Original Article

Restorative Effect of Thyroxine on Minocycline Induced Thyroid Gland Damage

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ABSTRACT

Objective: To evaluate the effects of thyroxine on minocycline induced thyroid gland in guinea pigs.
Study Design: Experimental study
Place and Duration of Study: This study was conducted at the anatomy of Basic Medical Sciences Institute (BMSI) of Jinnah Post Graduate Medical Center (JPMC), Karachi, from 1st October to 30th November 2015.
Materials and Methods: Thirty healthy, adult male guinea pigs weighing from 450-650 gram were selected. The animals were assigned to groups A, B & C, according to the experimental treatment. Group A was taken as control, group B received 0.02mg/g/day of Minocycline via nasogastric tube once daily, and group C was given same dose of Minocycline as in group B, with 0.5 µg/g/day thyroxine by same route, for the same duration. At end of study period, animals were sacrificed under ether anaesthesia. Blood samples were drawn from heart when animals were still breathing for levels of TSH and thyroid hormones. Thyroid gland was removed and processed. Tissue sections were stained with Mason Fontana stain to observe pigmentation in thyroid glands
Results: Serum TSH levels were raised in minocycline receiving group B when compared to control animals, whereas serum thyroid hormone levels were significantly reduced in group C where protection was provided with thyroxine. Marked pigmentation in thyroid tissue sections was observed in group B, although thyroxine had attenuated this effect in tissue sections from thyroid glands of Group C animals.
Conclusion: This study highlighted that minocycline caused thyroid gland damage, whereas thyroxine ameliorated this damage on thyroid gland.

Key Words: Minocycline, Thyroid gland, Thyroxine, Black pigmentation

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INTRODUCTION

Minocycline is a member of tetracycline group of antibiotics that exhibits antibacterial and anti-inflammatory outcomes[1]. Due to its high efficiency in controlling infections, it is widely used in dermatologic practice. Despite of its relevant outcome, studies have been reported that minocycline use can induce hyper pigmentation of various body tissues, including the skin, mucosa, teeth, conjunctiva, nail beds, bones and thyroid[2]. Adolescents treated for acne with minocycline also demonstrated thyroid abnormalities ranging from thyroid pigmentation to non-immune mediated thyroid dysfunction[3-5].

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The role of thyroxine in sequestrating the development of multiple organs has been observed for a long time. In recent years its involvement in maintaining body homeostasis and tissue regeneration has been extended to many organs like pancreas, liver and kidney.[6,7]
Although there are many studies which evidently established the influence of thyroxine on repair and regeneration of various body tissues,[6-8] however, scarce information is available regarding the role of thyroxine on black thyroid. To further explore the role of thyroxine, the present study investigated the effects of thyroxine on minocycline induced black pigmentation of the thyroid and the serum levels of TSH, T3 and T4 in model animals.

MATERIALS AND METHODS

This experimental study was undertaken in the anatomy department of BMSI, JPMC, Karachi, after obtaining ethical approval from ethical review committee of the institute, for 60 days from October-November 2015. Thirty healthy adult male guinea pigs were procured from the institute's animal house. The animals were segregated into three groups on the basis of the experimental treatment. The animals were observed for their food intake and weight gain for one week before the beginning of the study and maintained on 12 hours day and light cycle, to ensure their health. They were provided standard laboratory diet and water ad libitum.

They were weighed, numbered, and placed in propylene cages.

There were 10 animals in each group. Group A animals served as control. They were given standard laboratory diet only. Group B animals were treated with minocycline (Steifel Laboratories Pakistan (Pvt) Ltd, Steifel Laboratories Inc. Coral Gables, FL33134, USA) orally in a dose of 0.02mg/gram body weight/day9 via nasogastric tube. Group C animals were administered same dose of minocycline as in group B with thyroxine 0.5ug/gm body weight/day (Glaxo Smith Kline Pakistan Ltd, RN 000374, Ml 000017 & 000233) orally10, also by nasogastric tube.

After completion of the study period (60 days), the animals were sacrificed in a glass container under ether anaesthesia. The incision was made in the skin of neck from chin to sternum, then infrahyoid muscles were retracted to expose thyroid gland, and thoracic viscera were exposed to take blood sample by intra cardiac puncture when the animal was still breathing. 4ml blood was drawn and put in tubes without anticoagulant. The blood was then left for 10 minutes. Serum was obtained by centrifuging it at 4,000 rpm/minute for 10 minutes. It was stored at −20 °C until biochemical analysis was done for estimation of serum TSH, and serum thyroxine (T4), triiodothyronine (T3) levels11 by ELISA.

Thyroid gland was removed and washed using normal saline. Fixation was done by keeping the tissue in 10% buffered formalin for 24 hours12. Tissue was then kept overnight in 70% alcohol. It was then dehydrated in ascending grades of alcohol, starting from 80%, 90% and two changes of absolute alcohol (100%) for one hour each and cleared in two changes of xylene for one hour for each. Infiltration was done with paraffin at 58C°C in laboratory oven and paraffin block was made with paraffin embedding system12. Paraffin block was cut into 4 micron thick sections on rotary microtome and immersed in water bath at 42. They were taken on albumenized glass slides. Tissue sections were fixed on hot plate at 32°C and stained with Masson Fontana12.

Twenty observations were taken for each animal under 40X and 100X objective to observe the presence of pigmentation as sparse, densely packed or scattered in control and treated animals.

The statistical significance of differences in serum TSH and T3 & T4 levels, between control with minocycline and minocycline plus thyroxine treated guinea pigs was assessed by student 't' test. The difference was considered statistically significant if the 'P' value was equal to or less than 0.05. Computer software SPSS version 20 was used for calculations.

RESULTS

Serum thyroid stimulating hormone (TSH) levels (µl/ml) of all animals were estimated. The mean values of serum TSH in group A, group B and group C was 0.159 ± 0.01, 6.48 ± 0.26 and 0.736 ± 0.05 respectively (Table-1). There was a highly significant increase (P<0.001) in hormone level in group B when compared with group A animals. The serum levels of TSH decreased significantly (P<0.01) in group C animals in comparison to group B animals.

The levels of serum tri-iodothyronine (T3) and serum thyroxine (T4) were also determined in all animals for evaluation of thyroid function. The mean values of serum tri-iodothyronine (T3) and thyroxine (T4) levels in group A, B and C were 4.76± 0.31(ng/dl) & 34.5 ± 2.34µg/dl, 2.17± 0.15ng/dl & 15.8 ± 1.23µg/dl and 4.01 ± 0.28ng/dl & 29.3 ± 1.57µg/dl respectively (Bar chart-1). The animals of group B revealed a highly significant reduction (P<0.001) in serum levels of both T3 and T4 when compared with control group A animals. When these hormone levels were compared with group C, there was a significant increase (P<0.01) in both serum T3 and T4 levels in group C animals treated with thyroxine and minocycline as compared to minocycline treated group B animals (Table-1).

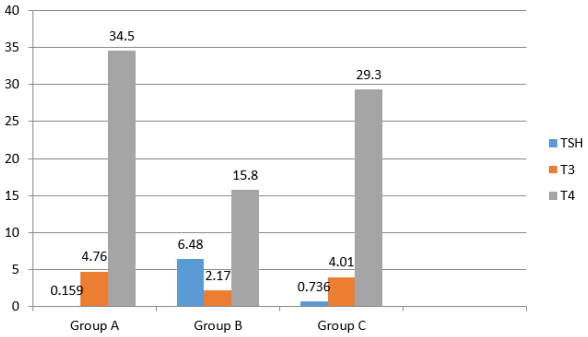

Figure No.1: Comparison of serum TSH, T3 and T4 between different groups of guinea pigs

Masson Fontana stained sections of control group A showed regular arrangement of thyroid follicles lined by a low simple cuboidal epithelium. The follicles were filled with colloid. Parafollicular cells were also visible among the follicles, arranged in clusters. There was no pigmentation in the thyroid follicular cells as well as colloid (figure-1)

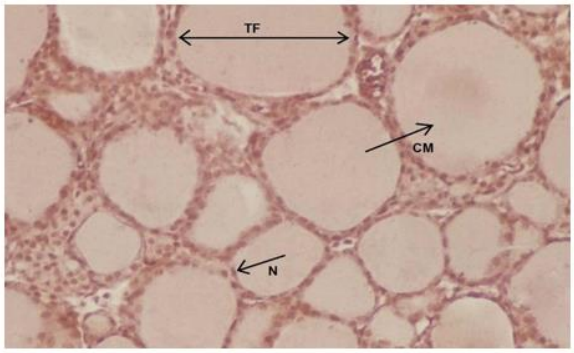

Figure No.1: Photomicrograph of 4 µm thick Masson Fontana stained section from group A (control) guinea pig showing normal architecture of thyroid follicles (TF) with flattened nuclei (N) and follicles filled with colloid material (CM). X400

Masson Fontana stained sections of group B animals showed that thyroid follicles were lined with by low simple cuboidal to squamous epithelium, with moderate deposition of black pigments. These pigments were mostly deposited in the follicular cells occupying area above the nucleus, facing the follicular lumen, and also inside the colloid of follicles in dispersed form. This pigment was black in color (figure-2 and 3).

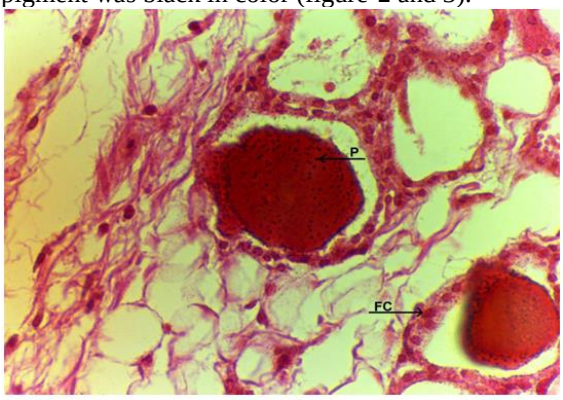

Figure No.2: Photomicrograph of 4 µm thick Masson Fontana stained section from minocycline treated group B guinea pig showing black pigments (P) inside the colloid material and follicular cells (FC). X400

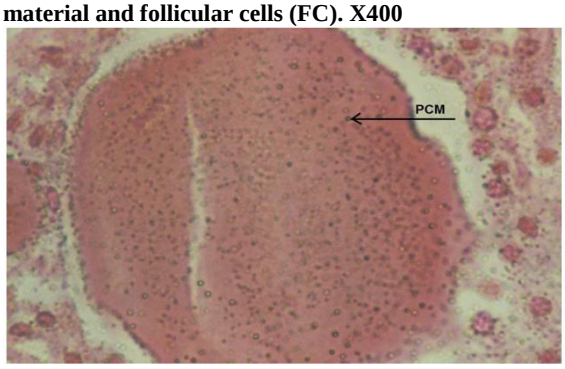

Figure No.3: Photomicrograph of 4 µm thick Masson Fontana stained section from minocycline treated group B guinea pig showing black pigments colloid material (PCM). X100

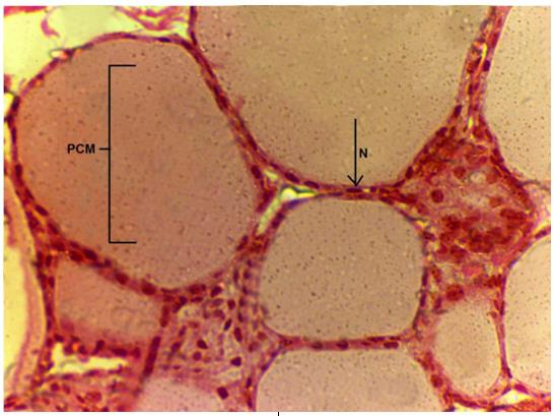

Figure No.4: Photomicrograph of 4 µm thick Masson Fontana stained section from minocycline with thyroxine treated group C guinea pig showing thyroid follicles filled with Pigmented colloid material (PCM) and lined with flattened follicular cells with flattened nuclei (N). X400

The Masson Fontana stained thyroid gland sections of group C revealed regular arrangement of thyroid follicles lined by simple squamous epithelium. There was mild pigmentation seen throughout the follicular cells as well as colloid, but the amount of pigment was less as compared to the group B animals (figure-4 & 5).

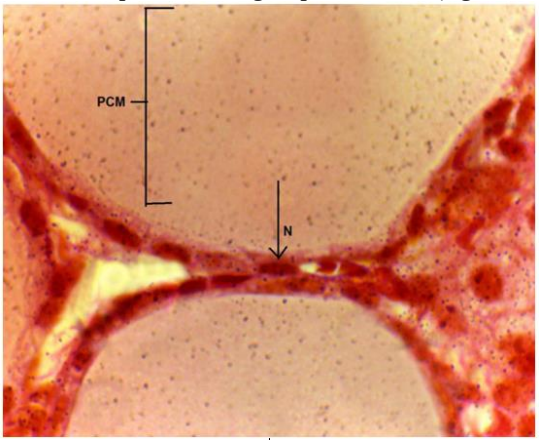

Figure No.5: Photomicrograph of 4 µm thick Masson Fontana stained section from minocycline with thyroxine treated group C guinea pig showing thyroid follicles filledwith Pigmented colloid material (PCM) and lined with flattened follicular cells with flattened nuclei (N). X1000

DISCUSSION

This study was planned to estimate the effects of minocycline on the thyroid gland and to observe the protection provided by thyroxine in the guinea pigs, as minocycline is a commonly used drug especially in cases of resistant acne vulgaris. Different studies on minocycline have proposed that it has strong antithyroid effects possibly by inhibiting the iodination of thyroglobulin and also by inhibiting the coupling of diiodotyrosine residues to form triiodothyronine and thyroxine[13,14]. Understanding the toxic effects of minocycline on thyroid gland and its associated hormonal changes is important in explaining not only the reason of hypothyroidism but also its amelioration.

Serum level of TSH was significantly raised in group B animals which were treated with minocycline. However, the levels decreased significantly when thyroxine was added to the treatment in group C animals. According to Hall,[15] the increased thyroid hormone inhibits secretion of TSH by the anterior pituitary gland mainly by a direct effect on the anterior pituitary gland itself. This was also in accordance to Davoren[16] who had shown that decreasing serum levels of TSH indicate adequacy of the dose of thyroxine in hypothyroid patients.

Results of the study highlighted a significant decrease in the levels of T3 and T4 in minocycline treated group B. This was most likely due to the inhibition of iodination of thyroglobulin by minocycline during thyroid hormone synthesis, as was observed by Taurog

et al (1996)[17]. They reported that the patients taking minocycline presented with clinical and laboratory evidence of thyroid hypo-function (decreased serum T4 and increased serum TSH).

The present study demonstrated a significant increase in serum T3 and T4 levels in group C who received minocycline along with thyroxine. Chao et al (2009)[18] stated that the treatment with thyroxine (T4) is likely safer than the treatment with a combination of T4 and T3. Therefore, thyroxine (T4) alone is the most appropriate therapy for patients with hypothyroidism. The data supports results of previous study[11] who demonstrated normalization of T3 and T4 levels in hypothyroid rats when L-thyroxine was added to the treatment regimen.

Masson Fontana stained sections of minocycline treated group B animals showed deposition of black pigment in the apices of the follicular cells and also inside colloid of the follicles in scattered form. This was most likely because of oxidation of minocycline to reactive species by thyroid peroxidase, resulting in formation of dark pigment in the thyroid[19]. Pantanowitz and Tahan (2003) demonstrated that minocycline was accumulated in the follicular epithelial cells as well as colloid in benign and hyperplastic tissue due to the oxidative action of the enzyme thyroid peroxidase on the drug[20]. This finding augments the results of Hecht et al[21] (1999) who observed black and granular pigments in the cytoplasm of epithelial follicular cells, and the pigment was also visible within the lumen of thyroid follicles as black deposits mixed with the colloid. Onyia et al[22] (1996) showed that these granular pigments, in the follicular cells as well as in the colloid were strongly positive by Masson Fontana stain. They were also non-birefringent, and negative by iron stain. Experimental studies on other animals with minocycline also recognized dark brown discoloration of the thyroid gland in rats, dogs, and monkeys after giving the drug for one month to these animals[23] so there could be corresponding rise in pigment formation with long term use of minocycline. Minocycline seemed to enhance the onset of black pigmentation of the thyroid gland.

The present study revealed decreased pigmentation in thyroid glands of group C animals treated with thyroxine along minocycline due to the protective effect by thyroxine. The findings were similar to Bowels[13] (1998) who used ascorbic acid at a dose of 0.1% of the diet in rats in addition to the minocycline for a duration of 6 weeks. This group of rats showed no sign of pigmentation similar to the control, as compared to the group which received minocycline only which demonstrated extensive deposits of black pigment within the thyroid follicles.

CONCLUSION

In light of the results of the present study, it is concluded that use of minocycline for a period of sixty days resulted in damage to the thyroid gland as revealed by the elevated levels of serum TSH, and decreased serum T3 and T4, as well as appearance of black pigmentation in the thyroid gland in the guinea pigs of the minocycline treated group. However, these effects were ameliorated to a significant extent by the concomitant use of thyroxine in the experimental animals.

Author's Contribution:

Concept & Design of Study:	Naheed Gohar
Drafting:	Imran Ishaque
Data Analysis:	Sarah Sughra Asghar, Imran Ishaque
Revisiting Critically:	Naheed Gohar, Imran Ishaque
Final Approval of version:	Naheed Gohar

Conflict of Interest: The study has no conflict of interest to declare by any author.

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