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Research paper

Concomitant and Pre-emptive Melatonin Administration Improves Thyroid Hormones Level and Spleen T-cells Proliferation in Propyl-thiouracil (PTU) Induced Hypothyroid Condition in Swiss Albino Mice

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ABSTRACT

Hypothyroidic development is associated with the disruption of thyroid secretions along with noticeable deterioration of body defense mechanism. The epiphyseal hormone melatonin was detected as an ameliorator of many unsuitable situations of mammalian physiology. In such context the current study was planned out to observe the role of melatonin in thyroid secretions and proliferation of immune cells in experimentally induced hypothyroid condition in mice. The levels of thyroid hormones were checked by ELISA kits. Proliferation of immune cells were assayed through analyzing the spleen T-cells proliferation. The level of thyroid hormones and spleen T-cells proliferation rate were significantly deteriorated in hypothyroid condition. Both concominant and pre-emptive melatonin administration significantly recovered the level of thyroid hormones in hypothyroid condition. Whereas, elevation of spleen T-cells proliferation was recorded only in pre-emptive treatment of melatonin in hypothyroid condition. This performed investigation delineated the concomitant and pre-emptive therapeutic potentiality of melatonin in the improvisation of disturbed thyroid activity and immune responses in hypothyroid condition.

1. Introduction

Hypothyroidism is a clinical syndrome caused due to decreased thyroid activity, associated with a sub-metabolic state with lowered energy and oxygen metabolism (Weitzel et al., 2003). Perinatal disruption of thyroid function leads disorders in physiological networks, including the central nervous system (Porterfield and Hendrich 1993) and the immune system (Stagi et al., 2005). Propyl-thiouracil (PTU) induced hypothyroid condition exerts temporal immunomodulatory effects on the developing immune system (Nakamura et al., 2007). Investigations in this field also disclosed the risk factors for hypothyroidism as immune system associated adverse events linked with programmed cell death-1 inhibitors (Shimada et al., 2021). Development of hypothyroidism through the ingestion of PTU to maternal rats resulted in the transfer of this drug to the offspring induces several immunological changes including a relative increase in the proportion of T cells in the spleen (Nakamura et al., 2007).

Melatonin (N-acetyl-5-methoxytryptamine), an indoleamine secreted from the pineal gland is mainly involved in the regulation of circadian rhythm as well as thyroid physiology of mammals. Rats induced to hyperthyroidism have higher levels of this indoleamine in plasma, suggesting a relationship between the thyroid disorders and the pineal secretion (Belviranli and Baltaci 2008) (Baltaci and Mogulkoc 2017). Likewise, Bondarenko et al. (2011) demonstrated that signs of hypothyroidism in rats with low levels of melatonin due to exposure to constant light were reversed with their exogenous application. Melatonin performs the synergistic, cumulative or antagonistic effects through which it deprogramming the effects of thyroid deficiency in the neonatal period of rat (Thakkar et al., 2011). This hormone also shows the protective effects on mitochondrial injury and neonatal neuron apoptosis induced by maternal hypothyroidism (Hidayat et al., 2019). Besides these, a large body of evidence also supports its immune stimulatory role; *in vitro* administration of melatonin enhances the proliferative ability of lymphoid tissues (Drazen et al., 2001) (Kriegsfeld et al., 2001) and elevates the mitogenic response of peripheral blood T lymphocytes (Kliger et al., 2000).

In this study we attempted to investigate another aspect of melatonin by administering its dose in concomitant and pre-emptive way in hypothyroid condition induced by PTU. As hypothyroid condition is linked with deprivation of thyroid hormone and immune responsiveness according to many study. So our effort was to delineate the response of thyroid and spleen after the invasion of exogenous melatonin was noted in experimentally induced hypothyroidism. This study will definitely include some knowledge of dose dependent stimulation of melatonin in thyroid problem.

2. Materials and Methods

All the experiments on the animals were conducted in accordance with institutional practice and within the framework of the revised Animal (Specific Procedure) Act of 2007 of Govt. of India on animal welfare. The study was approved by Institutional Animal Ethics Committee (IAEC) with ethical clearance no. TU/IAEC/2013/V/5-3.

2.1 Animal procurement and maintenance

Healthy Swiss albino mice colonies were housed at animal house in ambient laboratory conditions having temperature of 25±2°C with alternative maintenance of light/dark cycle (12L:12D). Mice were kept in groups of seven (n=7) in polycarbonate cages (43cm x 27cm x 14cm) to avoid the crowding effect and fed with mice feed and water *ad libitum*.

2.2 Experimental Design

For observing the effects of melatonin in thyroid secretions and spleen T-cells proliferation of Propyl thiouracil induced hypothyroid condition, mice were divided into five groups having 5 mice in each group as follows:

Control (Con) group: Mice of this group were received subcutaneous injection of ethanolic saline (0.01% ethanol), 0.1 ml/ day for consecutive 30 days.

Melatonin (Mel) group: Mice of this group were received subcutaneous injection of melatonin (Sigma-Aldrich Chemicals, St. Louis, USA), 25µg/100g BW/day for consecutive 30 days at evening (16:30-17:00) hours.

Propylthiouracil (PTU) group: Mice of this group were received 5-propyl-2-thiouracil, PTU (Sigma-Aldrich Chemicals, St. Louis, USA) in the drinking water, 60 µg/g BW/day for consecutive 18 days (Klecha et al., 2000).

(PTU+Mel) group: Mice of this group were received PTU and melatonin simultaneously for 18 days considered as concomitant melatonin treated group.

(Mel+PTU) group: Mice of this group were received melatonin for consecutive 30 days and were also received PTU for last 18 days of the experimental period of melatonin treatment considered as pre-emptive melatonin treated group.

2.3 Sample collection and processing

After 24 hours of last administration, experimental mice were sacrificed under anaesthesia (pentobarbital, 15mg/Kg, intraperitoneal injection). Trunk blood was collected in heparinized tube. Blood serum was separated and stored at -20°C till hormonal analyses. The spleen was dissected out from each experimental group and immediately processed for single cell suspension preparation for the study of spleen T-cells proliferation.

2.4 Hormonal analysis

Serum T3 and T4 hormones analyses were done by commercial ELISA Kits (Diagnostic AutomatationInc, CA, USA). For T3, detection range 0-10ng/mL, specificity 96.30% and sensitivity was 0.2ng/mL. For T4, detection range 0-30µg/dL, specificity 96.30% and sensitivity was 0.05µg/mL.

2.5 Splenocytes Proliferation

Spleens of experimental mice were dissected out on chilled PBS and processed for preparation of single cell suspension of splenocytes. Erythrocytes of splenic cell suspension were lysed with 1:10 solution of cold 0.5% Tris and 0.84% NH4Cl (pH 7.2). Cell suspension was washed thrice with chilled PBS. Cell viability was determined by trypan blue exclusion method. Viable cells (which exceeded 95%) number was adjusted to

1 X 10^7 cells/ml in culture medium [RPMI-1640 medium supplemented with Streptomycin (100 µg/ml), Penicillin (5000U/ml), L-glutamine (2 mM/ml), 0.1% 2-mecaptoethanol (5 X 10^{-2} mM/ml) and 10% FCS). 100 µl splenocytes suspension was added to the wells of sterile flat bottom 96 well culture plates. Mitogen concanavalin-A (Con A; T cell mitogen; Sigma- Aldrich Chemicals, St. Louis, USA) solution was prepared at the concentration of 5 µg/ml in the culture medium. 50 µl mitogen solution was added to the wells containing splenocytes suspension and yielded a volume of 150 µl/ well (in duplicate). Finally, a volume of 200 µl/well was made by adding complete culture media 50 µl/well in mitogen containing and 100 µl/well in without mitogen containing wells of culture plate. Culture plate was incubated in a humidified 5% CO_2 containing chamber at 37°C for 44 h. 20 µl MTT solution [3- (4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, SRL, Mumbai, India, 5 mg/ml in PBS) was added in each well and incubated at 37°C in 5% CO_2 for additional 4 h. After 4 h 100 µl of acidified propanol (0.04 mol/l HCl in isopropanol) was added in each well and the optical density was determined with a microplate reader (ECIL, India) at 570 nm wavelength (Ahmad and Halder 2010). Mean OD values for each set of duplicates were used in subsequent statistical analysis. Response was calculated as percent (%) stimulation index representing the ratio of absorbance of the mitogen stimulated cultures to control cultures.

2.6 Statistical analysis

Statistical analysis of the data was performed with one way Analysis of Variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) multiple range test. The differences were considered significant when p< 0.05. Microsoft Excel program and Statistical Package for the Social Sciences (SPSS) were used for calculation and graph preparation.

3. Results

3.1 Serum T3 Level

T3 hormone level was significantly (p<0.01) decreased in both melatonin treated group and PTU treated group in comparison with control group. The level of T3 hormone was significantly (p<0.01) increased in (PTU+ Mel) group as well as in (Mel + PTU) group in comparison with PTU group (Fig. 1).

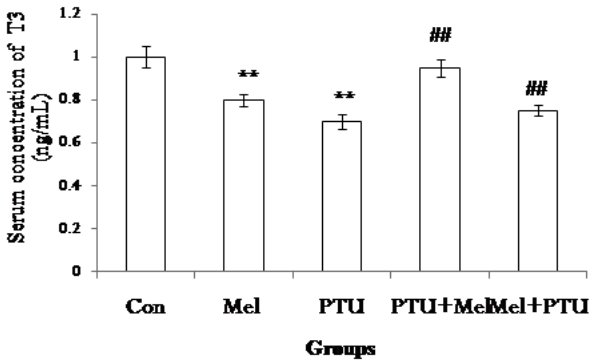

Fig. 1 Serum T3 concentration in experimental groups of mice. Histogram represents Mean ± SEM. The differences were considered significant when p< 0.05. **p<0.01: Con vs Mel, Con vs PTU; ## p<0.01: PTU vs PTU+Mel, PTU vs Mel + PTU

3.2 Serum T4 Level

Strong suppression in T4 hormone level was noted in PTU treated mice whereas significant (p<0.01) decrease inT4 hormone level was also noted in melatonin treated group in compare to control group. Significantly (p<0.01) increased T4 hormone level was noted in (PTU+ Mel) group and (Mel +PTU) group in comparison with PTU group (Fig. 2).

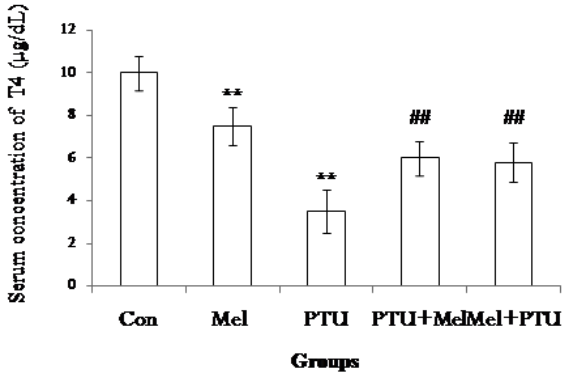

Fig. 2 Serum T4 concentration in experimental groups of mice. Histogram represents Mean ±SEM. The differences were considered significant when p< 0.05. **p<0.01: Con vs Mel, Con vs PTU; ## p<0.01: PTU vs PTU+Mel, PTU vs Mel+PTU

3.3 Effect of melatonin on splenocyte stimulation index (%SR)

Splenocyte stimulation index (% stimulation ratio) was significantly (p<0.01) increased in melatonin treated group and significantly (p<0.01) decreased in hypothyroid (PTU) group in comparison with control group. In (PTU+Mel) group, stimulation index was remained same whereas in (Mel+PTU) group, % stimulation ratio was significantly (p<0.01) increased as compare to hypothyroid (PTU) group (Fig. 3).

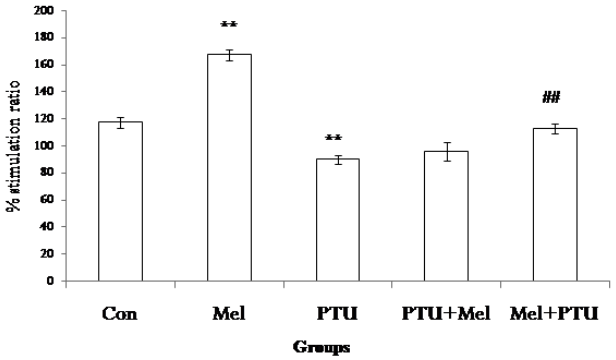

Fig. 3 Percent stimulation ratio (%SR) of splenocyte. Histogram represents Mean ± SEM. The differences were considered significant when p< 0.05. **p<0.01: Con vs Mel, Con vs PTU; ## p<0.01: PTU vs PTU+Mel, PTU vs Mel+PTU

4. Conclusions

The relationship between the thyroid gland and the pineal hormone melatonin has for long time been a subject of intensive research. In this study we have observed the deterioration of thyroid hormones (T3 and T4) after introducing the melatonin. Our previous laboratory investigations in mice were reflected the same pattern of impact of melatonin over thyroid secretions (Laskar and Singh 2020). Melatonin administration suppresses mitotic activity and due to its strong inhibition of thyroid gland function was reported (Wajs and Lewinski 1992) (Sewerynek, Wiktorska, and Lewinski 1999). Treatment of 5-propyl-2-thiouracil (PTU) decreased T3, T4 hormones levels as it is well known anti-thyroid drugs caused hypothyroid condition. In (PTU+Mel) group or concomitant melatonin treated group as well as in (Mel+PTU) group or pre-emptive melatonin treated group, both T3 and T4 hormones levels were increased as compare to hypothyroid (PTU) group. Melatonin treatment to PTU induced hypothyroid mice helps to counteract the PTU caused suppression in the level of both T3 and T4 hormones. The reverse signs of hypothyroidism in hypothyroid rats with the administration of exogenous melatonin were also reported (Bondarenko et al., 2011). Beside this report, in the same year Thakkar and his co-workers (2011) also suggested that melatonin treatment reversed the neonatal hypothyroidism induced negative impacts on thyroid function.

Splenocytic proliferation is used as an index of immune function in many clinical applications. In this study, splenocytes proliferation was found to be increased in melatonin treated mice as compare to control mice. The immune stimulatory activity of melatonin through elevation of spleen T-cell proliferation was documented by some researchers (Demas and Nelson 1998). The immune modulatory role of melatonin and its application in the control of various diseases is supported by the synthesis of melatonin by lymphocytes (Mohammed et al., 2013). In PTU induced hypothyroid group, stimulation index of spleen T-cell proliferation was decreased. The mechanism of PTU action on immune suppression was proposed to be indirect through decreased production of thyroid hormones (Volpe 2001). Reports also suggested that anti-thyroid drugs might have immunomodulatory effects on the developing immune system (Nakamura et al., 2007). In (PTU+Mel) group, simultaneous or concomitant treatment of melatonin along with PTU could not cause any change in T-cell proliferation as compared to hypothyroid group. But in (Mel+PTU) group or pre-emptive group where mice were received 30 days melatonin treatment along with PTU given for last 18 days of the experimental period of melatonin injection, significant elevation of spleen T-cell proliferation was observed in comparison to hypothyroid group. These results indicate that pre-emptive treatment of melatonin may counteracts the PTU induced suppression in spleen cells proliferation.

The concomitant and pre-emptive administration of melatonin in PTU induced hypothyroisism was found to be effective in improving the levels of thyroid secretions, T3 and T4 hormones i.e., thyroid related complications in the physiology. In spleen cells, T cells proliferation was significantly elevated due to pre-emptive treatment of melatonin. This observation indicates that pre-emptive exposure of melatonin was more effective than the concomitant dose of melatonin in recovering the immune response of hypothyroid mice. Thus, all these above findings concluded that pineal hormone melatonin helps to regulate the normalize the activities of thyroid and lymphoid (spleen) tissues in distorted hypothyroid condition.

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Declaration of Conflict

The authors declare that there are no conflicts of interest regarding publication of this paper.

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