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EFFECT OF ANTITHYROID AGENT (CARBIMAZOLE) ON THE DIURNAL MITOTIC RHYTHM OF THYROID FOLLICULAR CELLS OF GUINEA PIG

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ABSTRACT

Effect of carbimazole on the diurnal rhythm in mitotic activity of thyroid follicular cells of guinea pig was noted. Twelve male guinea pigs were kept under natural light and dark condition. These animals were given carbimazole treatment orally for 30 days. On the last day of treatment all the animals were sacrificed at selected periods of time at 15:00 hours during day time and at 03:00 hours during night time. Vincristine sulphate as a metaphase arrest agent was used. The mitotic nuclei in the cells of centrally placed follicles were observed and the mitotic rate was expressed as number of metaphases per one thousand cells.

No diurnal rhythm in mitotic activity of treated guinea pig was observed, the absence of diural rhythm might be due to decreased mitotic activity as has been observed.

INTRODUCTION

Administration of antithyroid drug (Carbimazole) in rats has produces a large rapid and persistant increase in mitotic activity in thyroid follicular cells which abolishes normal diurnal rhythm in mitotic activity (Redmond and Tuffery 1979).

Effect of antithyroid drugs (Thiouracil and Propyl thiouracil) causing hypertrophy and hyperplasia in the thyroid gland of guinea pig were reported (D'Angelo et al., 1950, 1951, 1953, 1954). But the effect of antithyroid drug (Carbimazole) on the diurnal mitotic rhythm of thyroid follicular cells in guinea pig has not been found in literature. Though the present authors have studied the presence of a typical diurnal rhythm with maximum mitotic activity during dark period of time and minimum mitotic activity during light period of time in thyroid follicular cells of normal guinea pig (Fatima and Khan 1998). Therefore it would be of great interest to observe the fate of diurnal mitotic rhythm whether it persists or not after carbimazole treatment in guinea pig. The degree of hypothyroidism induced in guiea pig either by antithyroid agent or by thyroidectomy is relatively mild as compared to rat which is five time more active (Rand et al., 1951).

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MATERIALS AND METHODS

Twelve young male guinea pigs of J.P.M.C. breed weighing between 200 and 300 grams were used for this study. All the animals were kept under natural light and dark conditions. Only four animals per cage were kept. The animals were maintained on carrots and Lucern adlibitum.

The animals were given Carbimazole treatment orally (0.13 mg/0.13 ml of solution per 300 gm body weight) for 30 days.

To see the effect of carbimazole on the diurnal rhythm in mitotic activity of thyroid follicular cells these animals were sacrificed in two groups having six animals each.

Group I *(treated day group):* six animals were killed during day time at 15:00 hours.

Group II *(treated night group):* six animals killed during night time at 03:00 hours.

On the last day of treatment metaphase arrest agent vincristine sulphate (1 mg/kg) was given intraperitonealy to each animals, three hours before sacrifice (Redmond and Tuffery, 1979, Fatima and Khan 1998).

All the animals were killed by an over dose of ether anaesthesia, thyroid glands were removed, fixed in Bouin's fixative, dehydrated with ethyl alcohol, infiltrated and embedded in paraffin. Five micron thick, sections were cut, every fifth section was selected and stained with Feulgen's stain (Bancroft & Harry, 1994).

OBSERVATIONS AND RESULTS

Observations were based on:

(i) Microscopic study
(ii) Wet weight of thyroid gland

(i) Microscopic study were carried out by observing only one high power field in every fifth section and the nuclei of central follicles were examined since the peripheral follicle differ from the more cental follicles in size and possibly also in sensitivity to carbimazole (Bloom and Fawcett 1986; Tuffery 1976). The mitotic rate was expressed as the number of meta phases per one thousand cells. Standard error (S.E.) of the mean was used (Swinscow 1976) to test for significant differences between mean values. Probably values less than 0.05 were considered significant.

(ii) Wet weight of thyroid gland of each animal was noted by torsion balance before fixation.

Note:

The results of normal healthy guinea pigs used to study the diurnal rhythm in mitotic activity of thyroid follicular cells by the same authors were used in this study as control to compare the results of Carbimazole treated guinea pigs because both the studies were carried out simultaneously.

Group I (treated day group): six animals killed at 15.00 hours. The mean mitotic rate was 7.33±1.64 metaphases per thousand cells. This value was slightly higher than the control day group (6.57+1.17 metaphases/1000 cells) the difference was statistically not significant ($p > 0.7$, table 2).

Group II (treated night group): six animals killed at 03:00 hours. The mitotic rate was 6.46 ± 1.48 metaphases per thousand cells. This value was much less than the control night group (13.36 ± 2.44 metaphases/1000 cells) the difference was statistically significant ($P < 0.05$, table 2). As compared with treated day group the difference was statistically not significant ($P > 0.7$, table 1).

Weight of thyroid gland

Group I (treated day group): the mean weight of thyroid gland was 73.0±4.06 mg. This value was higher than the control day group (43.66 ± 3.32 mg) and the difference was statistically significant ($P < 0.01$, table 4).

Group II (treated night group): the mean weight of thyroid gland was 83.80 ± 2.82 mg it was higher than the control night group (47.16 ± 1.27). The difference was statistically significant ($P < 0.01$, table 4), though the mean thyroid weight was higher than the treated day group but the difference was statistically not significant ($P > 0.05$, table 3).

DISCUSSION

The results of normal healthy guinea pigs found during the study of diurnal rhythm in mitotic activity of thyroid follicular cells by the same authors (Fatima and Khan, 1998) were used as control to compare the results of Carbimazole treated guinea pigs, because the animals in both studies were kept under same experimental environment.

The treated guinea pigs which were given Carbimazole treatment orally for 30 days did not show any sign of the presence of diurnal rhythm, in mitosis, though a typical diurnal rhythm in mitotic activity of thyroid follicular cells in guinea pigs has been witnesed by the same authors in other experiment (Fatima and Khan, 1998). The result obtained from treated group showed a statistically significant decrease in mitotic rate of treated night group when compared with control night group, and were found to be insignificantly less than treated day group.

The absence of diurnal rhythm in mitotic activity was due to decreased mitosis. Whereas Redmond and Tuffery (1979) reported absence of diurnal rhythm in mitosis due to three fold increase in mitotic activity in thyroid follicular cells of rat after three weeks carbimazole treatment.

The difference of mitotic activity in the present study and that of previous study (Redmond and Tuffrey, 1979) might be due to species difference between rat and guinea pig in response to the antithyroid drug and the pituitary thyroid system.

The thyroid hormones are regulated by the thyroid stimulating hormone (TSH) released by pituitary gland, the relationship of thyroid hormones to TSH is inversely proportional. Whereas the action of antithyroid drugs on thyroid gland is to block the synthesis of thyroid hormones cause reduction in the amount of circulating thyroid hormones hence indirectly increases the output of TSH which stimulate the thyroid gland to undergo hypertrophy and hyperplasia (Guyton, 1991). There should be a balance between hormone production and its peripheral utilization and TSH secretion.

In the absence of thyroid activity the duration of the euthyroid state would be inversely proportional to the rate of Peripheral utilization of the hormone. The time lag before stimulation of pituitary TSH production and the subsequent appearance of the goiter should be proportional to the rate at which thyroid hormone was utilized (Rand et al., 1951). In rat the rate of thyroid hormone synthesis is very much greater than guinea pig and the peripheral hormone utilization and equivalent levels of hypothyroidism occur five times more than guinea pig (Rand et al., 1951) the time lag for TSH production and goitrogenesis was much faster and earlier in rat (Redmond and Tuffery, 1981). Therefore 3 weeks carbimazole treatment induced hyperplasia with 3 fold increase in mitotic activity (Redmond and Tuffery, 1979).

In guinea pig the hormone synthesis, the peripheral hormone utilization is very slow (Rand et al., 1951). The blood titre of TSH in guinea pig as compared to rat and bird (Pigeon) was found to be low (D'Angelo and Gordon, 1950). The degree of hypothyroidism induced in guinea pig either by antithyroid agents or by thyroidectomy is relatively mild. Slight decrease in metabolic rate, inactive histologic picture of thyroid, low concentration of TSH in blood and pituitary all suggest that under ordinary laboratory condition the thyroid pituitary system of the guinea pig function at a relatively low level (D'Angelo et al., 1954).

Maximum histological changes in hyperplasia in thyroid gland of guinea pig after a prolonged continuous propylthiouracil treatment was observed by (D'Angelo et al., 1954) the thyroid hyperplasia is cumulative response to a sustained effective concentration of circulating TSH after a prolonged propylthiouracil treatment for 2-200 days in guinea pig. In present study the period of goitrogen (Carbimazole) treatment was very short (30 days) to induce marked hyperplasia with appreciable increase in mitotic activity of thyroid follicular cells, though hypertrophy of follicular cells was evidenced but that would be an immediate response to the inhibitory effect of carbimazole, as has been observed by D'Angelo et al. (1951) in some guinea pigs after 8 days of goitrogen administration.

The decrease in mitotic activity is thought to be due to the failure of thyroid follicular cells to under go mitosis because of inhibition of thyroid hormone production, which is thought to exert a significant mitogenic effect with intact pituitary gland. Because growth hormone and thyroxine are the two major growth promoting factors which may play a role in the growth response of the organism (Leblond and Carriere, 1955).

Weight of Thyroid Gland

The mean weight of thyroid gland in treated guinea pigs is significantly higher as compared to that of untreated normal (control) guinea pigs. The increase in weight of thyroid gland after treatment with antithyroid agents has been observed by various workers. As it is known that an inverse relation exists between the synthesis of thyroid hormone and its regulation by the TSH (thyrotropin) from anterior pituitary, the antithyroid drug inhibits the formation of thyroid hormone causes a decline in the level of circulating thyroid hormone which stimulate the output of TSH from anterior pituitary. The initial response of the thyroid gland to the rise in the level of thyrotropin (TSH) hormone in the blood is an increase in follicular cell height and the release of stored colloid (Uhleuhuth and Schwartzbech, 1927, 1928; Junkman and Scholler, 1932 both cited by Santler, 1957). This colloid is not replaced to any extent during the period of treatment with antithyroid drug (thiouracil) but loss of weight due to loss of colloid has only been reported as a very slight temporary effect (Adams and Buss, 1952).

The increase in weight and volume of the thyroid gland of rat that occurs during the administration of thiouracil is made up of an increase in the individual volume of the cells of the thyroid follicular cells, increase in the amount of blood vessel space and an increase in the number of follicular cells and the cells of the blood vessel walls and connective tissue (Santlar, 1957).

The increase in thyroid weight of guinea pig was observed approximately 1000 percent of normal control after six months treatment with propylthiouracil (D'Angelo et al., 1951).

An increase in the weight of thyroid gland in carbimazole treated guinea pigs in present study was found to be almost double the weight of control guinea pigs. This increase was made up by hypertrophy of thyroid follicular cells, increase in blood vessels spaces and an increase in amount of connective tissue elements. These present results confirmed the results of our previous study showing hypertrophy of thyroid follicular cells with a significant decrease in diameter of lumen of follicles, amount of colloid, increase in the diameter of follicles measured and an increase in connective tissue elements and vascularity of the gland, but all these changes were not very extensive due to short term (30 days) treatment of carbimazole in such an animals whose thyroid hormone metabolism is very slow with delayed TSH response to alter changes in histological picture of thyroid gland (Fatima and Khan, 1998).

Table 1
Mean Mitotic rate in treated animals

Animal Group	No. of Animals	*Mean Mitotic Rate per 1000 cells	Statistical Significance
I Treated Day Group	6	7.33 ± 1.64	P > 0.7 Not significant
II Treated Night Group	6	6.46 ± 1.48	

*Mean ± Standard Error.

Table 2
Mean Mitotic rate control and treated animals

Animal Group	No. of Animals	*Mean Mitotic Rate per 1000 cells	Statistical Significance
Control Day Group	6	6.57 ± 1.17	P > 0.7 Not significant
I Treated Day Group	6	7.33 ± 1.64	
Control Night Group	6	13.36 ± 2.44	P > 0.5 Significant
II Treated Night Group	6	6.46 ± 1.48	

*Mean ± Standard Error.

Table 3
Mean weight of thyroid gland in treated animals.

Animal Group	No. of Animals	*Mean weight of thyroid gland (mg)	Statistical Significance
I Treated Day Group	6	73.0 ± 4.06	P > 0.05
II Treated Night Group	6	83.6 ± 2.83	Not significant

*Mean ± Standard Error.

Table 4
Mean Weight of thyroid gland in control and treated animals

Animal Group	No. of Animals	*Mean Weight of Thyroid Gland (mg)	Statistical Significance
Control Day Group	6	43.66 ± 3.32	P > 0.01
I Treated Day Group	6	73.00 ± 4.06	Significant
Control Night Group	6	47.16 ± 1.27	P > 0.01
II Treated Night Group	6	83.60 ± 2.83	Significant

*Mean ± Standard Error.

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