant Difference (LSD) test.

Group II is compared with Group I (a p <o.oo1).

 Group III is compared with Group II (p < 0.001).

Group IV is compared with Group I; ns not significant

Elevated levels of TBARS have been observed in the liver tissue of ammonium acetate treated rats indicating the increased levels of lipid peroxidation. It is a well-established fact that ammonia intoxication enhances lipid peroxidation and generates free radicals (Kosenko et al. 2000; Dakshayani et al.2002; Vidya et al.2003). The levels of TBARS in ammonium acetate and melatonin treated rats were significantly decreased when compared to group 2 rats. This suggests that melatonin could offer protection against lipid peroxidatio (Lastra et al.1997).

The non-enzymatic antioxidant glutathione is a scavenger of hydroxyl radicals and singlet oxygen (Halliwell and Gutteridge 1999). It has been reported that ammonia intoxication induces depletion of glutathione and an increase in lipid peroxidation (Kosenko et al. 1999). Reports have also shown that ammonia intoxication leads to the increased formation of nitric oxide which results in the oxidation of glutathione (GSE) to glutathione disulphide (GSSG) and to mixed glutathione disulphides (GSSR) resulting in depletion of GSH and increased free radical formation (Luperchio et al.1996). Group 3 rats compared to group 2 rats showed elevated levels of glutathione. This is because, under hyperammonemic conditions, melatonin increases the levels of glutathione, an important intracellular antioxidant, by stimulating its rate-limiting enzyme, ϒ-glutamylycysteine synthase (Urata et al. 1999).

In our study, the decreased activities of antioxidant enzymes (SOD, CAT, GPx) in the ammonium acetate treated group may be due to the inhibition of these enzymes by nitric oxide. It is known that ammonia-induced inhibition of antioxidant enzymes is mediated by the activation of NMDA receptors that leads to increased intracellular calcium levels, which in turn activate neuronal nitric oxide synthase, leading to the formation of nitric oxide which inhibits the activities of antioxidant enzymes (Kosenko et al.2000) Under hyperammonemic conditions, melatonin causes an increase in the gene expression and activities of the antioxidant enzymes such as glutathione peroxidase, glutathione reductase and superoxide dismutase (Barlow et al. 1995; Antolin et al. 1996) which results in the elevated levels of these enzymes in group 3 rats. The elevated levels of these enzymes might protect against oxidative damage caused by the free radical formation (Reiter et al. 2003).

Melatonin directly scavenges hydrogen peroxide to form *N*1 –acetyl-*N*2 –forms *N*1 –acetyl-5-methoxy Kynuramine (Tan et al. 2000). These biogenic amines could also scavenge hydroxyl radicals and reduce lipid peroxidation. Ammonium acetate may deplete the levels of α-KG and other Krebs cycle intermediates (Yamamoto 1989) and thus elevate the levels of acetyl coenzyme A. The elevated levels of acetyl coenzyme A may increase the levels of lipid profile variables (free fatty acids, triglycerides, phospholipids and cholesterol) as observed in our study. The decreased α-KG levels in rats treated with ammonium acetate might be reversed during treatment with melatonin, since melatonin was found to reduce these levels (Baydas et al. 2002).

Our results suggest that melatonin could control the oxidative stress caused by hyperammonemia by (i) directly scavenging a variety of radicals and reactive oxygen species (ii) inducing antioxidative enzymes which reduce steady state levels of reactive oxygen species and by (iii) stabilizing cell membranes which assist them in reducing oxidative damage.

Table 3: Changes of lipid profiles in liver tissue

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | GroupI | GroupII | GroupIII | GroupIV |
| Free fatty acid(mg/100g tissue) | 596.76 ± 57.53 | 910.75 ± 73.13xxx | 748.06 ± 54.69xxx,a | 573.30 ± 61.07ns |
| Phospholipids(mg/100g tissue) | 950.18 ± 102.82 | 1618.84 ± 57.37xxx | 1299.87 ±98.98xxx,a | 941.01 ± 100.33ns |
| Triglycerides(mg/100g tissue) | 342.84 ± 29.04 | 653.06 ± 32.84xxx | 422.78 ± 30.14xxx,a | 458.83 ± 33.75xxx,a |
| Cholesterol(mg/100g tissue) | 348.62 ± 32.67 | 565.86 ± 55.47xxx | 458.83 ± 33.75xxx,a | 340.62 ± 30.41ns |

Statistical significance was evaluated using ANOVA followed by Least Significant Difference (LSD) test.

 Group II is compared with Group I (a p <o.oo1). Group III is compared with Group II (p < 0.001).

Group IV is compared with Group I; ns not significant.

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