Zinc sulphate following the administration of iodine-131 on the regulation of thyroid function, in rats

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Abstract

Hyperthyroidism in men is often treated with high doses of iodine-131 (^{131}I), which may induce radiation side effects to patients and their environment. These therapeutic doses of ^{131}I could be decreased, if the ^{131}I uptake of the thyroid gland of the patients could be increased. Zinc sulphate has been considered to exercise a protective role by maintaining the cellular integrity of the thyroid under various pathological states. *The aim* of our study was to study in Wistar rats whether zinc sulphate can after treatment of the thyroid gland with ^{131}I: a) increase the uptake of ^{131}I in the thyroid and b) stabilize the function of the follicular cells. If such a stabilization finally exists in men we could have favorable results like fewer cases of hypothyroidism after ^{131}I treatment of hyperthyroidism. *To carry out* these investigations, rats were divided into four groups comprising of eight animals each. Group I animals served as normal controls. Group II animals received a dose of 3.7 MBq of ^{131}I. Group III animals were supplemented with zinc (227 mg/L of drinking water) and animals in Group IV were given ^{131}I together with zinc sulphate as above. *Our results* showed that in Group II, serum levels of tetra-iodo-thyronine (T_4) and tri-iodo-thyronine (T_3) decreased significantly as a function of time following ^{131}I treatment. An increase in the levels of serum thyroid stimulating hormone (TSH) was noticed one week after ^{131}I treatment, becoming less pronounced with time. In Group II, thyroid uptake at 2h and at 24h was significantly decreased. In the same Group biological half life (T_{biol}) of ^{131}I in the thyroid gland, was significantly elevated four weeks after the administration of ^{131}I and decreased eight weeks after. In Group IV animals, zinc sulfate after four weeks, induced normalization of elevated serum TSH levels and a further increase in the T_{biol} of ^{131}I. After eight weeks in these animals, serum T_3 became normal and TSH remained at normal levels. Thyroid ^{131}I uptake at 2 and 24 h was increased as compared to Group II. Group III animals showed some increase in the levels of Na^+K^+ATPase and type 1,5'-deiodinase (5'-DI) as compared to normal rats of Group I. *In conclusion*, this study suggests the protective potential of zinc sulphate in the disturbed after ^{131}I treatment, thyroid function, thyroid hormones and TSH while the ^{131}I uptake was reduced. Thus, if this result is further confirmed, zinc sulphate may show to be a promising radioprotective agent for the thyroid gland.

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Introduction

Hyperthyroidism is a rather common clinical condition, with an up to 5% lifetime risk in women [1]. Surgery as a treatment is often not recommended and since many patients treated with antithyroid drugs will remit, the majority of patients are treated with iodine–131 (^{131}I) [2-5]. Hypothyroidism which often complicates ^{131}I this treatment [6, 7] and is also seen in rats [8]. It would be useful to identify agents that could increase the uptake and retention of ^{131}I in the rat thyroid, thus increasing the radiation dose delivered to the thyroid gland and reducing the overall dose of radioactivity administered to the patients. In a study from our laboratory, lithium has been shown to increase the uptake and retention of ^{131}I in the rat thyroid gland [9]. Zinc is an essential trace element in men, relatively nontoxic [10], ubiquitous in sub-cellular metabolism and essential component of catalytic sites of enzyme classification [11, 12]. It has been shown in men that zinc has an antioxidant effect and stabilizes cell membranes [13, 14]. Another putative mechanism of zinc action is in thyroid hormone metabolism, where it is required for thyroid hormones attachment to their receptor [15, 16]. Thyroid hormone receptors in men require zinc ion [17, 18], which facilitates their folding into an active shape [19]. The present study was planned to elucidate the role of zinc on the thyroid function of ^{131}I treated rats.

Materials and methods

Animals

Female *Wistar* rats weighing 145-160 g were used in this study. The principles of animals care as laid down by the National Institute of Health (NIH publication no. 85-23, revised in 1985), were strictly followed. The animals were procured from the Central Animal House, Panjab University and were acclimatized in the departmental animal quarters for one week, before subjected to various treatment schedules.

Experimental design

Animals were segregated into four groups. Each group comprised of eight rats and was subjected to different treatments for a period of eight weeks. Animals in Groups II and IV were given a dose of 3.7 MBq of carrier-free ^{131}I, intraperitoneally [20]. Animals in Group IV additionally received zinc sulfate as $ZnSO_4.7H_2O$ at a dose level of 227 mg/L added to their drinking water for a total duration of eight weeks as was previously reported by us [14, 21, 22]. Animals in Groups I and III served as untreated normal and zinc treated controls, respectively. Animals in Groups III and IV received the same zinc treatment.

Thyroidal radioiodine uptake measurements

Animals of Groups II and IV were injected intraperitoneally with 0.37 MBq of carrier-free ^{131}I procured from BRIT-BARC (Mumbai, India). ^{131}I uptake measurements over the thyroid were performed at 2 h, 24 h at 4 and 8 weeks by using a well-type gamma-sensitive probe (ECIL, Hyderabad, India). For these measurements, the rats were under light ether anesthesia and held over a suitably shielded gamma sensitive probe, in such a way that only the neck embracing the thyroid was exposed to the probe through a hole of 1.7 cm in diameter in the lead shield. The lead shield had a length of 18.0 cm, width of 10.5 cm and thickness of 1.5 cm, and a hole in one corner. This shield was kept over the probe serving the dual purpose of exposing the thyroid to the detector and preventing the background (bg) body radiation from reaching the detector [23]. Furthermore, a glass sheet of 0.5 cm was placed above this shield to avoid fecal/urinary contamination of the lead shield during the uptake measurements. During the course of recording the radioactivity, five sets of measurements of 20 sec each were recorded, in order to minimize the statistical error, which was found to be 1.4%. The standard activity of ^{131}I equivalent to that injected in each animal, was also measured each time under similar conditions and geometry, to account for the physical decay of the radioisotope, the possible instrument error during the study and to calculate the percentage uptake values of ^{131}I by the thyroid at 2 h and 24 h.

In order to determine the biological half life (T_{biol}) of ^{131}I in the thyroid gland, the percentage of ^{131}I uptake values at different time intervals from 24 h onward, were calculated by taking the 24h uptake as 100%. Biological bg was subtracted. The percent thyroidal ^{131}I uptake values were plotted on the log scale y-axis and the time interval on the linear scale x-axis of the semi log paper. Further, the T_{biol} of ^{131}I was interpolated from the semi-log plot and was calculated by taking the difference on the x-axis of any two points, where the percentage uptake was bisected [24].

Estimation of serum total T_3, T_4 and of serum TSH

Animals were anaesthetized using mild ether anesthesia and blood samples were drawn from all groups at different time intervals by puncturing the ocular vein, (retro-orbital plexus). Serum was separated by centrifugation and stored at –20°C until analysis for the levels of the above hormones. Serum concentrations of total triiodothyronine (T_3) and thyroxine (T_4) were determined by radioimmunoassay and thyroid sfimulating hormone (TSH) concentrations by immunoradiometric assay. The kits were procured from BRIT, BARC, Mumbai (India).

Biochemical estimations

Animals from all groups were sacrificed after subjecting them to ether anesthesia at the end of the eight weeks study. Thyroids were removed and homogenized with a Potter Elvenjhem homogenizer. For the estimation of the enzymes activity, homogenates 0.5%, were prepared in tris HCl buffer (pH 7.5). In the tissue homogenates Na^+K^+ATPase activity was estimated by the method of Wallech and Kamat (1966) [25]. Type 1, 5'-deiodinase activity was estimated by the method of Behne et al (1990) [26].

Statistical analysis:

The statistical significance of the data has been determined using one-way analysis of variance (ANOVA) and a multiple post-hoc test (Student's Newman Keuls) with 5% considered significance. The results were represented as mean ±SD. ANOVA tests the hypothesis of no differences between the treated groups but does not determine which groups are different, or the size of these differences. So this multiple comparison test was done in order to isolate these differences by running comparisons between the experimental groups.

Results

Serum levels of total T_3 and T_4 and of TSH in animals of all groups at different treatment intervals of one, four and eight weeks, are presented in Tables 1, 2 and 3, respectively.

In Group II but not in Group IV the levels of total T_3 and T_4 were found to be significantly decreased at different time intervals as compared to normal controls. In Group IV a significant increase in total T_3 was found after eight weeks of zinc sulphate treatment (Table 1).

In Group II one week after ^{131}I treatment, there was a highly significant increase in the levels of TSH ($P< 0.001$; 65.62%), however there was no significant difference after four and eight weeks of ^{131}I treatment. The levels of TSH in Group IV were significantly decreased as compared to those of Group II when zinc sulpahte was supplemented for one week and became normalized after four and eight weeks (Table 3).

Table 1. *Serum total T_3 levels in all four Groups (ng/ml)*

Groups	Time interval		
	One week	Four weeks	Eight weeks
I	1.37 ± 0.36	1.04 ± 0.09	1.22 ± 0.13
II	0.58 ± 0.09a	0.46 ± 0.07a	0.76 ± 0.07a
III	1.08 ± 0.12b	1.13 ± 0.29	1.22 ± 0.08
IV	0.58 ± 0.13a	0.53 ± 0.07a	0.99 ± 0.07a, d

a: P <0.001 and b: P <0.01 by Newman-Keuls test when the indicated values were compared with those of Group I and
d: P <0.001 when the indicated values of Group IV were compared with those of Group II.

Table 2. *Serum total T_4 levels in all four Groups (μg/ml)*

Groups	Time interval		
	One week	Four weeks	Eight weeks
I	11.55 ± 0.96	9.32 ± 1.50	9.68 ± 0.58
II	9.25 ± 1.02a	4.28 ± 0.54a	7.92 ± 0.43a
III	10.87 ± 0.78	10.41 ± 0.45c	8.58 ± 0.50a
IV	10.26 ±1.46c	5.40 ± 0.58d	6.92 ± 0.23a,d

a: P <0.001 by Newman-Keuls test when the indicated values were compared with those of Group I.
c: P <0.01 and d: P <0.001 when the indicated values of Group IV were compared with those of Group II.

Table 3. *Serum TSH levels in all four Groups (ulU/ml)*

Groups	Time interval		
	One week	Four weeks	Eight weeks
I	0.64 ± 0.04	0.65 ± 0.03	0.62 ± 0.04
II	1.06 ± 0.08a	0.77 ± 0.07b	0.51 ± 0.07b
III	0.72 ± 0.06c	0.76 ± 0.05b	1.01 ± 0.05a
IV	0.80 ± 0.07a,d	0.66 ± 0.07e	0.66 ± 0.07 f

c: P <0.05, b: P <0.01 and a: P <0.001 by Newman-Keuls test when the indicated values were compared with those of Group I.
f: P <0.05, e: P <0.01 and d: P <0.001 by Newman-Keuls test when the indicated values of Group IV were compared with those of Group II.

Table 4. *The 2 h and 24 h percentage thyroid uptake of ^{131}I in all Groups*

Groups	2 h		24 h	
	Four weeks	Eight weeks	Four weeks	Eight weeks
I	38.51±6.30	37.03±6.32	54.48±10.2	60.69±2.50
II	16.04±3.11a	16.81±3.90a	23.28±6.60a	34.16±3.43a
III	25.85±4.73a	43.28±2.09 b	69.97±8.92a	67.41±3.22a
IV	18.92±2.90a	25.92±5.74a,d	27.83±2.91a	38.40±3.24d,e

b: P <0.001 and a: P <0.01 by Newman-Keuls test when the indicated values were compared with control Group I.
e: P <0.05 and d: P <0.001 when the indicated values of Group IV were compared with those of Group II.

Table 5. *The effect of zinc sulphate on the biological half-lives of ^{131}I in the thyroids of all four Groups of rats.*

Groups	T_{biol} (days)	
	Four weeks	Eight weeks
I	3.31 ± 0.17	3.81 ± 0.25c
II	3.87 ± 0.30b	3.45 ± 0.10
III	3.70 ± 0.29b	3.54 ± 0.21c
IV	4.21 ± 0.28d,a	3.61 ± 0.10e

c: P <0.05, b: P <0.01 and a: P <0.001 by Newman-Keuls test when the values indicated are compared with those of Group I.
e: P <0.05, and d: P<0.001 by Newman-Keuls test when the values indicated of Group IV were compared with those of Group II.

Table 6. *The effect of zinc sulphate on the Na^+K^+ATPase and type 1,5'-deiodinase thyroid activity in all four Groups of rats.*

Groups	Na^+K^+ATPase (nmol of Pi liberated /min/mg protein)	Type 1,5'-deiodinase (μg of T_3 produced/mg protein)
I	7.33 ± 0.63	0.90 ± 0.16
II	3.28 ± 0.81a	1.04 ± 0.08
III	8.73 ± 0.61c	1.44 ± 0.08a
IV	4.87 ± 1.01a,d	1.17 ± 0.07e

c: P<0.05 and a: P<0.001 by Newman-Keuls test when the indicated values are compared with those of Group I.
e: P<0.01 and d: P<0.001 by Newman-Keuls test when the indicated values of Group IV were compared with those of Group II.

In Group II statistically significant decrease of the 2 h and the 24 h ^{131}I uptake was noticed after four and eight weeks of ^{131}I treatment (Table 4). On the contrary, the 24 h ^{131}I uptake in Group III as compared to Group I rats showed significant elevation both at four and eight weeks (28.4%, P<0.001) and (11.0%, P<0.001) respectively, as compared to their normal controls (Group I). However the 2 h thyroid ^{131}I uptake in Group III rats showed significant depression up to four weeks and significant elevation after eight weeks. Interestingly, in Group IV, zinc and ^{131}I treatment for different periods of time, resulted in higher uptake values than in Group II (Table 4).

The T_{biol} of ^{131}I increased significantly in Group II after four weeks of ^{131}I treatment (9.44%, P<0.01). Group IV rats after four weeks of treatment resulted in a significant increase in $T_{(biol)}$ of ^{131}I (8.78%, P<0.001) as compared to Group II rats. Also, Group III rats had a significant increase (11.78%, P<0.01) in T_{biol} after four weeks of zinc sulfate treatment (Table 5).

Enzyme activity of Na^+K^+ATPase and of type-1 5'-deiodinase are presented in Table 6. Na^+K^+ATPase activity in Group II rats as compared to Group I was found to be depressed by 57.5% (P<0.001). In Group IV, supplementation of zinc sulphate significantly attenuated as compared to Group II the levels of Na^+K^+ATPase by 48.4% (P<0.001). Type-1 5'-deiodinase activity showed an insignificant increase in Group II rats.

Discussion

A significant reduction of total serum T_3 and T_4 was observed as a function of time following ^{131}I administration after four weeks and a relative increase after eight weeks. Following ^{131}I treatment, the levels of circulating thyroid hormones have been reported to decrease both in humans [27, 28] and in animals [29]. Following this reduction, an increase in the levels of TSH was observed that decreased with time and even got slightly below normal levels after eight weeks of ^{131}I treatment indicating a feedback mechanism. However, zinc sulphate supplementation to ^{131}I treated Group IV showed a significant decrease of TSH. It has been reported that zinc brings TSH levels to normal limits [30]. However these animals also showed recovery in the levels of T_3, which tended to become normal as zinc treatment continued. This effect could possibly be due to increased formation of T_3 from T_4 with the support of iodothyronine 5'-deiodinase. Others have also reported that oral zinc supplementation could increase plasma levels of TSH in uremic patients under peritoneal dialysis [31]. Our previous studies have shown that zinc administration to hepatotoxic animals restored serum total T_3 levels to normal [17].

The significant reduction of thyroid uptake of ^{131}I after the administration of ^{131}I (Group II) is in agreement with earlier studies in humans [31-34] and rats [35]. Interestingly, zinc supplementation alone to normal rats Group III showed significant elevation of the 24 h ^{131}I uptake that may be due to the increased level of TSH, which in our opinion seems to induce increased Na^+/K^+ ATPase enzyme activity. There are also reports that exposure to zinc alone afforded a 20% increase in Na^+/K^+ ATPase enzyme activity of cultured rat cortical cells [36]. Zinc treatment to ^{131}I treated rats (Group IV) for a period of eight weeks as in the present study, also resulted in a significantly higher 2h and 24h thyroid ^{131}I uptake values which also could be due to the increased TSH levels, in these animals.

^{131}I treatment resulted in a significant increase in T_{biol} after four weeks of ^{131}I treatment but not after eight weeks. This could be due to damage of the histo-architecture thereby affecting the release of ^{131}I from the thyroid gland [37]. However, this increase decreased with time, which could be due to the recovery of the damaged thyroid follicles as a function of time. Zinc administration for a period of eight weeks to the ^{131}I treated rats (Group IV), normalized the elevated T_{biol} of ^{131}I. Zinc has earlier been shown in rats to improve the altered histopathology of liver in stress-induced conditions [21].

Na^+K^+ATPase is the membrane spanning protein complex, responsible for the extrusion of Na^+ and the absorption of K^+ by most of the animal cells. A decrease in the levels of Na^+K^+ATPase was found in our ^{131}I treated (Group II) that could be elaborated to the decreased thyroid uptake observed in our study. However, zinc treatment to ^{131}I treated rats, significantly increased the Na^+K^+ATPase activity, which was still far from being normal. Others have also observed the protective effect of zinc against abeta toxicity, which was attributed to the enhancement of Na^+K^+ATPase in cultured rat cortical cells [36]. This effect prevents the disruption of calcium homeostasis and of cell death associated with abeta toxicity.

An insignificant increase in type 1,5'-deiodinase activity was observed in the ^{131}I treated group. Interestingly, zinc supplementation also showed an insignificant increase in enzyme activity suggesting the contribution of zinc to the conversion of the T_4 to T_3 [38]. Others have stated in men, a decrease in type 1,5'-deiodinase activity in case of zinc deficiency [39].

In conclusion, this study suggests the protective potential of zinc sulphate in the disturbed after ^{131}I treatment, thyroid function -thyroid hormones and TSH while the ^{131}I uptake was reduced. Thus, if this result is further confirmed, zinc sulphate may show to be a promising radioprotective agent for the thyroid gland.

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