PYRROLOQUINOLINE QUINONE HAS THE POTENTIAL TO AMELIORATE PTU INDUCED LIPID PEROXIDATION AND OXIDATIVE DAMAGES IN MICE

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ABSTRACT

Objective: This investigation was aimed to examine the hitherto unknown potential of pyrroloquinoline quinone (PQQ) in ameliorating propylthiouracil (PTU) induced lipid peroxidation and in oxidative damages of the different tissues including muscle, testis and brain of adult mice.

Methods: Out of 5 groups of animals 4 groups received PTU (0.05%) in drinking water) for 35 days and were rendered hypothyroidic. While group one, receiving normal drinking water served as control, group III, IV and V were treated with 1, 5 and 10 mg/Kg of PQQ respectively for last 6 days along with PTU, after which alterations in tissue lipid peroxidation (LPO) and in enzymatic activities of superoxide dismutase (SOD) and catalase (CAT) and in glutathione (GSH) content were evaluated in different organs such as muscle, testis and brain. Simultaneously, concentrations of serum glucose, total cholesterol, serum glutamate oxaloacetate transaminase (SGOT), creatinine and urea were measured.

Results: PTU administration enhanced the tissue LPO, serum SGOT, total cholesterol, creatinine and urea with a parallel decrease in serum glucose levels. All the animals treated with different amounts of PQQ, these adverse effects were ameliorated. However, PQQ at 10 mg/kg was found to be the most effective concentration, as it reduced the tissue LPO, with a parallel increase in cellular antioxidants in a better way.

Conclusion: PQQ appears to have the potential to mitigate PTU-induced oxidative damage in muscle, testis and brain of mice, suggesting that it can reduce hypothyroid induced oxidative stress.

Keywords: PQQ, PTU, LPO, SGOT, Antioxidant system.

INTRODUCTION

Normal levels of thyroid hormones are required for proper body functions including muscle contraction, lipid and carbohydrate metabolism, oxygen consumption, reproduction and neural function [1]. Studies have shown that thyroid hormone deficiency, a state of hypothyroidism leads to marked delay in sexual maturation and development [2]; testicular atrophy, decrease in sperm count and sperm motility resulting in male infertility [3, 4, 5]. However, exact mechanisms by which the thyroid hormones are able to control testicular metabolism are still uncertain. Their receptors are highly expressed in neonatal testis cells, and a testis as such is an important thyroid hormones target [6].

Thyroid dysfunction is also postulated to be closely related to the generation of reactive oxygen species (ROS), which might account for thyroid hormone-induced tissue damage. Increased generation of ROS, particularly in hypothyroidism, has been reported both in humans [7, 8] and in rats [9]. Zamoner et al. 2008 [10] showed that PTU induced congenital hypothyroidism depletes antioxidant defenses in rat testes. It is also known that thyroid dysfunction increases LPO reactions and ROS [11, 12]. Lipid peroxidation (LPO) is an autocatalytic mechanism leading to oxidative destruction of cellular membranes [13]. Such destruction can lead to cell death and to the production of toxic and reactive aldehyde metabolites called free radicals, where malondialdehyde (MDA) is the most important product. It is further known that ROS leads to the oxidative damage of biological macromolecules, including lipids, proteins and DNA [14]. Because of the fact that hypothyroidism is associated with enhanced LPO, it was thought that a compound having known antioxidant property may counteract the hypothyroid induced oxidative damage.

Pyrroloquinoline quinone (PQQ), an anionic water soluble compound is believed to be an essential nutrient, because animals given PQQ deficient diets display a variety of illnesses [15]. It is also attributed with multiple physiological functions including regulation of electron transport system and stimulation of the production of nerve growth factor [16]. Both in vivo and in vitro studies have shown that PQQ can protect against several types of oxidative damage [17, 18] stroke damage [19] and irradiation injury [20]. It is believed that PQQ scavenges ROS and protects cells from oxidative stress-induced damage, primarily by improving the activities of free radical scavenging enzymes and decreasing the level of LPO [21, 22]. PQQ was also found to be neuroprotective in a rodent stroke model [23, 24], presumably by scavenging peroxynitrite [25]. Furthermore, PQQ could prevent oxidative stress-induced neurotoxicity and neuronal death [26, 27]. In vitro studies showed that PQQ protects isolated liver mitochondria from damage after oxidative stress and scavenges superoxide radicals [28]. Previous in vivo studies of I/R injury in rats indicated that PQQ reduces myocardial infarct size and improves cardiac function [23]. Despite all these beneficial actions of PQQ, no attempt was made so far by any worker to evaluate its role in the regulation thyroid dysfunctions.

Some reports indicate the development of oxidative stress and cardiovascular diseases with hypothyroidism [11, 12, 14, 29]. Since PQQ is known to regulate oxidative damage and hypothyroidism is associated with oxidative stress [11, 30], it was thought that the compound may ameliorate PTU induced oxidative damage also. Because of this presumption and keeping in mind the paucity of the scientific studies on the role of PQQ in thyroid dysfunctions, this study was designed to evaluate whether the PQQ can act as potential ameliorative agent in PTU induced oxidative damage and associated problems or not.

MATERIALS AND METHODS

Animals

Adult Swiss albino mice, weighing 30 ± 2 gm were housed in polypropylene cages in a standard photoperiod (14 h light; 10 h dark) and temperature (27 ± 1°C) controlled room with the provision of laboratory feed (Gold Mohur feed, Hindustan Lever Limited, Mumbai, India) and water ad libitum. Animals were maintained in accordance with the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Social justice and Empowerment, Govt. of India. (Ref. No. 779)
Chemicals
PTU was obtained from Sigma-Aldrich chemicals (St. Louis, MO, USA), while PQQ was purchased from Quality of life lab, USA. Ellman’s reagent, m-phosphoric acid, thio-barbituric acid (TBA), sodium dodecyl sulphate, tri carbaylic acid (TCA), hydrogen peroxide (H₂O₂) were obtained from E. Merck Ltd, Mumbai, India. Kits for the estimation of different lipids, glucose, urea, creatinine and glucose were procured from Transasia Bio-Medicals Ltd, Solan, India. All other chemicals were of reagent grade and obtained from Sisco Research Laboratories Pvt. Ltd, Mumbai, India.

Experimental design

Thirty-five adult healthy mice were divided into 5 groups of 7 each. Group I animals receiving simple drinking water served as control, whereas those of group II, III, IV and V received only PTU (0.05%) in drinking water for 5 weeks to render them hypothyroidic [12, 30, 31]. On 30th day, animals of group III, IV and V received different doses of PQQ (1, 5 and 10 mg/kg/day) for 6 days respectively, along with PTU as administered to group II animals. Experiment was continued for 5 consecutive weeks. On the day of termination (36th day), over night fasted animals were sacrificed under mild anaesthesia, blood from each animal was collected and serum was separated for the estimation of different biochemical parameters including serum concentration of urea (NO₃), glucose, total cholesterol, γ-glutamyltransferase (GGT), creatinine, and total cholesterol. After exsanguinations, muscle, testis and brain tissues were removed quickly, washed with phosphate buffered saline (PBS) and total cholesterol. After exsanguinations, muscle, testis and brain tissues were removed quickly, washed with phosphate buffered saline (PBS) and processed for the estimation of lipid peroxidation (LPO), super-oxide dismutase (SOD), catalase (CAT) activities and glutathione (GSH) content.

Biochemical estimations

Muscle, testis and brain tissues were homogenized in PBS (0.1M, pH 7.4), centrifuged at 15,000 g for 30 min. at 4°C and the supernatant was used for subsequent analyses.

Lipid peroxidation (LPO)

Lipid peroxidation level in the tissues was measured by the method of Ohkawa et al. 1979 [32] which is based on the TBA reaction with MDA, a product formed due to the peroxidation of membrane lipids. The amount of MDA was measured by taking the absorbance at 532 nm (extinction coefficient, E =1.56x10⁵), using a Shimadzu UV-170 spectrophotometer. LPO was finally expressed as nM MDA formed/h/mg protein.

Super-oxide dismutase (SOD) assay

Activity of SOD was determined following the pyrogalol auto-oxidation inhibition assay method of Marklund & Marklund 1974 [33]. The rate of auto-oxidation is calculated from the increase in absorbance at 420 nm. The enzyme activity was expressed as units/mg protein and 1 unit is defined as the enzyme activity that inhibits auto-oxidation of pyrogalol by 50%.

Catalase (CAT) assay

Catalase activity was estimated following the method of Aebi 1983 [34] that is based on the decomposition of H₂O₂ which is measured spectrophotometrically from the changes in absorbance at 240 nm which was expressed as μM of H₂O₂ decomposed/min/ mg protein.

Glutathione (GSH) assay

For the estimation of tissue GSH content the method of Ellman 1959 [35] was followed in which the –SH group of GSH reacts with DTNB to produce a yellow-colored 2-nitro-5-mercaptobenzoic acid and the absorbance was taken at 412 nm. The GSH content is expressed as μM GSH/mg protein.

Protein, glucose, total cholesterol, urea and creatinine estimations

Protein estimation was done by the routine method of Lowry et al. 1951 [36] using bovine serum albumin as standard and fasting serum glucose concentration was measured by glucose oxidase / peroxidase method based on the protocol of Trinder 1969 [37], where 4-amino antipyrine and phenol reacts with glucose to produce a pink colored quinoneminine dye. The intensity of the color developed is proportional to glucose concentration in the sample, while estimation of serum total cholesterol was done using spectrometric methods of Alain et al., 1974 [38]. Urea and creatinine were estimated using the commercially available kits and protocols of Transasia bio-medicals Ltd, Solan, India.

SGOT assay

The method used here is of Reitman Frankel, 1957 [39] colorimetric end point reaction method. SGOT catalyses transfer of amino group from L-aspartate to α-ketoglutarate with formation of oxaloacetate and glutamate. The oxaloacetate, so formed, is allowed to react with 2,4-DNPH (2,4 dinitro phenyl hydrazine) to form 2,4 dinitro phenyl hydrazone derivative, which is brown in color in alkaline medium. The absorbance of this hydrazone derivative is correlated to SGOT activity by plotting a calibration curve using pyruvate standard. The colored complex is read at 505 nm.

Statistical analysis

Data are expressed as means ± SEM. For the statistical evaluation, analysis of variance and Student t test were used [40]. A p value of 0.05 or less is considered as the level of significance.

RESULTS

Results of the present study clearly indicated marked differences in the body weight (B wt.) of PTU and PQQ treated animals (Table 1). While body weight was increased following PTU administration, it was decreased after PQQ treatments. With respect to LPO, PTU enhanced it in all test tissues, which was decreased by simultaneous administration of PQQ. However, out of the three doses (1, 5 and 10 mg/kg/d) of PQQ, 10 mg/kg was found to be most effective in all tissues and the percentage decreases were found to be 74%, 86% and 69% in muscle, testis and brain respectively.

Table 1: Effects of PTU and PQQ on change in percentage (% body weight in different groups of mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Body weight (g)</th>
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<tbody>
<tr>
<td></td>
<td>(Initial)</td>
<td>(Final)</td>
</tr>
<tr>
<td>Control</td>
<td>30.72 ± 0.788</td>
<td>34.36 ± 0.929</td>
</tr>
<tr>
<td></td>
<td>(10.6 %↑)</td>
<td>(10.6 %↑)</td>
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<tr>
<td>PTU</td>
<td>28.91 ± 0.622</td>
<td>36.82 ± 0.87</td>
</tr>
<tr>
<td></td>
<td>(21.48 %↑)</td>
<td>(21.48 %↑)</td>
</tr>
<tr>
<td>PTU+1mg</td>
<td>29.18 ± 0.687</td>
<td>32.11 ± 0.781</td>
</tr>
<tr>
<td></td>
<td>(10.83 %↑)</td>
<td>(10.83 %↑)</td>
</tr>
<tr>
<td>PTU+5mg</td>
<td>29.87 ± 0.567</td>
<td>31.17 ± 0.997</td>
</tr>
<tr>
<td></td>
<td>(6.34 %↑)</td>
<td>(6.34 %↑)</td>
</tr>
<tr>
<td>PTU+10mg</td>
<td>29.37 ± 0.786</td>
<td>30.14 ± 1.705</td>
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<tr>
<td></td>
<td>(2.55 %↑)</td>
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PTU administration increased the LPO (Fig. 1) in all tissues significantly (p<0.001 for testis and p<0.01 for muscle and brain), with increased SOD in muscles (p<0.01). It also decreased CAT activity in all tissues.
significantly (p<0.05 for testis & brain; p<0.01 for muscle). The significant decrease in GSH level after PTU administration was seen only in brain (p<0.05). With respect to SOD activity, the highest dose, i.e. 10 mg/kg clearly appeared to be most effective (p<0.01 for muscle; p<0.05 for testis) (Fig. 2) and the percentage increase was found to be 56% and 79% in muscle and testis respectively.

With respect to serum parameters (Fig. 5), a significant increase was found in the level of total cholesterol, SGOT, creatinine and urea in PTU treated animals (p<0.01, p<0.001, p<0.001 and p<0.001 respectively). However, administration of PQQ at a dose of 10 mg/kg markedly reduced all these indices and the percentage decreases were found to be 46%, 55%, 42% and 44% in total cholesterol, SGOT, creatinine and urea respectively. By the administration of PTU, level of serum glucose decreased significantly (p<0.05 by 25%); while by the simultaneous treatment of 5 mg and 10 mg/kg of PQQ it increased significantly (p<0.001 for both, 26% & 32% respectively).

DISCUSSION

Results of this experiment clearly revealed that, PQQ has the potential in regulating the PTU induced oxidative damage in muscle, testis and brain tissues of mice and other associated problems including serum glucose, cholesterol, creatinine, urea and SGOT.

Thyroid hormones are known to be involved in almost all body functions, including metabolic, respiratory, cardiovascular, nervous and reproductive phenomena. Obviously hypothyroidism affects all these functions. PTU is an anti-thyroid drug which inhibits both the synthesis of thyroid hormones in thyroid gland [41] and the conversion of thyroxine (T4) to its active form, triiodothyronine (T3), in peripheral tissues [42]. Thyroid abnormalities are also considered as physiological modulators of cellular oxidative stress. It is now well known that deficiency of thyroid hormones can lead to an oxidative stress condition in the skeletal muscles, testis and brain tissues with a consequent lipid peroxidative response [43, 44, 45]. In the present investigation, administration of PTU, significantly increased lipid peroxidation with a concomitant decrease in the antioxidants such as SOD and CAT in the similar manner as reported earlier by others [9, 10, 11, 30, 46]. However, in PTU-induced animals, PQQ reversed these effects, suggesting that it has the potential to ameliorate hypothyroid induced adverse effects. Some ambiguity exists on the relationship between hypothyroidism and peroxidative system. Hypothyroidism-induced dysfunction of the respiratory chain in the mitochondria causes accelerated production of free radicals (i.e., superoxide anion, hydrogen peroxide, and hydroxyl radical as well as lipid peroxides) which consequently leads to oxidative stress [44, 47, 48]. On the other hand, some workers suggest that hypothyroidism protects tissues against accelerated LPO, although the data concerning oxidation / antioxidant in hypothyroidism are incomplete and contradictory [11, 49]. In fact, Dariyerli et al. 2004 [50] did not find any change in the content of MDA in rats with thiamizole-induced hypothyroidism. Hypothyroidism specifically reduces cellular thiol reserves in most tissues and alters glutathione content. GSH is a well-known antioxidant that provides the major protection against cellular oxidative damages and maintains SH level in proteins [51]. In this experiment, GSH level remained unchanged in PTU administered group in comparison to control as observed earlier by some other

Fig. 2: Effects of PQQ on SOD (units/ mg protein). Data are mean ± S.E.M. (n=7). * p<0.01 compared to the respective control values. † p<0.05 as compared to the PTU treated group. PQQ, Propylthiouracil, SOD, Superoxide Dismutase and PQQ. Pyrroloquinoline Quinone

Fig. 3: Effects of PQQ on CAT (µM H2O2 decomposed/ min/ mg protein). Data are mean ± S.E.M. (n=7). † p<0.01 † p<0.05 compared to the respective control values. * p<0.001, † p<0.01 and † p<0.05 as compared to the respective value of PTU treated group. PQQ, Propylthiouracil, CAT, Catalase and PQQ. Pyrroloquinoline Quinone

In case of GSH content, PTU exhibited no significant change in muscle and testis. However PQQ increased the GSH content significantly (p<0.001) at a dose of 10 mg/kg in both these tissues with a percent increase of 53% and 142% respectively. PTU showed decrease in the GSH content only in brain (25%) where 5 mg and 10 mg/kg of PQQ significantly (p<0.05 and p<0.001 respectively) increased the same, latter concentration being the most effective one with a greater percentage (534%) (Fig. 4).

Fig. 4: Effect of PQQ on GSH content (µM GSH /mg protein). Data are mean ± S.E.M. (n=7). † p<0.05 compared to the respective control values. * p<0.001 and † p<0.05 as compared to the respective value PTU treated group values. PQQ, Propylthiouracil, GSH, Reduced Glutathione and PQQ. Pyrroloquinoline Quinone

Fig. 5: Effects of PQQ (1, 5, and 10 mg/kg/d, i.p.) for 6 days in serum total cholesterol (mg/dl), glucose (mg/dl), SGOT (IU/l), creatinine (mg/dl) and urea (mg/dl). Data are mean ± S.E.M. (n=7). † p<0.001, † p<0.01 compared to the respective control values. * p<0.001, † p<0.01 and † p<0.05 as compared to the respective PTU treated group values. PQQ, Propylthiouracil, PQQ. Pyrroloquinoline quinone, CAT Serum glutamate oxaloacetate transaminase.
workers [52, 53]. However, in brain a significant decrease in GSH level was observed. Interestingly, administration of PQQ increased the GSH level in all tissues, suggesting that the test compound has the potential to enhance cellular antioxidant status.

One of the important characteristics of PQQ is that it is highly electrophilic in nature and it forms stable adducts with carbonyl reagents. These characteristics provide PQQ the ability to oxidize the redox modulatory site, thus conferring protection against ROS-mediated cell injury [20]. The possibility of PQQ-induced reduction in intracellular ROS levels is consistent with the hypothesis that it acts directly or indirectly as a potent free radical scavenger. Probably for this reason LPO level was normalized in PQQ treated animals. In fact, the site of action of PQQ, either intra-mitochondrial or in the cytoplasm, or both, is yet to be determined [54]. The positive effects of PQQ were also reflected in the PTU induced animals with respect to other indices such as serum glucose, total cholesterol, creatinine and urea, particularly at a concentration of 10 mg/kg. The elevation of both serum urea and creatinine levels in response to PTU indicates possible renal and muscular damage. Creatinine and urea are considered as the major indices of impaired kidney functions [55] and their increased level in the serum of hypothyroid rats may be due to reduction in glomerular filtration rate (GFR) [56]. Interestingly, administration of PQQ to PTU treated mice resulted in declined level of both the indices as compared to PTU treated animals, again suggesting the protective effects of the test compound.

The beneficial effect of PQQ was also reflected in the alterations in the activities of SGOT. While in PTU administered animals there was an increase in this enzyme activity, it was reversed by simultaneous administration of PQQ. Enzymatic activities of SGOT are considered as sensitive serological indicators of kidney, muscle and brain tissues [57]. The positive effects of the test drug was again supported by the observation that following the administration of PQQ in PTU administered mice, serum SGOT levels were reduced to near normal values.

Most of the serum parameters were significantly altered by PTU, suggesting that PTU might cause critical injury to vital organs. These observations along with change in LPO and antioxidants indicated that hypothyroidism may lead to the overproduction of free radicals, which in turn exert deleterious effects on different organs. PTU administration increased the total cholesterol concentrations in animals, as reported earlier that chemical thyroidectomy by the treatment of thiourea or PTU causes either depletion or accumulation of liver lipids and variations in the serum total cholesterol is very often related to thyroid dysfunctions altering cholesterol biosynthesis [57]. It has been observed that hyperlipidemia results in the accumulation of cholesterol [58, 59] and hyperlipemia / hypercholesterolemia may result from increased mobilization of body fat reserves due to increased thyrotropic hormone level [60, 61]. In fact, low thyroidin level in hypothyroid animal triggers enhanced thyrotropin secretion, stimulates corticotrophin and adrenal steroids, and thereby increases lipid mobilization through overlapping endocrine axis [62]. An increase of lipid peroxidation attributed to dyslipidemia has also been observed in women with hypothyroidism [63]. Both qualitative and quantitative lipid disorders, hyperlipoproteinaemia and hypercholesterolemia have been described for a long time in association with hypothyroidism [64]. Since we also observed hypercholesterolemia and hyperlipidemia in other weight have been suggested as excellent indicators of decreased thyroid function [65]. Interestingly, in our study all these PTU induced adverse effects including increase in oxidative stress in different tissues were ameliorated by the simultaneous administration of PQQ, clearly suggesting the potential of test drug in ameliorating hypothyroidism.

The possible mechanism of its efficacy to ameliorate PTU induced oxidative stress could be the strong antioxidant properties of test compound, PQQ, as suggested in some previous studies, in which the free radical scavenging activity of PQQ has been highlighted [19, 15]. As decrease in tissue lipid peroxidation and increase in superoxide dismutase and catalase activities coincided with elevation in thyroid hormones, it is quite possible that these adverse effects of PTU might have been ameliorated by PQQ through the alterations in thyroid hormones, because thyroid hormones are known to reduce tissue lipid peroxidation and increase the levels of natural antioxidants [66].

PQQ was earlier known to regulate different abnormalities including liver and cardiac problems [23, 67] which are often related with thyroid dysfunctions. Despite this nothing was known on the role of PQQ in regulating PTU induced oxidative stress in muscle, testis and brain tissues and the present report appears to be the first one that indicates the efficacy of PQQ in regulating PTU induced LPO in the aforesaid tissues. Whatever may be the mechanism of action, from our present findings it is clearly evident that PQQ has the potential to ameliorate PTU induced oxidative stress and it may work against hypothyroidism. Further investigation will help in understanding the therapeutic properties of PQQ in regulating hypothyroidism.

CONCLUSION

Our findings revealed for the first time, that PQQ has the potential to ameliorate PTU induced oxidative damage in muscle, testis and brain of mice, indicating the possible beneficial effect of the test compound in regulating PTU induced oxidative damages. Of course out of three doses of PQQ (1, 5 and 10 mg /kg /d), 10 mg/kg body weight was found to be the most effective one.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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