Cimetidine administration decreases radiogenic damage on the thyroid gland in mice

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Abstract

Purpose: To study if Cimetidine administration could ameliorate the thyroid damage in external radiation.

Materials and methods: Forty healthy male adult mice were used in the present study. The animals were randomized into four groups. Untreated mice (Group 1) that received 1 mg/kg saline intraperitoneally (IP). Group 2 received a single 10 Gy gamma radiation dose with 1 mg/kg saline IP and group 3 were treated with Cimetidine IP. Group 4 was irradiated 1 hour after treatment with Cimetidine. The serum were assayed for the contents of triiodothyronine (T3), tetraiodothyronine (T4), thyroid stimulating hormone (TSH), Free T4 (FT4) and Cortisol using a radioimmunological technique 7 days following radiation. The thyroid tissue was processed and stained with hematoxylin and eosin (H&E), and periodic acid-Schiff (PAS) for histological examination. Data were statistically analyzed using Tukey’s post-hoc test and were considered significant at p < 0.05.

Results: External radiation resulted in weight loss and reduction of serum thyroid hormone levels. However, Cimetidine administration prevented marked changes. Histological study showed that Cimetidine injection to irradiated mice minimized the thyroid damage.

Conclusions: These findings suggested that Cimetidine administration 1 hour before radiation exposure was potent in ameliorating the thyroid damages.

Keywords: Cimetidine, thyroid gland, external radiation, mouse

Introduction

External radiation is extensively used in diagnosis and treatment of various disorders in patients. It can affect cells and tissues especially those possessing a greater reproductive activity including thyroid gland (Clifton 1986). The functional units of the thyroid gland are thyroid follicles that are lined by simple cuboidal epithelium. The primary products of the thyroid follicular cells are triiodothyronine (T3) and tetraiodothyronine (T4) (Banks 1993). There are numerous reports regarding the effect of irradiation on the thyroid functions in experimental animals and human. Lindsay et al. (1961) reported the degree of follicular atrophy and nuclear pleomorphism in rats in which thyroid glands had already been X-irradiated. Scott et al. (1984) investigated the presence and persistence of chromosome abnormalities in follicular epithelial cells after exposure to X-rays. Frantz et al. (1957) found follicular and papillary thyroid carcinomas in rats by X-irradiation. Irradiation has been implicated in the development of benign and malignant thyroid neoplasms in the human (Clark 1955, Simpson et al. 1955, Sheline et al. 1959). In this way, numerous studies have documented that radiation exposure is a well-established risk factor for the thyroid cancer (Ron 2003, Kleinerman 2006, Schonfeld et al. 2011, Cannizzaro et al. 2012).

Cimetidine, which is a histamine type 2 (H2) receptor antagonist used to treat patients with peptic ulcers and acid reflux (Jiang et al. 2010), has been demonstrated to have anticancer effects on those with colorectal cancer (Adams and Morris 1994, Morris and Adams 1995, Kelly et al. 1999, Sasson et al. 1999), renal cell carcinoma (Dexeus et al. 1990), malignant melanoma (Morton et al. 1987) and glioblastoma (Lefranc et al. 2006). In addition, it was shown that Cimetidine induced prolactin (PRL) result in thyroid disease is independent of the levels of thyroid hormone (Macaron et al. 1979). The short- and long-term effects of Cimetidine treatment on thyroid hormones were tested (Hugues et al. 1982, Knigge et al. 1982). The results suggested that Cimetidine administration in mice can modify the peripheral metabolism of thyroid hormones. Moreover, Perret et al. (1986) reported that Cimetidine affects the hepatic metabolism of thyroid hormones.

The present study aimed to investigate the probable effects of Cimetidine on reduction of radiologic damage on the thyroid gland in mice.
Materials and methods

Experimental animals
Thirty Balb/C healthy male adult mice weighing approximately 37 g were included in the present study. The animals were purchased from the Pasteur Institute, Tehran Iran. The mice were kept and utilized in accordance with the standard guide for the care and use of laboratory animals (Medical University of Tehran, Tehran, Iran. N. 91-01-159-18022). They were housed in a temperature of 23–25°C with a 12-h light cycle and were allowed standard mouse food (Pasteur Institute, Tehran, Iran) and water ad libitum.

Treatment and irradiation schedule
The animals were randomized into four groups of 10 animals each. Each mouse was weighed before and after experimental schedule. In group 1, mice received 1 mg/kg saline intraperitoneally (IP); in group 2, they received a single 10 Gy gamma radiation dose with 1 mg/kg saline IP. In group 3, the mice were treated with 1 mg/kg Cimetidine (Mahban Group, Tehran, Iran) IP. The mice of group 4 were irradiated with a single 10 Gy gamma radiation dose 1 hour after treatment with 1 mg/kg Cimetidine IP. Whole body irradiation was performed with cobalt 60 gamma radiation source (Theratron 780, Nordion, Ottawa, Ontario, Canada). Mice were placed in a ventilated Plexiglas cage and irradiated simultaneously. The distance of source to skin was 80 cm with a dose rate of 0.5 Gy/min⁻¹ at room temperature (23 ± 2°C) and the animals were irradiated with a total dose of 10 Gy gamma rays. This dose allowed us to follow the radiogenic damages and radioprotective effects of Cimetidine without fast losing the mice.

Hormone analysis
The animals were anesthetized using chloroform and blood samples were collected from hearts of all subjects on day 7 after treatment and irradiation, and then collected in vacutainers and sera were separated by centrifugation at 500 g for 15 min and were frozen at −20°C. After collection of all specimens, serum levels of T3, T4, thyroid stimulating hormone (TSH), Free T4 (FT4) and cortisol were measured by radioimmunoassay technique.

Histomorphometrical evaluation
After collecting thyroid tissues, they were vigorously flushed out in saline. The specimens were subsequently fixed in 10% buffered formalin. The tissues were routinely processed for light microscopy and embedded in paraffin. The paraffin-embedded blocks were cut into 5 μm semi-thin sections and stained with hematoxylin-eosin (H&E) and periodic acid-Schiff (PAS). After that, the tissue sections were dehydrated in alcohol, cleared in xylene, and mounted in a resins mountant (Entellan New, Merck, Darmstadt, Germany). The slides were observed under light microscope (YS2-T, Nikon, Tokyo, Japan) equipped with camera and the Axiovision software (Carl Zeiss, Oberkochen, Germany). The images were processed using Adobe Photoshop Cs (Adobe System, San Jose, CA, USA).

The total number of follicles, the number of large and small follicles, the diameter of large and small follicles, the number of large and small follicle’s cell, height of epithelial cells of follicles and also amount of colloidal substance in the follicles were measured. For each animal, every kind of measurements was made at 8 representative sections in each thyroid gland.

Statistical analysis
All values were reported as mean ± standard error. One-way analysis of variance followed by Tukey’s post-hoc test was used for statistical analysis. A value of p < 0.05 was considered significant.

Results

Body weight evaluation
The difference between the body weights before and after treatment in each group is indicated in Table I. There was a significant difference in values between different groups (p < 0.05). The mice treated with Cimetidine had a smaller body weight loss difference than those of animals without Cimetidine treatment before irradiation.

Serum concentration of hormones
Mean values of serum T3, T4, FT4, TSH and cortisol concentrations in the different groups are shown in Table II. Serum TSH concentration was decreased in the mice of group 2 compared with the relevant values in the animals of the other groups. Examination of FT4 serum level showed that values were decreased significantly in the mice of group 2 in comparison with those in the animals of groups 1 and 3 (p < 0.05). Serum FT4 concentration in the irradiated mice after Cimetidine treatment was higher than in the irradiated mice without Cimetidine treatment but the difference was not significant. Examination of cortisol serum level showed that values were decreased in the mice of group 2 in comparison with those in the animals of the other groups.

Table I. Analysis of the body weight parameters in experimental groups. Group 1 received 1 mg/kg saline intraperitoneally (IP). Group 2 received 1 mg/kg saline by IP with a single 10 Gy gamma radiation dose. Group 3 was treated with Cimetidine IP. Group 4 irradiated 1 hour after treatment with Cimetidine IP.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
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<tbody>
<tr>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td>Body weight before experiment (g)</td>
<td>36.5 ± 1.5a</td>
</tr>
<tr>
<td>Body weight after experiment (g)</td>
<td>37.8 ± 1.1a</td>
</tr>
<tr>
<td>Body weight differences (g)</td>
<td>1.3 ± 0.5a</td>
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</table>

The measurements from 10 animals are expressed as mean ± standard error values. Different superscript letters indicate a significant difference between all data in the same rows, p < 0.05.
Histomorphometrical characteristics

The comparison of the results from the histomorphometrical study of the thyroid gland in the four groups is summarized in Table III. The average follicular cell height in groups 1 and 3 was significantly different from those of the other groups (p < 0.05). The thyroid follicular cells in irradiated mice with Cimetidine treatment were higher than in irradiated mice without Cimetidine treatment, however, the difference was not significant. There was no significant difference between the total numbers of follicles in different groups. Treatment of the mice with Cimetidine before radiation resulted in an increase in the number of small follicles and a decrease of large follicles in comparison with the animals of group 2. The size of small and large follicles was increased significantly with irradiation while a lesser enlargement occurred in the mice treated with Cimetidine before irradiation (p < 0.05).

We further examined the histological changes of the thyroid gland in the mice of the different groups. The group 1 animals displayed a normal thyroid tissue in which the thyroid follicles were varied in size and location. The peripheral follicles were lined by flattened cuboidal epithelial cells while those of the central follicles were taller. The cytoplasm was eosinophilic and the nuclei were round. The colloid was uniform and appeared pale. The interfollicular connective tissue was normal without any vascular lesion (Figures 1A and 2A).

The irradiated mice of group 2, however, contained larger follicles than the mice of the control group (p < 0.05). Other points of interest were the loss of colloid, irregularity of arrangement and shape of follicles in thyroid glands of these animals. Also the basement membrane disappeared around some thyroid follicles (Figures 1 and 2B).

Approximately the histological features of thyroid tissues in the mice of group 3 were similar to those of group 1 (Figure 1C). The overlap of histological features of thyroid gland in radiated and non-radiated animals was seen in animals treated with Cimetidine before irradiation (Figure 1D).

Discussion

The findings of the present study suggested that Cimetidine administration at 1 hour before whole body irradiation could ameliorate damage on the thyroid gland in mice.

Whole body irradiation leads to body weight loss similar to what has generally been stated in previous studies (Anwar et al. 2013, Patel et al. 2013). Considering that the animals treated with Cimetidine before irradiation had a lesser body weight loss than those of irradiated animals without Cimetidine treatment before irradiation, the general radioprotective role of Cimetidine is obvious.

Serum levels of thyroid hormones are commonly used as reliable indicators of the thyroid function in humans and experimental animals (Clifton 1986, Badiei et al. 2009, Lombardo Grifol et al. 2013). The synthesis, storage and secretion of thyroid hormones occur in the follicular epithelium of thyroid gland. These reactions are controlled by pituitary thyroid stimulating hormone (TSH) which acts on follicular cells in thyroid gland. Pituitary TSH secreting is controlled by a negative feedback mechanism modulated by the circulating level of free T4 and free T3 and also by conversion of T4 to T3 in the pituitary thyrotropic cells. TSH secretion is also influenced by thyrotropin releasing hormone (TRH) synthesized in hypothalamus (Clifton 1986, Badiei et al. 2009).

In the present study serum concentrations of T4, FT4 and TSH were decreased in the radiated animals in group 2. Also Song and Evans (1968) showed gradual suppression of thyroid hormone synthesis after whole body X-irradiation with an exposure of 1000 R. Beyer and Schulze (1985) noticed the decrease of the total concentration of thyroid hormones after applying X-ray contrast media. They also implied that neither in the early phase within the first hour, nor in the late phase within 10 days after application of contrast media,

Table II. Analyses of the serum contents of triiodothyronine (T3), tetraiodothyronine (T4), thyroid stimulating hormone (TSH), Free T4 (FT4) and Cortisol in experimental groups. Group 1 mice received 1 mg/kg saline intraperitoneally (IP). Group 2 received 1 mg/kg saline IP with a single 10 Gy gamma radiation dose. Group 3 was treated with Cimetidine IP. Group 4 was irradiated 1 hour after treatment with Cimetidine IP.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
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</thead>
<tbody>
<tr>
<td>TSH (mIU/l)</td>
<td>0.33 ± 0.05a</td>
<td>0.13 ± 0.01b</td>
<td>0.29 ± 0.08a</td>
<td>0.28 ± 0.9a</td>
</tr>
<tr>
<td>T3 (nmol/l)</td>
<td>0.99a</td>
<td>0.97b</td>
<td>0.96a</td>
<td>0.9a</td>
</tr>
<tr>
<td>T4 (nmol/l)</td>
<td>93.21a</td>
<td>71.25b</td>
<td>94.95a</td>
<td>97.5a</td>
</tr>
<tr>
<td>FT4 (Pmol/l)</td>
<td>11.0 ± 1.5a</td>
<td>5.9 ± 0.5b</td>
<td>11.0 ± 0.5a</td>
<td>9.1 ± 0.5b</td>
</tr>
<tr>
<td>Cortisol (mg/dl)</td>
<td>6.9a</td>
<td>3.1b</td>
<td>5.8a</td>
<td>5.1a</td>
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</tbody>
</table>

The measurements from 10 animals are expressed as mean ± standard error values. Different superscript letters indicate a significant difference between all data in the same rows, p < 0.05.

Table III. Comparative analysis of the Histomorphometrical values in experimental groups. Group 1 mice received 1 mg/kg saline intraperitoneally (IP). Group 2 was received 1 mg/kg saline IP with a single 10 Gy gamma radiation dose. Group 3 was treated with Cimetidine IP. Group 4 was irradiated 1 hour after treatment with Cimetidine IP.

<table>
<thead>
<tr>
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<th>Group 4</th>
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<tbody>
<tr>
<td>Follicular cell height (μm)</td>
<td>31.3 ± 2.2a</td>
<td>7.2 ± 0.1b</td>
<td>25.3 ± 1.2a</td>
<td>9.6 ± 0.9b</td>
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<tr>
<td>Number of cells in small follicles (n)</td>
<td>8.2 ± 0.7a</td>
<td>7.0 ± 0.9a</td>
<td>8.4 ± 0.1a</td>
<td>8.5 ± 0.1a</td>
</tr>
<tr>
<td>Number of cells in large follicles (n)</td>
<td>21.5 ± 4.7a</td>
<td>26.0 ± 1.9b</td>
<td>27.2 ± 3.8a</td>
<td>30.2 ± 2.8a</td>
</tr>
<tr>
<td>Diameter of the small follicles (μm)</td>
<td>179.0 ± 2.0a</td>
<td>396.5 ± 23.0b</td>
<td>215.0 ± 5.0a</td>
<td>350.4 ± 35.7b</td>
</tr>
<tr>
<td>Diameter of the large follicles (μm)</td>
<td>458.0 ± 96.9a</td>
<td>1686 ± 121.4b</td>
<td>595.0 ± 90.9a</td>
<td>1520 ± 118.7b</td>
</tr>
<tr>
<td>Proportion of the small follicles to total follicles (%)</td>
<td>1.92a</td>
<td>16.37b</td>
<td>2.72a</td>
<td>4.53a</td>
</tr>
<tr>
<td>Proportion of the large follicles to total follicles (%)</td>
<td>26.29a</td>
<td>6.03b</td>
<td>23.06a</td>
<td>13.60a</td>
</tr>
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Radiation damage to thyroid reduced by Cimetidine

...protein synthesis occurs (Clifton 1986). Nadol’nik et al. (2003) studied the effects of single-dose external gamma irradiation on rat thyroid status during the year. It was concluded that the decreased concentration of blood thyroid hormones was not due to the activation of their peripheral metabolism; however, probably, to inhibition of their biosynthesis in thyroid cells under conditions of radiation-induced activation of oxidative stress. Hugues et al. (1982) suggested that Cimetidine administration can modify the peripheral metabolism rather than the secretion of thyroid hormones.

It is important to note that in this study serum level of TSH and thyroid hormones in animals treated with Cimetidine

blood samples should be collected for determination of thyroid function parameters, since otherwise misleading results may be obtained. Laway et al. (2012) stated that radiation could induce thyroid two kinds of damage, i.e., subacute damage and late damage. The former develops in almost all patients; however, the incidence of the latter varies considerably among different reports. On the other hand, there is little or no evidence of acute impairment of T3 and T4 production at low doses X-rays (Clifton 1986).

It is explained that iodinated protein release immediately after low doses of external radiation, occurs as a result of damage to the colloid endocytosis-hydrolysis processes whereas in higher doses, inhibition of iodide uptake and protein synthesis occurs (Clifton 1986). Nadol’nik et al. (2003) studied the effects of single-dose external gamma irradiation on rat thyroid status during the year. It was concluded that the decreased concentration of blood thyroid hormones was not due to the activation of their peripheral metabolism; however, probably, to inhibition of their biosynthesis in thyroid cells under conditions of radiation-induced activation of oxidative stress. Hugues et al. (1982) suggested that Cimetidine administration can modify the peripheral metabolism rather than the secretion of thyroid hormones.

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It is important to note that in this study serum level of TSH and thyroid hormones in animals treated with Cimetidine
before irradiation was higher than those of animals without Cimetidine treatment before irradiation. It demonstrated that administration of Cimetidine was also potent in amelioration of the radiogenic damage on thyroid cells. Radio-protective effects of Cimetidine in mouse bone marrow cells has previously been reported (Mozdarani and Gharbali 1993). Kojima et al. (2002) stated the protective effects of Cimetidine on radiation-induced micronuclei and apoptosis in human peripheral blood lymphocytes via OH radical scavenging and an intracellular antioxidation mechanism.

The histological features observed in the mice thyroid glands irradiated with X-rays closely resemble those observed previously in human and experimental animals (Lindsay et al. 1954, Doniach 1974, Nadol'nik et al. 2004, Berges et al. 2010). Tarnowska et al. (2003) reported diminishing of the follicle lining cells with the obliteration of the follicle structure of thyroid after radiotherapy which became more intense with aging. Histological changes in thyroid follicles, i.e., colloid separation, adhesion of follicles and number of large follicles, were decreased with Cimetidine treatment before irradiation. Although the average follicular cell height in groups 2 and 4 was significantly decreased in comparison with those of the control group, however, treatment of the mice with Cimetidine showed an increase in the follicular cell height in comparison with the irradiated animals without Cimetidine. As mentioned above, in the present study the TSH serum level was also decreased due to irradiation in group 2. Decreased height in follicular cells can be easily illustrated because TSH receptors are located on the basal surface of the follicular cells and induces its growth.

Conclusion

In conclusion, Cimetidine administration is helpful in minimizing the thyroid damage. Our results offer a baseline for future detailed studies with different doses of Cimetidine treatment and radiation exposure in various experimental animals.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Radiation damage to thyroid reduced by Cimetidine


