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EVALUATION OF THE ANTIPEROXIDATIVE EFFECTS OF MELATONIN IN AMMONIUM ACETATE-TREATED WISTAR RATS

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The efficacy of melatonin (MLT) against ammonium acetate-induced neurotoxicity was biochemically studied in the experimental rats. The activities of serum transaminases and the levels of thiobarbituric acid reactive substances were significantly increased in ammonium acetate-treated rats. These levels were significantly decreased in MLT and ammonium acetate-treated rats. Further, non-enzymatic (vitamin C and E) and enzymatic (superoxide dismutase and catalase) antioxidants were significantly decreased in ammonium acetate-treated rats and were increased in MLT and ammonium acetate-treated rats. These biochemical alterations during MLT treatment could be due to its ability to: (i) scavenge a variety of radicals and reactive species, (ii) induce antioxidative enzymes which reduce steady state levels of reactive species, (iii) inhibit nitric oxide synthase which generates nitric oxide and (iv) stabilize cell membranes which assists them in reducing oxidative damage and, thus, prevents the oxidative stress in rats.

Key words*: melatonin, ammonium acetate, antioxidants, lipid peroxidation*

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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 [23]. Hyperammonemia, caused by insufficient removal of ammonia in the liver [16] or portacaval shunting [4], leads to an increase in ammonia level in the brain [4], which is responsible for development of hepatic encephalopathy [1, 3]. Ammonia intoxication impairs mitochondrial function [10], which could lead to decreased ATP synthesis and also to increased formation of free radicals [11]. The major toxic effects of ammonia likely involve changes in cellular pH and the depletion of certain citric acid cycle intermediates, in particular α-ketoglutarate. It has been reported that sustained hyperammonemia in mice leads to increased lipid peroxidation in liver and brain, reflecting an oxidative stress condition.

Melatonin (N-acetyl-5-methoxy-tryptamine) is the chief secretory product of the pineal gland. It is present in virtually all organisms ranging from bacteria [15] to mammals [24]. Recently, it has been reported that a variety of other tissues including retina [22], Harderian gland [17], ovary [8], testes [37] and bone marrow [5, 35] may also synthesize melatonin.

Melatonin is an endogenous free radical scavenger [33] and a broad spectrum antioxidant [26]. It detoxifies a variety of free radicals and reactive oxygen intermediates including the hydroxyl radical, peroxynitrite anion, singlet oxygen and nitric oxide [27]. Melatonin, which shows extreme diffusibility through membranes, is important for its scavenging action, since it could enter all cells and every subcellular compartment.

However, the antioxidant potential of melatonin during hyperammonemia has not been investigated so far. In the present study, the antioxidant potential of melatonin has been evaluated by estimating the activities of transaminases, levels of thiobarbituric acid reactive substances (TBARS), non-enzymatic antioxidants (vitamin C and E) and enzymatic antioxidants [superoxide dismutase (SOD) and catalase (CAT)] in experimental rats.

MATERIALS and METHODS

Adult male Wistar rats (weighing 180–220 g), obtained from National Center for Laboratory Animal Sciences, Hyderabad, were kept at room temperature (32 ± 2°C) at L:D (12:12) cycles. All studies were conducted in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" [18]. Animals were randomized and separated into four groups (Group I – control, Group II – ammonium acetate-treated, Group III – ammonium acetate- and melatonin-treated, Group IV – melatonin-treated; n = 6 in each group). Food pellets (Kamadhenu Agencies, Bangalore, India) and water were available *ad libitum* to animals.

Melatonin (salt form) was purchased from Sisco Research Laboratories Private Limited, Mumbai, India. Ammonium acetate and all other chemicals used in this study were of analytical grade. Group I animals served as controls. Group II animals were administered with ammonium acetate intraperitoneally (*ip*) (100 mg/kg) every day for 45 days [7]. Group III animals were treated with ammonium acetate as Group II animals along with melatonin (5 mg/kg) (*ip*) [14]. Group IV animals received melatonin (5 mg/kg) (*ip*) for 45 days.

Biochemical determinations were performed after 45 days of ammonium acetate and/or melatonin administration. At the end of experimental period (45 days) animals from all the groups were sacrificed by cervical dislocation.

Blood samples were collected from each group of rats. Biochemical analyses were performed in blood, serum hemolysate and plasma samples. The activities of aspartate transaminase [AST] and alanine transaminase [ALT] were analyzed in serum [28], vitamin C content was determined in blood [29], the levels of TBARS [20], vitamin E [2], ammonia [6] and urea [38] were measured in plasma, and in hemolysate the activities of CAT [32] and SOD [9] were evaluated. Analysis of variance followed by Least Significant Difference test was carried out to detect the significant differences between control and experimental groups.

RESULTS

There was no significant change in the body weight of the experimental animals when compared to control (Tab. 1). The concentration of ammonia and urea was significantly increased in ammonium acetate-treated group (Tab. 1). The group treated with ammonium acetate and melatonin showed significantly lower levels of ammonia and urea when compared to the ammonium acetate-

Table 1. Body weight changes and the levels of ammonia and urea in the rats treated with ammonium acetate and/or melatonin

Group	Body weight (g)			
	Initial body wt	Final body wt	NH_3 (μmol/l)	Urea (mg/dl)
Normal	184 ± 15	194 ± 16	88.27 ± 7.82	10.68 ± 0.65
Ammonium acetate	206 ± 17	220 ± 19	331.11 ± 17.04^{a}	22.60 ± 1.19^{a}
Ammonium acetate + melatonin	190 ± 18	202 ± 17	166.76 ± 16.86^{b}	13.29 ± 1.04^{b}
Melatonin	185 ± 16	196 ± 18	83.92 ± 7.25^{ns}	11.62 ± 0.96^{ns}

Statistical significance was evaluated using ANOVA followed by Least Significant Difference (LSD) test. Group II is compared with Group I (^{a} $p < 0.001$). Group III is compared with Group II (^{b} $p < 0.001$). Group IV is compared with Group I; ns – not significant

Table 2. Levels of TBARS, ALT and AST

Group	TBARS (nmol/ml)	Aspartate amino transferase (IU/l)	Alanine amino transferase (IU/l)
Normal	2.21 ± 0.20	105.93 ± 7.47	34.60 ± 3.57
Ammonium acetate	3.79 ± 0.33^{a}	176.07 ± 14.25^{a}	75.43 ± 4.44^{a}
Ammonium acetate + melatonin	2.74 ± 0.18^{b}	139.17 ± 9.76^{b}	53.33 ± 3.65^{b}
Melatonin	2.17 ± 0.23^{ns}	106.74 ± 7.49^{ns}	32.10 ± 3.39^{ns}

Statistical significance was evaluated using ANOVA followed by Least Significant Difference (LSD) test. Group II is compared with Group I (^{a} $p < 0.001$). Group III is compared with Group II (^{b} $p < 0.001$). Group IV is compared with Group I ; ns – not significant

Table 3. Levels of non-enzymatic and enzymatic antioxidants

Group	Vitamin C (mg/dl)	Vitamin E (mg/dl)	SOD (50% inhibition of NBT reaction/mg of protein)	CAT (mmoles /dl)
Normal	1.64 ± 0.07	1.62 ± 0.05	2.54 ± 0.28	34.60 ± 3.57
Ammonium acetate	0.55 ± 0.03^{a}	0.58 ± 0.03^{a}	0.75 ± 0.19^{a}	75.43 ± 4.44^{a}
Ammonium acetate + melatonin	1.55 ± 0.05^{b}	1.58 ± 0.03^{b}	1.70 ± 0.29^{b}	53.33 ± 3.65^{b}
Melatonin	1.66 ± 0.06^{ns}	1.67 ± 0.06^{ns}	2.68 ± 0.24^{ns}	32.10 ± 3.39^{ns}

Statistical significance was evaluated using ANOVA followed by Least Significant Difference (LSD) test. Group II is compared with Group I (^{a} $p < 0.001$). Group III is compared with Group II (^{b} $p < 0.001$). Group IV is compared with Group I; ns – not significant

treated group. The melatonin-treated group showed near normal levels of ammonia and urea when compared with the controls.

The concentration of TBARS in plasma was significantly increased in the ammonium acetate-treated group (Tab. 2). The ammonium acetate- and melatonin-treated group showed significantly lower levels of TBARS when compared to the corresponding ammonium acetate-treated group (Tab. 2). The melatonin-treated group showed near normal levels of TBARS when compared with the controls. The activities of the liver marker enzymes (ALT and AST) (Tab. 2) were also altered in a similar manner between the groups.

The levels of vitamins (C and E) (Tab. 3) were significantly increased in ammonium acetate- and melatonin-treated group, when compared with the corresponding ammonium acetate-treated group. Melatonin-treated group showed near normal levels of antioxidant vitamins (C and E) when compared with the controls. The alterations in the levels of the enzymatic antioxidants such as CAT and SOD (Tab. 3) were similar to that of vitamin C and E.

DISCUSSION

Body weight changes

There were no significant changes in the body weights of the experimental animals when compared to controls (Tab. 1).

Ammonia and urea

In the liver, ammonia is removed either in the form of urea in periportal hepatocytes and/or as glutamine in perivenous hepatocytes [19]. Elevated levels of ammonia and urea in ammonium acetate-treated rats may be due to the liver damage caused by ammonia-induced free radical generation. Reports have shown that excess ammonia induces nitric oxide synthase which leads to enhanced production of nitric oxide, leading to oxidative stress and liver damage [13, 30]. The decrease in urea and ammonia in melatonin-treated rats may be due to the antioxidant potential of melatonin. Melatonin has been proved to be an effective free radical scavenger [26, 33], by inhibiting the pro-oxidant enzyme nitric oxide synthase [25].

Lipid peroxidation

Ammonia intoxication enhances lipid peroxidation and leads to the formation of free radicals [11, 39]. This might account for the increased levels of TBARS (which is a measure of lipid peroxidation and an index of membrane oxidative damage) and decreased vitamin C and E levels in ammonium acetate-treated rats. Ammonia intoxication depletes the level of glutathione (GSH) [12]. Since the regeneration of vitamin C requires GSH, a deficiency of GSH might cause the reduction of vitamin C and E in the plasma, which was observed in our study. The protective capability of antioxidants against free radical-induced damage is increased if the scavenging molecule can be recycled. Mahal et al. (1999) reported regeneration of melatonin from the one-electron oxidized melatonyl radical by both ascorbate and urate. Melatonin directly scavenges hydrogen peroxide to form N^1-acetyl-N^2-formyl-5-methoxykynuramine, which, by the action of CAT forms N^1-acetyl-5-methoxykynuramine [34]. These biogenic amines could also scavenge hydroxyl radical and reduce lipid peroxidation.

Transaminases

Aspartate and alanine aminotransferases are sensitive indicators of liver cell injury [40]. Enhanced activities of ALT and AST in ammonium acetate-treated rats might be related to the devastation of the liver tissue [31, 40]. Melatonin abolishes ammonia effects, by inhibiting the inducible nitric oxide synthase expression and stimulating the glutathione peroxidase, glutathione reductase and SOD, whereby it decreases the oxidative stress and tissue damage.

In our study, the decreased activities of antioxidant enzymes (SOD and CAT) in 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 of nitric oxide synthase and formation of nitric oxide which inhibits the activities of antioxidant enzymes [13].

The indole moiety of the melatonin molecule is the reactive center of interaction with oxidants due to its high resonance stability and low activation energy barrier towards the free radical reactions. The methoxy and amide side chains also contribute significantly to melatonin's antioxidant capacity. The methoxy group in C_5 appears to keep melatonin from exhibiting pro-oxidative capacity [36].

Receptor-dependent actions of melatonin, e.g. an antioxidative enzyme induction, seem to contribute to the overall antioxidant protection by melatonin [21]. Melatonin has the ability to scavenge up to 4 or more reactive species, which makes melatonin a potent antioxidant and a free radical scavenger. It can be concluded that melatonin could control the oxidative abuse 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, (iii) inhibiting nitric oxide synthase which generates nitric oxide and (iv) stabilizing cell membranes which assist them in reducing oxidative damage.

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