IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
ISSN: 2278-3008. Volume 5, Issue 3 (Jan. – Feb. 2013), PP 12-18
www.iosrjournals.org

Ciprofloxacin induced hormonal changes in the thyroid gland of rats and Anti-oxidant vitamin A, C and E as rescue agents

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Abstract: *Ciprofloxacin is a widely prescribed antimicrobial drug belonging to a class of drugs called quinolones. The first 1 – cyclopropyl theroquinolone ciprofloxacin was synthesized in 1983 (Wentland, 1990) and this drug was remarkable among the quinolones for its high activity. Currently, the most important benefit claimed for vitamins A, C and E is their role as antioxidants, which are scavengers of particles known as oxygen free radicals (or oxidants). According to Goldberg (2005) without sufficient vitamin A, thyroid gland cannot produce thyroxine. Vitamin A is a key nutrient to produce thyroxine (Gary Null, 2005), Vitamin C is necessary for tyrosine metabolism and it is quite a delicate balance (http://www.what reallyworks.co.uk /start / fact sheets.asp? article ID = 410). Vitamin E works with iodine for proper thyroid function (Earl Mindell, 2005). Development of the thyroid gland is controlled by the thyroid stimulating hormone (TSH) secreted by the hypothalamus; TSH is also called thyrotropin (The Medicine Journal, 2000). The main function of the thyroid gland is to synthesis the iodothyronine, hormone 3, 5, 3′, 5′ tetraiodothyronine (T_4, thyroxine) and 3, 5, 3′ triiodothyronine (T_3). These are peptides containing iodine. The two most important hormones are T_3 and T_4, and these hormones are essential for life and have many effects on body metabolizing growth and development (Thyroid foundation of Canada, 1999).NSAIDs produce abnormal thyroid functions and there by alter the synthesis, transport and metabolism of thyroid hormones (Davis and Franklyn, 1991). They alter TSH response to TRH and thereby influence TSH release (Lim et al., 1995), and also compete for T_3 binding sites in serum and at the cell surface, which act as T_3 antagonists. Disturbances of the gastro intestinal tract are the most frequent side effects observed with fluoroquinolones and occur in about 5% of treated patients. Microsomal metabolism of ciprofloxacin generates free radicals. Free radicals formation might play a role in the mechanism of producing adverse effects. Microsomal metabolism of ciprofloxacin generates free radicals. Free radicals formation might play a role in the mechanism of producing adverse effects. Ciprofloxacin is a potent inhibitor of the thyroid hormone regulated P 450 enzyme system in the liver since ciprofloxacin and enoxacin have shown the greatest inhibitory capacity (Clinical Toxicology Review, 1997).*

I. Introduction

Taking ciprofloxacin can spur germs to mutate so that future bacterial infections become untreatable. During the last decades a dramatic increase in bacterial strains multi resistant to antibiotics, particularly CIPRO, has been reported, ciprofloxacin no longer can be used to treat gonorrhea because the disease has become resistant to the drug. (Coronado et al., 1995; Smith et al., 1999 and CDC Special Report, USA). Currently, the most important benefit claimed for vitamins A, C and E is their role as antioxidants, which are scavengers of particles known as oxygen free radicals (or oxidants). These chemically active particles are by-products of many of the body's normal chemical processes. Their number is increased by environmental assaults, such as smoking, chemicals, toxins, and stress. The simple act of living also produces them, as we breathe in oxygen constantly. Vitamins protect us against a variety of diseases such as heart disease, Alzheimer's diseases, respiratory diseases and infectious diseases by boosting our immunity on eyes and skin function. Maintaining proper vitamin intake is quite simple with the help of a healthy diet and a high quality liquid multi vitamin taken daily.

In relation to the physiology of the endocrine glands, hormones and their levels are the best indicators of glandular activity. The TSH, T_3 and T_4 level in the sera picturize the drug and hormone interaction effectively. To verify if any rescue agents minimize / maximize the drug effect three excellent antioxidants vitamins, C, and E were given to animals of separate groups. To learn the recovery of the drug effect withdrawal group was maintained for each dose and durations. To observe the difference in drug effect for a short course and a long course, two different experimental periods were charted as short duration (7 days) and long duration (30 days).

II. Methodology

Animals

Healthy, adult male albino rats of Wistar strain weighing 260-300 grams were used for the present investigation. The animals were housed in proper ventilated animal house with constant 12 ± 1 hour light and 12 ± 1 hour dark schedule. The cages were cleaned thrice daily. Experimental animals were provided with standard diet and clean drinking water *ad libitum.*

Experimental protocol

The animals were weighed and divided into three groups of five animals each.

Group I: Control:

The healthy rats were selected and treated as control and they received saline orally. A separate batch of five rats was maintained for vitamin supplementation groups and received gingelly oil orally.

Group II: Short duration

The animals selected for short duration treatment were treated with ciprofloxacin at twelve hours interval for seven days.

Group III: Long Duration

Here the animals were treated with ciprofloxacin at twelve hours interval for thirty consecutive days.

Group II and Group III was further sub-divided into six groups, each group consisting of five animals. The animals received the following regimen of treatment and all the treatments were designed on the basis of adult human dosage prescribed by the physicians and interpolated to the body weights of rats.

a. Low dose

The animals selected for short duration treatment were treated with ciprofloxacin at twelve hours interval for seven days.

b. High Dose

The animals received 400mg of ciprofloxacin / 60kg body weight as an oral dose.

c. High Dose + Vitamins A

The animals received 400mg. of ciprofloxacin followed by 7.5mg of vitamin A / 60kg body weight, as an oral dose.

d. High dose + Vitamin C

The animals received 400mg of ciprofloxacin followed by 500mg of vitamin C / 60kg body weight as an oral dose.

e. High dose + Vitamin E

The animals received 400mg of ciprofloxacin followed by 600mg of vitamin E / 60kg body weight, as an oral dose.

f. High dose withdrawal

The experimental animals received 400mg of ciprofloxacin as an oral dose and were allowed a withdrawal period of the drug for further seven days for short duration and one month for long duration

Suitable controls were maintained for each duration of treatment. However, as there was no difference in any parameter among control group, a common control was employed in the present study

Hormone Analysis

Enzyme linked Immunosorbent Assay (ELISA of Hormones) of TSH, T_3 and T_4 l were carried out using kits.

Specimen collection and preparation

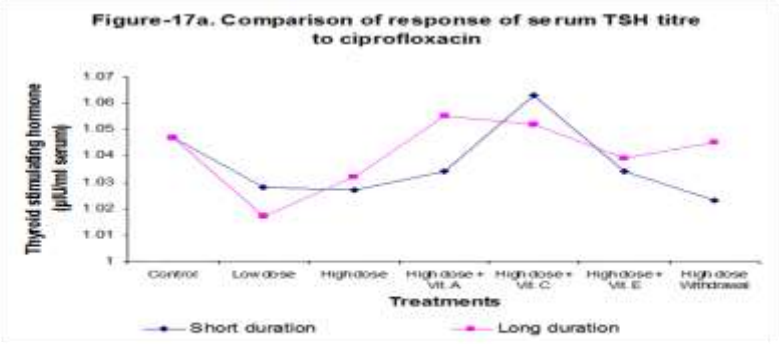
For all hormone assays fresh serum samples were acquired without additives. Serum must be stored between 2^0C and 8^0C for up to 48 hours prior to testing. For longer storage the serum should be stored at -20^0C and the same can be preserved for up to one year. Thawed samples must be mixed prior to testing.

Calculation of results

Calculate the mean absorbance value (A_{450}) for each set of standards and specimens. Construct a standard curve by plotting the mean absorbance from each standard against its concentration in μlU ml on graph paper, with absorbance values on the Y – axis and concentrations on the X – axis. Using the mean absorbance values for each sample, the concentration of TSH in μlU/ml is determined from a standard curve. The T_3 and T_4 values were expressed in ng/ml

III. Result

Effect on thyroid stimulating hormone (TSH) (Figure - 17a)



Each value is the mean ± SE of five animals
* Control Vs Treatment, Significant at 5% level by ANOVA
** Control Vs Treatment, Significant at 1% level by ANOVA
Means ± SE followed by a common letter are not significantly different at the 5% level by DMRT

Short duration treatment

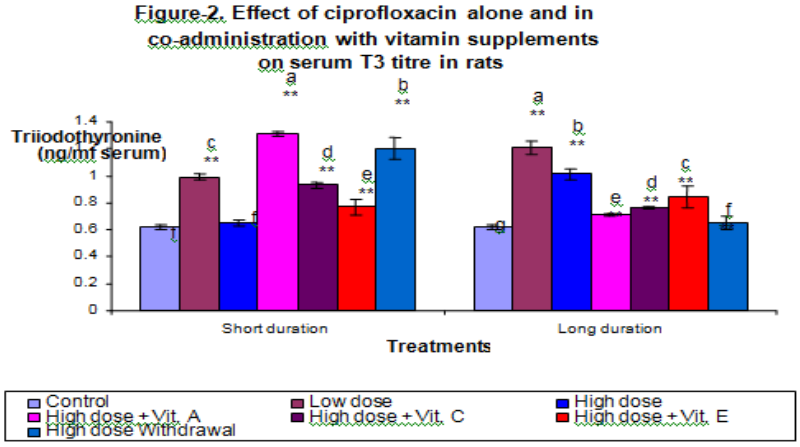
Ciprofloxacin administration did not bring about any significant changes in the TSH levels at the doses used. Vitamins supplementations had no marked effects on this hormonal level. Similarly withdrawal of the drug also had no marked effect on the TSH titres.

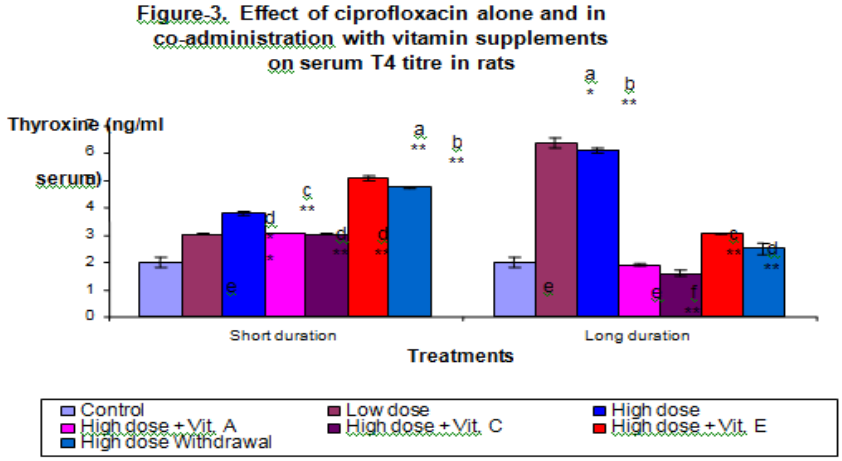
Long duration treatment

Similar to the short duration administration vitamins supplementations as well as withdrawal of the drug elected a similar response (i.e.) no marked effect on the TSH titres.

Effect on triiodothyronine and thyroxine (T_3 and T_4) (Figure- 2 and 3).

Short duration treatment





Low dose of ciprofloxacin had induced a significant increase in T_3 (16%) and T_4 (53%) levels. However, high dose of the drug brought about a marked (91%) increase in T_4 level only. Supplementation with vitamin A to the high dose drug treated animal could not maintain the T_4 levels (54%) but had a marked influence on T_3 titres as it raised it by 112%. On the other hand, vitamin C was effective on raising the T_3 levels by 50% but only by 54% of the T_4 levels. Unlike the two-vitamin, supplementation vitamin E exerted a positive effect by raising the T_4 level by 155% of the control and more than 64% of the high dose groups. But this vitamin group could raise the T_3 levels only by 24% compared to vitamin A and C. Withdrawal of the drug was effective in raising the T_3 (93%) and T_4 (137%) when compared to the controls, and 46% and 90% compared to the high dose group.

Long duration treatment

Though ciprofloxacin administration for the longer duration, stimulated the response elicited by the short durational drug administration the magnitude of response was higher. The T_4 levels were raised by 220% and T_3 by 95%, whereas, high dose could raise the T_4 levels by 205% and T_3 by 63% only. Vitamins supplementations appeared to lower the raised T_3 and T_4 levels like the drug withdrawal group. While vitamin A brought down the T_3 levels by 48% and the T_4 levels were drastically reduced to normal. Vitamin C supplementation however could bring only 23% decrease in T_3 titres but the T_4 titres were considerably reduced nearly 20% compared to controls. On the other hand vitamin E restored the control values and caused a 152% decrease in the T_4 titres. Drug withdrawal induced a 180% decrease of T_4 compared to high dose group and restored the T_3 levels to near normalcy.

Comparison of T_3 and T_4 response to ciprofloxacin.

T_3 and T_4 levels were raised by the ciprofloxacin treatment and especially more effectively in the long durational groups. Longer durational vitamin supplementations could restore the T_3 and T_4 levels more effectively than the short durational treatments. Similarly, drug withdrawal of longer durational treatment could restore the T_3 levels to normalcy and T_4 levels to near normalcy.

IV. Discussion

Effect on serum thyrotropin and thyroid hormones (TSH, T_3 and T_4)

Many goitrogenic xenobiotics that increase the incidence of thyroid tumors in rodents exert a direct effect on the thyroid glands to disrupt one of several steps in the biosynthesis and secretion of thyroid hormones (Capen, 1994). The mammalian thyroid gland is composed of two distinct endocrine cell populations concerned with the synthesis of two different classes of hormones. One is the follicular cells, which secrete the metabolically active iodothyronines, and C- (parafollicular) cells, which produce calcitonin. The synthesis of metabolic thyroid hormones is different than in other endocrine glands because the final assembly of hormone occurs within the follicular lumen. This extracellular synthesis of thyroid hormones is made possible by thyroglobulin, a glycoprotein synthesized by follicular cells. The secretion of thyroid hormones, under the influence of pituitary thyrotropin (TSH) from stores in the luminal colloid is initiated by elongation of microvilli and formation of pseudopods. D and C Red No.3 are tetraiodinated derivatives of fluorescein which in lifetime studies increases the incidence of thyroid follicular cell adenomas in male rats (Capen and Martin, 1989)).

In a normal thyroid gland the follicles secrete T_3 and T_4 after an elaborate synthesis that involves iodide trapping, oxidation of iodide to iodine and its condensation with tyrosine molecules attached to thyroglobulin. This results in the formation of monoiodothyrosine (MIT) and an iodination at the 5^{th} position forms diiodothyrosine (DIT). Two DIT molecules then undergo on oxidative condensation to form T_4 or DIT and MIT couple to form T_3. The hormone remains unbound to thyroglobulin until secretion. When they are decreased, colloid is ingested by the thyroid cells, the peptide bonds are hydrolysed and free T_3 and T_4 are discharged into the capillaries (Ganong, 1995).

The secretion of TSH by the adenohypophysis is partially determined by the level of circulating thyroid hormones and partially by neural influences transmitted from the hypothalamus by a thyrotropin releasing factor which reaches the pituitary through the hypothalamic-pituitary portal system of veins (Volpe,1979). TSH exerts profound effects on many aspects of thyroid gland function such as increase in gland size and vascularity. It also increases iodine uptake, iodide clearance from the plasma, formation of iodotyrosine and iodothyronine, thyroglobulin proteolysis, and T_4 and T_3 release from the thyroid gland (Ragevendra Puri, 2003).

In the present investigation with ciprofloxacin, the TSH, T_3 and T_4 were altered by the drug. The drug had increased the T_3 and T_4 levels in long duration of its treatment with no change in TSH titres. Tricyclic anti depressants (TCAs) interface with the hypothalamic – pituitary – thyroid (HPT) axis via the serotonergic and nor adenergic systems, this lead to a decrease in TSH (Beckett *et al.*, 1993).. The same study also observed that, by long term administration of clomipramine decrease T_4, fT_4 (free T_4) and rT_3 (reverse T_3).

Many drugs affect tests of thyroid function through alterations in the synthesis, transport and metabolism of thyroid hormones as well as via influences on thyrotropin (TSH) synthesis and secretion (Davis and Franklyn, 1991). Lower dose of proadrenomedullin N-terminal peptide had significantly lowered blood TSH concentration, without affecting the total, free T_3 and T_4 concentrations in rat (Ginda *et al.,* 2000). Morris and Gasca (1985) found no significant effect on mean basal plasma concentration of thyroxine or T_3 among horses given phenyl butazone. In a study with neurotensin, the thyroid gland weight was observed to be increased, but the serum T_4 level was decreased with no changes in serum TSH and T_3 levels (Malendowicz and Miskowiak, 1990). These workers also observed that neuropeptide – Y had no effect on thyroid gland function. Nishrimura *et al* (2002) observed a low dose of 2, 3, 7, 8- tetrachlorodibenzo-p-dioxin, to affect thyroid hormone regulation especially that of TSH in the pituitary and of T_4 in the thyroid gland of rat. A long-term administration of salicylate depressed serum levels of both T_4 and T_3 and rT_3 to about 75% as well serum as TSH concentrations (McConnel, 1999).

NSAID's are reported to produce adverse effect on endocrine systems, especially cause abnormality on thyroid function. In the absence of clinical features thyroid dysfunction is noticed by alterations in the synthesis, transport and metabolism of thyroid hormones (Davies and Franklyn, 1991). NSAID attenuate TSH response to TRH and thereby influence TSH release (Lim *et. al.,* 1995). Several drugs and supplements can affect thyroid hormone absorption. An interaction of potentially much greater clinical significance recently reported is " the effect of calcium carbonate on thyroxine absorption". (Paauw, 2000). NSAIDs compete for T_3 binding sites in serum. In cytosol NSAIDs were stronger competitors than in the nuclear extract (Barlow *et.al.,* 1994). Diclofenac sodium inhibits binding of T_3 and T_4 in the protons (Lim*et.al.,*1988; Topliss *et.al.,* 1988 and Aoyama *et,al.,* 1990). NSAID administration increase the risk of hypoaldosteronism (E_1 – Deiry et.al., 1997) and also alter the hypothalamic – pituitary – adrenal axis(HPA) response (Hall *et.al.,* 1994).

The increased excretion of thyroid hormones causes decrease in serum T_4 and promote TSH release from the pituitary gland thereby decreasing T_3 and T_4 in the serum and increasing TSH, by the administration of diniconazole (Hosokawa, 1993). The raised and lowered levels of these hormones are controlled by iodine, which controls the thyroid hormone synthesis and metabolism. The low dose of ciprofloxacin might have triggered the hydrolysis of thyroglobulin, so the T_3 level of low dose drug treated group increased significantly in the present study of both long and short duration. A same effect with T_4 secretion in long duration of ciprofloxacin treatment was also seen.

The thyroid gland secretes mostly T_4 and very little T_3. Under certain conditions the conversion of T_4 to T_3 decreases and more reverse T_3 is produced from T_4 (Metabolic Health.com 1999). TSH and thyroid hormone levels vary during the day and from day to day during the week, and the levels of TSH and T_4 were within the normal range at some time and the levels were abnormal at other times (Metabolic Health.com 2000).

Diclofenac competes for T_3 and T_4 binding sites in serum at the cel lsurfaces (Bishnoi *et al.,* 1994). But in the present study, ciprofloxacin did not compete for T_3 and T_4 but it induces the gland to secrete both the hormones to maximum and was dosage dependent. The TSH is known to stimulate both differentiation and proliferation of thyroid follicle cells and its decreased level affects the above said processes of thyroid follicle cells (Pietrzik *et al.,* 1998). The decrease in metabolism of T_4 and T_3 appears to be responsible for the decrease in the level of TSH.

Effect of Antioxidant vitamins

An adequate host defense activity critically depends upon the micronutrient status of an individual among which the cellular oxidant – antioxidant balance is an important determinant. Oxidative burst is part of the physiological function of phagocytes connected to a massive production and release of reactive oxygen intermediates. At the same time maintenance of the functional capacity of the host defense system in fighting against foreign antigens is significantly affected by the various reactive oxygen species. To compensate this critical condition there must be a high intracellular concentrations of antioxidants (Peake,2003).

Vitamin E is considered the most important radical scavenging vitamin of the lipid soluble compartment and vitamin A comprises all rational with properties like trans-rentinol, and the individual surfactant proteins are selectively regulated by retinoic acid (Bauernfeind, 1998). The antioxidative vitamins, ascorbic acid and the tocopherols, are important not only for limiting tissue damage but also in preventing increased cytokine production, and also exerting anti-inflammatory effects in studies in man and animals (Bland, 1998). In the present study also the three antioxidants have prevented the tissue damage caused by ciprofloxacin especially when administrated in high dose during the long duration study. Stimulation of gap junctional communication and intercellular communication are some of the main activity of carotenoids, which increase the oxidative property of vitamin A (Helmut Sies and Whilhelm Stahl, 1997).

In the present study also vitamins helped to bring back the normal status of T_3 and T_4 production which was raised by ciprofloxacin administration to maximum level. Vitamin C, vitamin E and the B complex vitamins may help in the treatment of hyperthyroidism and hypothyroidism because they play key roles in

improving the overall healthy function of the thyroid gland and the immune system (http://www.readers.digest.co.uk/health/thyroid disease.htm). Vitamin E and coenzyme Q play some role as scavengers in thyroid follicular cell hyper function or dysfunction (Mano *et al.*, 1998). In the present study also vitamin E showed its stimulant effect by restoring T_3 and T_4 levels from the elevated levels caused by the drug. The drug withdrawal effectively restored the hormone status by 85%-99%, in a duration dependent manner. This may be due to the readopting of the thyroid tissue to its normal condition in due course of time. Deivendran (2004) observed a similar reversal of male reproductive hormones with ciprofloxacin on drug withdrawal.

V. Conclusion

Ciprofloxacin administration did not bring any significant changes in the TSH levels in the doses used at both the duration of treatments. Neither vitamins nor drug withdrawal could bring any marked changes on the TSH titres. On the other hand T_3 and T_4 levels were raised especially in the long durational groups. Vitamins supplemented along with the drug were found to restore the T_3 and T_4 levels to control values more effectively in long duration treatment groups than in the short duration groups. By drug withdrawal the T_3 level was restored to normalcy and T_4 level to near normalcy.

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