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

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\(_{1/2}\)) 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\(_{1/2}\) 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.

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 $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ 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 ($T_{\text{bio}}$) 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 $T_{\text{bio}}$ 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 $-20^\circ\text{C}$ 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 stimulating 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 Elvenjem 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 Wallich 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 sulphate was supplemented for one week and became normalized after four and eight weeks (Table 3).
Interestingly, in Group II, zinc and $^{131}$I uptake values showed a significant depression up to four weeks and significant elevation after eight weeks. In Group III, the levels of Na$^+$K$^+$ATPase and of type-1 5'-deiodinase activity showed an insignificant increase in Group II rats as compared to Group I. Also, significant increase (11.78%, $P<0.01$) in T$_{3}$ concentration was observed in Group II rats after four weeks of zinc sulfate treatment (Table 2).

The $T_{bol}$ of $^{131}$I increased significantly in Group II after four weeks of $^{131}$I treatment (9.44%, $P<0.01$). Group IV rats showed a significant increase in the type-1,5'-deiodinase thyroid activity in all four Groups of rats.

The 2 h and 24 h percentage thyroid uptake of $^{131}$I in all Groups are presented in Table 4. Na$^+$K$^+$ATPase and of type-1 5'-deiodinase activity in all four Groups of rats.

Table 1. Serum total T$_3$ levels in all four Groups (ng/ml)

<table>
<thead>
<tr>
<th>Groups</th>
<th>One week</th>
<th>Two weeks</th>
<th>Eight weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.37 ± 0.36</td>
<td>1.04 ± 0.09</td>
<td>1.22 ± 0.13</td>
</tr>
<tr>
<td>II</td>
<td>0.58 ± 0.09a</td>
<td>0.46 ± 0.07a</td>
<td>0.76 ± 0.07a</td>
</tr>
<tr>
<td>III</td>
<td>1.05 ± 0.12b</td>
<td>1.13 ± 0.29</td>
<td>1.22 ± 0.08</td>
</tr>
<tr>
<td>IV</td>
<td>0.58 ± 0.13a</td>
<td>0.53 ± 0.07a</td>
<td>0.99 ± 0.07a, d</td>
</tr>
</tbody>
</table>

Table 2. Serum total T$_4$ levels in all four Groups (µg/ml)

<table>
<thead>
<tr>
<th>Groups</th>
<th>One week</th>
<th>Two weeks</th>
<th>Eight weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>11.55 ± 0.96</td>
<td>9.32 ± 1.50</td>
<td>9.68 ± 0.58</td>
</tr>
<tr>
<td>II</td>
<td>9.25 ± 1.02a</td>
<td>4.28 ± 0.54a</td>
<td>7.92 ± 0.43a</td>
</tr>
<tr>
<td>III</td>
<td>10.87 ± 0.78</td>
<td>10.41 ± 0.45</td>
<td>8.58 ± 0.50a</td>
</tr>
<tr>
<td>IV</td>
<td>10.26 ± 1.46c</td>
<td>5.40 ± 0.58d</td>
<td>6.92 ± 0.23a,d</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>One week</th>
<th>Two weeks</th>
<th>Eight weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.64 ± 0.04</td>
<td>0.65 ± 0.03</td>
<td>0.62 ± 0.04</td>
</tr>
<tr>
<td>II</td>
<td>1.06 ± 0.08a</td>
<td>0.77 ± 0.07b</td>
<td>0.51 ± 0.07b</td>
</tr>
<tr>
<td>III</td>
<td>0.72 ± 0.06c</td>
<td>0.76 ± 0.05b</td>
<td>1.01 ± 0.05a</td>
</tr>
<tr>
<td>IV</td>
<td>0.80 ± 0.07a,d</td>
<td>0.66 ± 0.07e</td>
<td>0.66 ± 0.07f</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Two hours</th>
<th>Eight hours</th>
<th>Two hours</th>
<th>Eight hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>38.51±6.30</td>
<td>37.03±6.32</td>
<td>54.48±10.2</td>
<td>60.69±2.50</td>
</tr>
<tr>
<td>II</td>
<td>16.04±3.11a</td>
<td>16.81±3.90a</td>
<td>23.28±6.60a</td>
<td>34.16±3.43a</td>
</tr>
<tr>
<td>III</td>
<td>25.85±4.73a</td>
<td>43.28±2.09b</td>
<td>69.97±8.92a</td>
<td>67.41±3.22a</td>
</tr>
<tr>
<td>IV</td>
<td>18.92±2.90a</td>
<td>25.92±5.74a</td>
<td>27.83±2.91a</td>
<td>38.40±3.24a,d</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Four weeks</th>
<th>Eight weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3.31 ± 0.17</td>
<td>3.81 ± 0.25c</td>
</tr>
<tr>
<td>II</td>
<td>3.87 ± 0.30b</td>
<td>3.45 ± 0.10</td>
</tr>
<tr>
<td>III</td>
<td>3.70 ± 0.25b</td>
<td>3.54 ± 0.21c</td>
</tr>
<tr>
<td>IV</td>
<td>4.21 ± 0.28da</td>
<td>3.61 ± 0.10e</td>
</tr>
</tbody>
</table>

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 $T_{bol}$ of $^{131}$I increased significantly in Group II after four weeks of $^{131}$I treatment (9.44%, $P<0.01$). Group IV rats showed a significant increase in the type-1,5'-deiodinase thyroid activity in all four Groups of rats.

After four weeks of treatment resulted in a significant increase in $T_{bol}$ 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 $T_{bol}$ 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 T₃ and T₄ was observed as a function of time following ¹³¹I administration after four weeks and a relative increase after eight weeks. Following ¹³¹I 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 went slightly below normal levels after eight weeks of ¹³¹I treatment indicating a feedback mechanism. However, zinc sulphate supplementation to ¹³¹I treated Group IV showed a significant decrease of TSH. It has been reported that zinc brings normal as zinc treatment continued. This effect could possibly resulted in a significantly higher 2h and 24h thyroid function -thyroid hormones and TSH while the ¹³¹I 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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Bibliography

18. Sustrova M, Strbak V. Thyroid function and plasma immunoglobulins in...
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21. Bandhu HK, Singh B, Garg ML et al. Hepatoprotective role of zinc indicated by hepatobiliary clearance of 99m-Tc-mebrofenin in protein defi-
23. Singh B, Dhanaw D. Effect of lithium on thyroidal 131I uptake, its clearance, and circulating levels of triiodothyronine and thyroxine in lead-
24. Sidhu P, Garg ML, Dhanaw DK. Effect of zinc on biological half-lives of 65Zn in whole body and liver and on distribution of 65Zn in different or-
29. Singh B, Dhanaw D. Effect of lithium on thyroidal 131I uptake, its clear-
ance and circulating levels Rad Environ Biophys 1999; 38: 261-266.
30. Licastro F, Mocchegiani E, Zannotti M et al. Zinc affects the metabolism of thyroid hormones in children with Down’s syndrome: Normalization of thyroid stimulating hormone and of reversal triiodothyronine plasma lev-
32. Larsen LG. Studies on radioiodine treatment of thyrotoxicosis with special reference to the behavior of the radioiodine tracer tests. Acta Radiol 1955; 126-135
36. Lovell MA, Xie C, Markeberg WR. Protection against amyloid beta pep-
38. Nishiyama S, Futagoishi Y, Suginohara Y et al. Zinc supplementation af-
39. Králík A, Eder K, Kirchgesser M. Influence of zinc and selenium defi-