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Research Article

DEXAMETHASONE INDUCED ALTERATIONS IN LIPID PEROXIDATION, ANTIOXIDANTS, MEMBRANE BOUND ATPASE IN WISTAR ALBINO RATS

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ABSTRACT

Dexamethasone is a synthetic adrenocortical steroid used to treat inflammatory and autoimmune conditions. It acts as an anti-inflammatory and immunosuppressant. It is more potent than hydrocortisone. Dexamethasone is used to treat ulcerative colitis, psoriasis, arthritis, allergic disorders and breathing related problems. However dexamethasone therapy is associated with a variety of side effects, among which nephropathy is the major one. Recent studies have suggested reactive oxygen species and oxidative stress as a cause of drug nephrotoxicity. Therefore, this study was designed to elevate the effect of dexamethasone administration on wistar rats with reference to lipid peroxidation, antioxidants and membrane bound ATPase enzymes. The adult male wistar albino rats were randomly assigned into two groups: Group I animals treated as normal control and group II rats received dexamethasone (0.7mg/kg/body weight). The results showed an increase in the kidney and liver weight and the significant decrease in body weight were observed in dexamethasone treated rats compared to control rats. Lipid peroxidation and ATPase activities were increased on dexamethasone administration. On the contrary the levels of antioxidant enzymes were decreased compared to control. These data suggested that long term exposure to dexamethasone treatment will have a devastating effect on the kidney and liver functions due to the fact that it can induce the lipid peroxidation and disrupt many metabolic pathways.

Keywords: Dexamethasone, Antioxidants, LPO, ATPase.

INTRODUCTION

Dexamethasone is a member of the glucocorticoid class of hormones, they are steroids but, unlike the "anabolic" steroids, these are "catabolic" steroids. Instead building up the body, they are designed to break down stored resources [fats, sugars and proteins] so they may used as a fuel at the time of stress. Glucocorticoid hormones are produced naturally by the adrenal glands and it is widely used for their influences on glucose and protein metabolism, since they are almost broadly in anti-inflammatory medications [especially for joint pain and itchy skin], immune suppression, cancer chemotherapy [especially in the treatment of lymphoma], and central nervous system disorders. Their unique therapeutic efficacy is in the treatment of numeral disease conditions due to their anti-inflammatory and immunosuppressive effects[1]. However their long term use has proved to induce changes in the thiobarbituric acid [TBA] reactant levels[2] and tissue/cell levels of the antioxidant enzymes[3, 4]. An increased TBA reactant level is an indication of reactive oxygen species production. Reactive oxygen species [ROS] are formed continuously in a controlled rate in physiological conditions. But their formation increases dramatically in conditions of oxidative stress induced by xenobiotics, pollutants, ionizing radiation and ultraviolet light[5, 6]. ROS plays a critical role in the pathogenesis of various diseases, such as cardiovascular injury associated with circulatory disturbance[7]. The ROS such as hydroxyl radicals might attack any type of molecule, but their main target emerge in the polyunsaturated fatty acid [PUFA], which is the precursor for lipid peroxide formation[8, 9]. The aim of the present study was to investigate the alterations in the TBA reactant level, antioxidant status and membrane bound ATPases in kidney and liver by the administration of dexamethasone, as this might pave the way for elucidation for the mechanism underlying the toxic effect of this drug against wistar albino rats.

MATERIALS AND METHODS

Drug and chemicals

Dexamethasone utilized in this study was procured from a pharmacy under the common brand name decadran. All other chemicals and solvents used in this study were of high purity and analytical grade.

Experimental design

Wistar albino male rats weighing 140±20 g used in this study were obtained from Central Animal House Facility, University of Madras, Taramani Campus, Chennai. They were housed in polypropylene cages with 12 h light/ dark cycle. Animals were fed with standard rat pellet and water ad libitum. The experiment was performed in accordance with the strict guidelines prescribed by the Institutional Animal Ethical Committee (01/05/2011). The rats were divided into two groups with six animals in each groups as follows: group I rats were served as control and the group II rats received dexamethasone at the dosage of 0.7mg/kg/body weight of decadran as an intraperitoneal injection for a total period of 9 days[10].

Tissue collection

Body weights of the rats were monitored at the start and end of the experimental period. At the 11th day, all the rats were sacrificed by cervical decapitation and the kidney and liver tissues were excised immediately and rinsed in ice-cold physiological saline. Tissues were homogenized in 0.01M Tris – HCl buffer (pH 7.4) and aliquots of this homogenate (10%) were used for the assays.

Assay of antioxidants and lipid peroxidation

The levels of protein was estimated according to the method of Lowry et al[11] and the antioxidants such as superoxide dismutase, catalase and lipid peroxidation were also estimated according to the methods of Marklund and Marklund[12], Sinha[13], Devasagayam[14] respectively. Estimation of protein thiols was determined according to the method of Sedlack[15].

Estimation of membrane bound ATPases

Na^+K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase activities were determined by the method of Bonting[16], Ohnishi et al[17], Hjerten and Pan[18] respectively.

Statistical Analysis

The results are expressed as mean ± standard deviation [SD] for six animals in each group. The results were statistically evaluated

using one-way analysis of variance [ANOVA] by SPSS 10.0 student version followed by Turkey's multiple comparison method to compare means of different groups. Significance at *P* values < 0.001, < 0.01, < 0.05 has been given respective symbols in the tables.

RESULTS

Table 1 portrays the significant loss of body weight during the dexamethasone treatment. On the contrary there was a significant increase in kidney, liver weight and protein content during the administration of dexamethasone.

The levels of TBA-reactants in kidney and liver showed a significant increase in the drug administered animal. There was a significant decrease in the levels of antioxidants such as catalase and superoxide dismutase [SOD] as shown in table 2.

Table 3 depicts the levels of the ATPases were noticeably decrease in their activity in the dexamethasone treated animals.

Table 1: Changes in the body weight, kidney weight, liver weight and protein content in control and dexamethasone treated rats.

Parameters	Group i (control)	Group ii (dexamethasone)
Body weight (g)	151 ± 4.85	123 ± 8.31 a***
Kidney weight (mg)	790 ± 0.26	993 ± 0.58 a***
Liver weight (g)	3.08 ± .0.32	4.12 ± 0.45 a***
Protein content in Kidney[1]	80.26 ± 11.96	89.25 ± 2.17 a***

Each value represents mean ± SD of six animals; a – Group II compared with Group I; ***P<0.001, **P<0.01, *P<0.05. [1]Expressed as mg/g wet tissue.

Table 2: Changes in the levels of TBA-reactants, thiol, activities of antioxidant enzymes in kidney and liver of control and dexamethasone treated rats.

Parameters	Kidney		Liver	
	Group i (Control)	Group ii (Dexamethasone)	Group i (Control)	Group ii (Dexamethasone)
LPO	2.87 ± 0.17	4.91 ± 0.26 a***	4.92 ± 0.26	10.16 ± 0.57 a***
THIOL	10.17 ± 0.22	6.88 ± 0.36 a***	15.23 ± 0.16	8.78 ± 0.47 a***
CATALASE	40.31 ± 1.67	33.53 ± 3.34 a***	74.23 ± 2.75	37.17 ± 3.92 a***
SOD	5.27 ± 0.15	4.05 ± 0.39 a***	9.13 ± 0.21	4.52 ± 0.33 a***

Each value represents mean ± SD of six animals. LPO [Lipid peroxidation] – nmoles/mg protein; Thiols - µg/mg protein; Catalase - µ moles of H_2O_2 consumed/mg protein/min; SOD - enzyme required to give 50% inhibition of pyrogallol; autooxidation (units/mg protein); a – Group II compared with Group I; ***P<0.001, **P<0.01, *P<0.05.

Table 3: The levels of membrane bound ATPases in kidney and liver of control and dexamethasone treated rats.

Parameters	Kidney		Liver	
	Group i (Control)	Group ii (Dexamethasone)	Group i (Control)	Group ii (Dexamethasone)
Na^+K^+ ATPase	0.676 ± 0.048	0.318 ± 0.021 a***	1.41 ± 0.041	1.56 ± 0.089 a***
Ca^{2+} ATPase	0.475 ± 0.010	0.285 ± 0.020 a***	1.81 ± 0.049	1.53 ± 0.109 a***
Mg^{2+} ATPase	0.518 ± 0.017	0.338 ± 0.026 a***	2.53 ± 0.072	2.03 ± 0.161 a***

Each value represents mean ± SD of six animals; Units: µ moles of inorganic phosphate liberated/mg protein/min; a – Group II compared with Group I; ***P<0.001, **P<0.01, *P<0.05.

DISCUSSION

The present study yields an additional support for the view that signifies dexamethasone administered morphological and biochemical alterations. Our findings clearly indicates the evidence to suggest that the long term use of dexamaethasone reduced the antioxidant capacity of the rat renal tissue and thereby leads to provide the chance to form the ROS. It was observed from the present study, the dexamethasone treated rats showed marked decline in the body weight and antioxidants such as catalase, and SOD and an increase in TBA-reactants, protein content, kidney and liver weight. Loss of body weight could be explained on the basis of detrimental effects of GCs on the gut[19, 20]. They have observed a 10% decrease in the non stretched part of the small intestine and a large decrease in protein synthesis in the smooth muscle of small intestine, which might have resulted in decreased food intake and loss of body weight. A significant loss of body weight, observed in this study is in agreement with the Nasjketti et al[21]. An increase in kidney and liver weights on dexamethasone administration was observed in this study concurs with the previous findings, in which it is reported that 39% increase in the weights of kidney and liver[22]. This increase in the organ weights has been attributing to the increase in the content of protein, as the dexamethasone does not induce fluid retention or edema[2]. Glucocorticoids are known to alter the levels of TBA-reactants[3] and antioxidant enzymes in different tissues[23, 24]. Dexamethasone induces increase in the levels of very low density lipoproteins [VLDL], thus accelerating the oxidation of VLDL by the arterial endothelial cells, the process which serves as the potential source of toxic ROS[25]. Dexamethasone increases triglyceride levels thus inducing an imbalance in lipid metabolism in kidney[2] and this could explain the reason for the increase in lipid peroxidation in these organs. As the ROS level in tissues depends upon the balance between the extent of lipid peroxidation and antioxidant status[26, 27]. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. There are several enzyme systems within the body that scavenge free radicals[28, 29]. Depletion in the activity of antioxidant enzymes can be due to an enhanced radical production during dexamethasone metabolism. Superoxide dismutase protects against the superoxide radical ($O_2^{\cdot-}$), which damages the membrane and its biological structure[30]. Catalase primarily decomposes hydrogen peroxide to H_2O at a much faster rate, sharing this function with glutathione peroxidase. The balance between these enzymes is important for the efficient removal of oxygen radicals from tissues[31, 32].

Therefore, reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and H_2O_2. ATPases are intimately associated with the plasma membrane and participates in the energy requiring translocation of sodium, potassium, calcium and magnesium[33]. The inhibition of sodium potassium ATPase can active the sodium-calcium exchange mechanisms in the myocardium. This sodium-calcium exchange mechanism in the myocardium may play an important role in regulating the cellular calcium level[34, 35]. In the present study the levels of sodium potassium ATPase, calcium ATPase, magnesium ATPase were decreased. Lipid peroxides have been shown to impair tissue membranes, which is a risk factor in

variety of diseases. Lipid peroxides are presumptive markers of free radical generation and development of oxidative stress. The decrease in the levels of ATPases could be due to an enhanced lipid peroxidation by the free radicals in the dexamethasone administered rats since the membrane bound enzymes are 'SH' group containing enzymes[36, 37].

CONCLUSION

The present study suggest that long term exposure to dexamethasone treatment was found to produce devastating effect on the kidney and liver functions due to the fact that it can induce lipid peroxidation by the production of ROS and disrupt many metabolic pathways.

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