Introduction

Ketoconazole (KTZ), a synthetic imidazole antifungal, is effective for superficial fungal infections, genital candidiasis and chronic mucocutaneous candidiasis, and has been used in immunocompromised patients and advanced prostatic carcinoma (4,9). High doses of the drug, however, can exert an inhibitory effect of gonadal and adrenal steroidogenesis in vitro and in vivo. This endocrine effect can be rapidly reversed by interrupting the administration of the drug (14). Its chemical structure, comprising several heterocyclic rings and electron donor groups, indicated that KTZ may possess antithyroid activity (2). Namer et al (12) reported that some patients with high doses of KTZ (1200 mg/kg/day) for breast cancer presented a biological hypothyroidism that required hormone replacement. Kitching (10) reported on two patients with severe mucocutaneous candidiasis and suggested that hypothyroidism might be a rare but not unlikely side-effect of KTZ therapy. In the previous study, we found that high dose of KTZ (60 mg/kg/day) had antithyroid effect in the rats (1). The purpose of this study was to determine whether low dose KTZ (20 mg/kg/day) had antithyroid effect in vivo.

Material and Methods

KTZ in powder form (Bilim Pharmaceuticals, Istanbul) was suspended in 0.3% Noble agar and administered orally to a group of 13 rats, twice daily (20 mg/kg/0.1 ml dose) at 0700 and 1700 h for thirty days (5,8). A control group received a placebo solution for thirty days. Twenty-six rats in two groups were included in this study. Control group; and study group; 13 rats treated with oral KTZ (20 mg/kg twice daily) for thirty days. Blood samples were drawn from the jugular vein to determine serum triiodothyronine (T3), thyroxine (T4) and thyroid-stimulating hormone (TSH) levels at the initial and on the 30th day of therapy. At the initial the rats were weighed. At the end of the treatment period, the rats were killed and weighed. The body weights were compared with those of the controls. Thyroid glands were removed and weighed, and compared with those of the controls. Cross-section of thyroid glands was cut and examined histologically after suitable fixation and staining. A single cross-section (including both lobes) was evaluated for each animal and > 20 follicles were measured by calculating the area enclosed by the apical surface of the follicular cells. The larger, more inactive, follicles along the periphery were excluded from the analysis. The activity of the gland was indicated by the appearance of the cells, and by the presence or absence of colloid. Hyperfunction was scored from + to ++++ as a function of the ratio of cylindrical to cubical cells (2,3,13).

- = normal activity (< 5% of cylindrical cells);
+ = moderate activity (>5% to <= 50% of cylindrical cells);
++ = clear activity (>50 to 75% of cylindrical cells);
+++ = strong activity (>75 to 90% of cylindrical cells);
++++ = extra-strong activity (>90% of cylindrical cells).

Summary: To determine whether low dose ketoconazole (KTZ) has antithyroid action, we studied thyroid function tests in the 13 rats treated with KTZ (20 mg/kg twice daily) for thirty days. Serum triiodothyronine and thyroxine levels were decreased ($P < 0.05$) and serum thyroid-stimulating hormone levels were mildly increased ($P > 0.05$) at the end of treatment. Histopathological analysis of the thyroid glands demonstrated an increase in cylindrical cells in study group, but the epithelial cells were mainly cubical in control group. These findings showed that low dose KTZ had antithyroid effect in rats. The responsible mechanisms may be direct effect of the drug on thyroid gland.

Key words: Ketoconazole; Thyroid gland; Rat

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The concentrations of $T_3$ and $T_4$ in the serum were assayed using the radioimmunoassay (RIA) kit supplied by Diagnostic Products Corporation (Los Angeles, USA) and rat TSH with the RIA kit supplied by Amerlite Diagnostics Ltd. (Amersham UK). Statistical analysis was performed by Mann-Whitney U - Wilcoxon Rank Sum W test.

**Results**

The body and the thyroid gland weights of the animals are shown in Table 1. There was no statistically significant difference in the body weight and thyroid gland weight between the control and study group. In histopathological analysis of the thyroid gland, the epithelium was mostly cubical cells in control animals. In study group, cylindrical cells were increased in analysis of the thyroid glands. The hypothyroid state of the rats treated with KTZ was indicated by hyperfunction of the thyroid gland as indicated by the presence of cylindrical thyrocyes. However, there was no significant difference between the two groups ($P > 0.05$). Serum $T_3$, $T_4$, and TSH levels in the control group and on the 30th day of therapy of study group are shown in Table 2. Serum $T_3$ and $T_4$ levels on the 30th day of therapy were decreased in study group compared with control group ($P < 0.05$). Serum TSH levels were mildly increased at the end of treatment; however, there was not a significant difference between the groups for serum TSH levels ($P > 0.05$). Serum thyroid hormone levels ($T_3$, $T_4$, and TSH) at the initial of therapy in the study group and TSH at the initial of therapy in the control group were as follows; $T_3$: 0.0833 $\pm$ 0.0227 $\mu$g/dl (0.0462-0.1139 $\mu$g/dl), $T_4$: 5.26 $\pm$ 1.14 $\mu$g/dl (3.09-6.95 $\mu$g/dl), and TSH 1.54 $\pm$ 0.45 $\mu$U/L (0.99-2.42 $\mu$U/L). There was not a statistically significant difference for serum $T_3$, $T_4$ and TSH levels between the initial values of the therapy in the study group and control group ($P > 0.05$).

**Discussion**

The effects of KTZ on different endocrine systems such as testosterone synthesis and cortisol production have been reported in-vitro and in-vivo. However, there is little information available on its action on thyroid gland and thyroid cells. Kohan et al (11) reported that KTZ (1-50 $\mu$mol/l) slightly increased the basal iodide uptake but, at higher concentrations (75-100 $\mu$mol/l), it sharply decreased iodide uptake below the basal levels. The present work is the first report of KTZ action in a normal rat thyroid cell line. De Pedrini et al (6,7) studied the effect of KTZ on thyroid function in five patients with thyrotoxicosis, five patients with hypothyroidism and ten normal subjects treated with KTZ at high dose (600 mg/daily in three 200 mg oral doses) for four weeks. They found no inhibitory effect on the synthesis of thyroid hormones and thyroid function and they did not detect any antithyroid activity after administration of KTZ to the hypothyroid patients and ten healthy subjects.

**Tab. 1: The weights of body and thyroid gland of the rats.**

| Parameters         | Control Group (n: 13) | Study Group (n: 13) | z    | P  |
|--------------------|-----------------------|---------------------|------|----|
| First body weight (g) | X ± SD (min-max) (0.0312–0.1098) | X ± SD (0.0301–0.0837) |      |    |
| Last body weight (g) | 188.4 ± 40.3          | 184.6 ± 30.5        | 0.20 | >0.05 |
| Thyroid weight * X ± SD |                      |                     |      |    |
| Control group      | 10.66 ± 1.60          | -1.50 >0.05         |      |    |
| Study group        | 12.19 ± 3.44          |                     |      |    |

| Parameters | Control Group (n: 13) | Study Group (n: 13) | z    | P  |
|------------|-----------------------|---------------------|------|----|
| $T_3$ (μg/dl) | X ± SD (min-max) (0.0312–0.1098) | X ± SD (0.0301–0.0837) |      |    |
|            | 0.0721 ± 0.0199        | 0.0500 ± 0.0149     | -2.89 | 0.003 |
| $T_4$ (μg/dl) | X ± SD (min-max) (3.47–9.11) | X ± SD (2.50–4.17) |      |    |
|            | 6.23 ± 1.63            | 3.31 ± 0.53         | -4.13 | 0.00001 |
| TSH (μU/L)  | X ± SD (min-max) (0.61–1.87) | X ± SD (0.88–2.71) |      |    |
|            | 1.28 ± 0.41            | 1.55±0.57           | -1.07 | 0.281 |

The normal ranges of $T_3$, $T_4$ and TSH levels were as follows; $T_3$ 0.0204–0.1447 μg/dl; $T_4$ 2.25–9.89 μg/dl; and TSH 0.25–2.05 μU/L.

In our study, there was mild decreased body weight and mild increased thyroid weight in the study group compared with control group. Comby et al (2) observed a fall in body weight with respect to controls, which may have been due to a loss of appetite induced by the drug, and they also observed thyroid weight increased, as in our study. Comby et al (2) suggested KTZ could inhibit 5'-desiodase, which is responsible for $T_3$ and $T_4$ des-iodation, so keeping $T_4$ levels higher than expected in the in-vivo study. Our previous and present studies showed that both high and low dose KTZ had antithyroid effect in the rats. The responsible mechanism may be direct effect of the drug on thyroid gland. Nonetheless, since it is well-known that the changes of total plasma can effect the capacity and affinity of plasma proteins for thyroid hormones and the food intake has a clear cut effect on conversion of $T_4$ to $T_3$, these factors may also responsible for our results.

The work has been carried out in Erciyes University Faculty of Medicine, Department of Pediatrics, Kayseri, Turkey

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