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Research Article

ANALYSIS OF THYROIDAL ENZYMES ACTIVITY IN ZINC SUPPLEMENTED LITHIUM TREATED RATS

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ABSTRACT

Lithium is used in the therapy of manic depressive disorder. It affects cell function via its inhibitory action on several intracellular enzymes. Zinc, an essential trace element, has an important role in several biological activities. Therefore, present study was aimed at investigating role of zinc on thyroidal enzyme activities following lithium administration. Control group animals were given standard feed and tap water *ad libitum*. Lithium treated animals were fed lithium ($LiCO_3$; 1.1g/kg body weight) through diet. Zinc treated rats were given zinc ($ZnSO_4.7H_2O$; 227 mg/L in drinking water). The treatments were given for 8 weeks. Monoamine oxidase activity was found to be significantly increased during lithium treatment. Thyroid peroxidase activity, however, was found to be significantly reduced in lithium treated group. However, combined lithium and zinc treatment showed a significant elevation in the enzyme activity. Co-administration of lithium and zinc also resulted in a significant depression in the Na^+/K^+ ATPase activity, thereby clearly indicating the effective role of zinc in regulating this activity. Zinc is playing an effective role in reducing the toxic effects of lithium in thyroid, possibly due to it's antioxidative nature.

Keywords: Zinc, Lithium, Thyroid, Monoamine oxidase, Peroxidase, Na^+/K^+ ATPase.

1. INTRODUCTION

Lithium is the main drug that is widely being used for the treatment of bipolar disorders and manic illness and has been seen to be associated with several endocrine complications [1]. Lithium carbonate has been shown to have numerous effects on the synthesis, release, inactivation and interaction with receptors of neurotransmitters [2, 3]. Lithium also influence the activity of a variety of enzymes including monoamine oxidase [4] and peroxidase [5] and it is possible that alteration in enzymatic processes is involved in its therapeutic actions. Monoamine oxidase [4] is the enzyme that is involved in metabolism of biogenic amines and peroxidase is involved in the biosynthesis of thyroid hormone [5].

Also, there is good evidence available now that thyroid peroxidase catalyzes the intermoleular coupling that is involved in iodothyronine formation. Na^+/K^+ ATPase is another enzyme located in the thyroid membrane that contributes to the accumulation of iodide, which is required for the thyroid hormone biosynthesis [6]. Several anti thyroid drugs have been found to inhibit iodide peroxidase activity [7]. Lithium has biphasic effects on the enzyme. At low concentration, it enhances and at high concentration, it inhibits the enzyme activity. The alteration of Na^+/K^+ ATPase activity plays an important role in the therapeutic action of lithium [8].

The implication of essential trace elements in endocrinological processes mainly thyroid function have been reviewed [9]. Most concerned elements in this field are iodine, selenium, copper and zinc. These minerals are powerful modulators of several physiological functions that can be considerably perturbed in deficient states. Of these, zinc has a clearly defined role in thyroid hormone metabolism [10]. Zinc supplementation has been proved to restore the active form of thyroid hormone, which contains one zinc ion/molecule and is associated to the improvement in thyroid function [11]. It has been reported that zinc influence the peripheral deiodination of T_4. It has been shown that zinc is required for the attachment of thyroid hormone to the receptors [12] and thus zinc status may be important for the full biological functioning of T_3 [13].

Further, research is required as the exact mechanism and the role of zinc, particularly in thyroid hormone metabolism, is still required to be explored under the given scenario. Therefore, information on thyroidal enzyme activity in zinc supplemented and lithium administration was designed. Moreover, the efficacy of zinc in regulating the thyroid functions in such conditions may be worth investigating.

2. MATERIAL AND METHODS

2.1. Animal Groups

Male Wistar rats weighing 150-195 grams were procured from the Central Animal House, Panjab University, Chandigarh. Animal care was done as per the principles laid down by the National Institute of Health (NIH publication no. 85-23, revised in 1985) strictly. Prior to the advancement of the experimental design, the approval was taken from institutional animal ethics committee (case no. 445/88-bph-16/Ph.D). The animals were divided into four groups; Group 1 animals were fed with Standard pellet feed and tap water *ad libitum*. Group 2 rats were fed with lithium in the form of lithium carbonate through diet at a concentration of 1.1g/kg body weight [14]. Group 3 animals received zinc treatment in the form of zinc sulphate ($ZnSO_4.7H_2O$) at a dose level of 227 mg/L mixed in drinking water of the animals [15]. Group 4 animals were given lithium and zinc in the same way as was given to the animals belonging to group 2 and 3 respectively. All these treatments were continued for 8 weeks. Blood samples were collected by ocular vein puncture under light ether anaesthesia. The samples were allowed to stand at room temperature fo 30 minutes, centrifuged at 1500 rpm and serum was carefully separated from the pellet and stored in the refrigerator.

2.2. Biochemical Estimations

The specific activity of monoamine oxidase was expressed as nmoles of benzaldehyde formed min^{-1} mg^{-1} protein by the method of Leyton [16]. Thyroid peroxidase activity was determined by the method of Harsog and Fahimi [17]. The activity of peroxidase was expressed as n moles of H_2O_2 decomposed min^{-1} mg^{-1} protein by using the extinction coefficient of 3.16 $mM^{-1}cm^{-1}$.The protein content was measured by the procedure of Lowry et al. [18]. A modified method of Sarkar [19] was employed for the estimation of Na^+/K^+ATPase. The enzyme activity was expressed as the amount of inorganic phosphorus liberated in μg mg^{-1} protein h^{-1}.

3. RESULTS AND DISCUSSION

Monoamine oxidase activity was found to be significantly increased ($p<0.001$) after 8 weeks in lithium treated group, in comparison with the normal controls. Rats given combined lithium and zinc treatment also indicated significant increase ($p<0.01$) in MAO activity in comparison to normal controls. However, it was not statistically different when compared with lithium treated rats (table 1).

Table 1: Effect of zinc on monoamine oxidase activity in thyroid following lithium administration

S. No.	Groups	nmoles of benzaldehyde formed/ min/mg protein
I	Normal Control	1.34±0.29
II	Lithium treated	$2.96±0.37^c$
III	Zinc treated	1.70±0.38
IV	Lithium + Zinc treated	$2.53±0.29^b$

Values are expressed as Mean ± S.D of 6 to 8 animals., $^bp<0.01$, $^cp<0.001$ by Newman-Keul's test when the values of groupie and IV are compared with those of group I.

Monoamine oxidase provides insight into abnormal neurotransmission function that may be present in affective disorders [20]. The present study indicated a significant elevation in monoamine oxidase activity after lithium administration for 8 weeks. This is in agreement with the reports by earlier workers [21]. Increased enzyme activity during lithium treatment provides an explanation for the efficacy of lithium in the management of mania. An accumulation of catecholamines, causing mania, would be reversed by lithium through its effect on monoamine oxidase. Monoamine oxidase is a stereo selective enzyme [22]. Lithium influenced the stereo selective index for monoamine oxidase, which suggested that lithium altered conformational features of the enzyme [23]. Zinc supplementation alone did not show any significant change in the enzyme activity as compared to the normal controls. But the activity was found to be reduced when lithium was given to the zinc treated group, which also suggests that zinc is trying to normalize the elevated enzyme activity either possibly by regulating the levels of catecholamines or at the level of receptor genes.

Thyroid peroxidase activity was found to be significantly reduced after 8 weeks in lithium treated ($p<0.001$), combined lithium and zinc treated ($p<0.01$) as compared to the normal control group. Combined lithium and zinc treated rats, however, showed a statistically significant elevation ($p<0.01$) in the enzyme activity as compared to the lithium treated rats (table 2).

Table 2: Effect of zinc on peroxidase activity in thyroid following lithium administration

S. No.	Groups	nmoles of H_2O_2 consumed/min/ mg protein
I	Normal Control	45.81±3.25
II	Lithium treated	30.09 ± 3.44^{c}
III	Zinc treated	45.96±6.12
IV	Lithium + Zinc treated	$38.24 \pm 3.96^{b,y}$

Values are expressed as Mean ± S.D of 6 to 8 animals. $^{b}p<0.01$, $^{c}p<0.001$ *by Newman-Keul's test when the values of groupie and IV are compared with those of group I,* ^{y} $p<0.01$ *by Newman-Keul's test when the values of groupie and IV are compared with those of group II.*

Thyroid peroxidase requires a source of H_2O_2 in in vitro systems for its actions [24]. It appears most likely that H_2O_2 production plays an essential role in thyroid hormone formation in vivo. Congenital hypothyroidism has also been shown due to the lack of thyroid peroxidase activity [25]. Vaisman et al. [26] conducted studies on patients with iodine organification defect and reported that the reduced activity of the peroxidase present in patients' goi-ter may be a responsible factor to result in some modifications in the thyroid structure (goiter formation). Szanto et al. [27] indicated that inactivation involves a reaction between the drugs and the oxidized heme group produced by interaction between thyroid peroxidase and H_2O_2. However, the effects of lithium salts on thyroid peroxidase have not been thoroughly investigated. Since, peroxidase is considered to be involved in the iodination phase and the present investigations indicate decrease in peroxidase activity following lithium treatment, it clearly defines interference in iodination by lithium or 131I. The reduction of thyroid peroxidase following lithium treatment probably leads to series of events right from inhibition of T4 and T3 iodo aminoacids within thyroid and which gets substantiated from the reduction of T3 and T4 in the systemic circulation as well as the PB131I. This depression in enzyme activity may also be due to decrease in H_2O_2 production after lithium treatment, which occurs due to increase in anti oxidative enzymes [28].

Na^{+} /K^{+} - activated adenosine triphosphatase (Na^{+}/K^{+} ATPase) is the membrane spanning protein complex responsible for extrusion of Na^{+}, and absorption of K^{+} by most animal cells. In epithelia, active transport mediated by the pump provides the driving force for the movement of solutes and water between serosal and luminal fluids [29]. In the present investigation, lithium administration showed a significant depression in Na^{+}/K^{+} ATPase activity as compared to the normal controls. Several other workers also suggested that lithium could alter cellular membrane [30] Na^{+}/K^{+} ATPase. Due to similarities in physical properties of lithium ions to Na^{+} and K^{+}, it is possible that some of its therapeutic effects could be due to interaction with ion fluxes across the membranes. The changes in Na^{+}/K^{+} ATPase activity are important in the therapeutic action of lithium [30]. Lithium may replace K^{+} ions for its transport into cell and alter extra cellular K^{+} concentration. The direct effects of lithium on Na^{+}/K^{+} ATPase under physiological conditions may vary depending on extra cellular K^{+} concentration. In the presence of low extra cellular concentration of K^{+}, such as those found in depression, lithium may enhance extra cellular potassium and stimulate the enzyme activity whereas at high concentrations of K^{+} lithium may inhibit the enzyme and decreases extra cellular $K^{+.}$ The ability of lithium to alter cellular transport mediated by Na^{+}/K^{+} ATPase indicates a potential locus of action for its effect on mood and behavior. Zinc has been known to play an important role in maintaining the normal activities of Na^{+}/K^{+} ATPase.

Zinc supplementation did not show any significant change in enzyme activity as compared to the normal controls. However, when lithium was administered to the zinc treated rats, the enzyme activity was found to be reduced, but the degree of reduction in comparison to lithium treated rats was significantly less, thereby clearly indicating the effective role of zinc in regulating this activity [31].

Lithium administration caused a statistically significant decrease ($p<0.001$) in Na^{+} K^{+}ATPase activity as compared to the normal controls. Zinc treatment, however, did not cause any significant change in the enzyme activity in comparison with the normal controls. Co-administration of lithium and zinc also resulted in a statistically significant depression ($p<0.01$) in the enzyme activity as compared to the normal controls and no significant change was observed as compared to the lithium treated rats (table 3).

Table 3: Effect of zinc on Na+/K+ ATPase activity in thyroid following lithium administration

S. No.	Groups	nmoles of Pi produced/min/ mg protein
I	Normal Control	50.49±3.28
II	Lithium treated	38.83±3.26[c]
III	Zinc treated	52.95±5.20
IV	Lithium + Zinc treated	42.34±4.23[b]

Values are expressed as Mean ± S.D of 6 to 8 animals. [b]$p<0.01$, [c]$p<0.001$ by Newman-Keul's test when the values of groupie and IV are compared with those of group I.

4. CONCLUSION

It can be concluded from the present study that zinc supplementation tend to normalise the adverse effects of lithium to some extent. This might be due to the fact that zinc is acting at the level of thyroid hormone synthesis, thereby improving the activities of the enzymes involved. The effect of zinc might also be due to its anti oxidative nature; causing elevation of antioxidant enzyme expression and thereby ameliorate the adverse effects of lithium. However, further studies are required to prove this in the present model.

Conflict of interest

Authors do not have conflict of interest in view of work carried out and results reported.

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