A subchronic oral toxicity study on pyrroloquinoline quinone (PQQ) disodium salt in rats

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ARTICLE INFO

Article history:
Received 4 June 2014
Accepted 7 November 2014
Available online 15 November 2014

Keywords:
Pyrroloquinoline quinone disodium salt
Subchronic toxicity
Rats
NOAEL

ABSTRACT

A subchronic oral toxicity study on pyrroloquinoline quinone (PQQ) disodium salt was performed in rats. Sprague-Dawley rats were randomly divided into four groups (10 rats/sex/group) and administered with PQQ disodium salt at doses of 0 (control), 100, 200 and 400 mg/kg bw/day by gavage for 13 weeks. Daily clinical observations and weekly measurement of body weights and food consumption were conducted. Blood samples were obtained on day 46 and day 91 for measurement of hematolog and serum biochemical parameters. Animals were euthanized for necropsy, selected organs were weighted and recorded. Histological examination was performed on all tissues from animals in the control and PQQ disodium salt treatment groups. No mortality or toxicologically significant changes in clinical signs, body weight, food consumption, necropsy findings or organ weights was observed. Differences between treated and control groups in some hematolog and serum biochemical examinations and histopatholog examination were not considered treatment-related. The no-observed-adverse-effect-level (NOAEL) of PQQ disodium salt in rats was considered to be 400 mg/kg bw/day for both sexes, the highest dose tested.

1. Introduction

In 1979, pyrroloquinoline quinone (PQQ) was identified as a novel coenzyme in several bacterial dehydrogenases (Salisbury et al., 1979). Since then, it has been detected in various foods and in tissues and body fluids of humans and rats (Kumazawa et al., 1992, 1995; Noji et al., 2007). PQQ can be synthesized by some microorganisms, the major source of PQQ in human is believed to be microbial sources from the diet (Kumazawa et al., 1992, 1995; Misra et al., 2012). PQQ plays an important nutritional role in both bacteria and higher organisms and is involved in numerous physiological and biochemical processes (Misra et al., 2012; Rucker et al., 2009). It has been reported to have benefits in rodents related to improvement of neonatal growth, reproductive performance and immune function, as well as cardioprotection and neuroprotection (Steinberg et al., 1994, 2003; Tao et al., 2007; Zhang et al., 2006).

In 2003, two scientists identified a PQQ-dependent dehydrogenase enzyme in mice and found that PQQ acted as a mammalian redox cofactor in lysine metabolism. Thus they concluded PQQ to be a newcomer to the B group of vitamins (Kasahara and Kato, 2003). However, the statement was subsequently doubted by other scientists who claimed that sufficient evidence was not available to conclude that PQQ performed an essential vitamin function in mammals (Felton and Anthony, 2005; Rucker et al., 2005). Nevertheless, PQQ did appear to have growth promoting properties in rodents and there are several patents on PQQ applications in different countries including the United States of America, Japan and China focusing on its commercial potential as human food and/or supplement ingredient (Gu et al., 2012; Sumi et al., 2012; Tsuji et al., 1998; Zhou et al., 2010).

For any new dietary ingredient intended to be added into foods or used as a dietary supplement, it is necessary and essential to conduct a safety assessment, especially a toxicological evaluation. However, there are only a few reports on the toxicity evaluation of PQQ. One study evaluated the potential genotoxicity of PQQ disodium salt using a battery of genotoxicity tests (Ames test, in vitro chromosomal aberration test in Chinese hamster lung cells, in vitro chromosomal aberration test in human peripheral blood lymphocytes and in vivo micronucleus assay in mice) and concluded that PQQ had no genotoxic activity (Nakano et al., 2013). In another study, functional and morphologic changes of kidneys were observed when PQQ was intraperitoneally injected into rats at a dose of 11.5 mg/kg (Watanabe et al., 1989). Subchronic toxicity studies can provide more information on the possible health hazards of test
substances from repeated exposure over a prolonged period of time; therefore, a 13-week subchronic oral toxicity test on PQQ disodium salt was conducted in rats in this study.

2. Materials and methods

2.1. Test substance

PQQ disodium salt, molecular weight 374.17, water-soluble reddish brown crystalline powder with mild hygroscopic property, purity >98%, was provided by Shanghai Med Co., Ltd., China.

2.2. Animals

Weanling Sprague-Dawley (SD) rats, specific pathogen-free grade, were purchased from Vital River Laboratory Animal Technology Co, Ltd (Beijing, China). Body weights of rats at receipt were 60–80 g. All animals were examined for clinical signs of ill health on receipt and observed within 5 days of arrival. Rats were housed in an environmental controlled room with the temperature at 23 ± 2 °C and the relative humidity within the range of 40–70%. Air was changed 10–15 times per hour. Light was set for a 12 hr light/dark cycle. Rats were individually housed in suspended stainless steel, open-mesh cages and allowed free access to pellet feed and tap water during the experiments.

2.3. Experimental design

Eighty healthy SD rats were randomly divided into four groups, 10 animals/sex/group. One group administered with water by gavage served as the control. An acute oral toxicity of PQQ disodium salt was previously conducted by Hoon’s method as described in the Chinese Toxicology Assessment Procedures and Methods for Food Safety (Chinese Standard GB15193.3-2003) in our lab, the dose selection for this study was based on the result of the acute oral toxicity (the LD50 of females and males was 5.01 and 3.69 g/kg, respectively), 400 mg/kg bw/day (around 10% of LD50) was selected as the high dose level, and 200 mg/kg bw/day and 100 mg/kg bw/day were chosen as the other two lower levels. PQQ disodium salt was administered to rats by gavage once daily for 13 weeks. Clinical observations were recorded daily. Body weights were measured weekly and the gavage volume (1 ml/100 g body weight) was adjusted accordingly. Blood samples were obtained in the middle of the study period.

2.4. Clinical observations

Each animal was observed twice daily for abnormalities, physical appearance and mortality throughout the study. Observations included, but were not limited to, changes in skin, fur, eyes, appearance, salivary gland secretions, oral mucosa, fecal characteristics, respiration and behavior.

2.5. Body weight and food consumption

The body weights of rats were measured pre-test, weekly thereafter and at sacrifice after fasting. Food consumption was measured once a week during the experiment period.

2.6. Hematology and serum biochemistry

On day 46 and day 91, rats were anesthetized with 3% sodium pentobarbital solution and blood samples were collected from the tail vein. Blood samples for hematology were collected into tubes containing ethylenediaminetetraacetic acid anticoagulant. A COULTER Ac.T diff2 Hematology Analyzer (Beckman Coulter Corporation) was employed to measure the following parameters: red blood cell count (RBC), hemoglobin (HGB), platelet count (PLT), white blood cell count (WBC) and WBC differential count of lymphocyte (LYM), granulocyte (GR) and monocyte (MO). Blood samples for serum biochemistry were collected into tubes containing no anticoagulant and centrifuged to obtain serum. An automatic analyzer (Hitachi 7080, Hitachi High-Technologies Corporation) was used to analyze the following parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), glucose (GLU), blood urea nitrogen (BUN), creatinine (CRE), cholesterol (CHO) and triglyceride (TG).

2.7. Pathology

All rats were humanely sacrificed at the end of the test, and a complete necropsy was performed including an examination of the external features of the carcass, external body orifices, the abdominal, thoracic, and cranial cavities, organs and tissues. Organ weights were obtained for the heart, kidneys, liver, spleen, testes and thymus. Paired organs were weighed together. Organ-to-body weight ratios (relative organ weights) were also calculated. In addition to the above-mentioned organs, the following tissues (when present) were sampled and fixed in 10% neutral-buffered formalin: cecum, colon, duodenum, esophagus, femur with bone marrow, ileum, jejunum, lacrimal gland, lung, lymph node, mammary gland, nasal turbinates, pancreas, pituitary gland, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle (thigh), skin, spinal cord, sternum with bone marrow, trachea, urinary bladder, vagina, ovary and adrenals.

All stored organs and tissues from each animal in the control group and PQQ disodium salt treatment groups were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and subjected to microscopic examination. Macroscopic lesions observed at necropsy were also examined from each animal in all groups.

2.8. Statistical analysis

Data were presented as mean ± SD. The SPSS Statistical System (SPSS for Windows 18.0, Chicago, USA) was used to analyze body weights, food consumption, hematology and serum biochemistry parameters, and organ weights, followed by testing for variance homogeneity. Homogeneous data were analyzed using the analysis of variance (ANOVA) and the significance of differences between control and treated groups were analyzed using Dunnett’s multiple comparisons. P < 0.05 was considered statistically significant.

3. Results

3.1. Mortality and clinical signs

No mortality or treatment related adverse clinical reactions were found during the study.

3.2. Body weight and food consumption

There were no statistically significant differences in body weights between the treatment groups and the control group in each week (Fig. 1). No significant differences were observed between the treatment groups and the control group for weekly food consumption of females and males (Fig. 2).

3.3. Hematology and serum biochemistry

There were some sporadic, statistically significant changes in some hematology and serum biochemical parameters (Tables 1 and 2).
Fig. 2. Mean food consumption of female and male rats received different levels of PQQ disodium salt for 13 weeks.

2). On day 46, compared with the control group, the percentage of granulocyte was significantly higher for females at 200 mg/kg bw/day group, and blood glucose level was significantly lower for male rats at 100 mg/kg bw/day group. No statistically significant differences were observed between the treatment groups and the control group for all other hematology and serum biochemistry parameters.

3.4. Organ weights and pathology

There were no statistically significant differences in all absolute organ weights and relative organ weights (organ-to-body weight ratios) between treatment groups and the control group for male and females (Table 3). No macroscopic pathology findings were observed in all males and females. Histopathological findings are summarized in Table 4. Histopathological findings showed various lesions, including slightly sporadic focal necrosis and spotty necrosis in liver, focal necrosis in heart, deposition of calcium salts in renal tubular in kidneys and testicular atrophy in testes. The incidences and/or severities of these changes did not differ between the controls and the PQQ disodium salt treatment groups. There were no histopathological lesions observed in the cecum, colon, duodenum, esophagus, femur with bone marrow, ileum, jejenum, lacrimal

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<th>Males</th>
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<td>WBC (×10^3/L)</td>
<td>6.57 ± 1.79</td>
<td>8.86 ± 2.62</td>
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<td>RBC (×10^6/L)</td>
<td>6.95 ± 0.39</td>
<td>6.60 ± 0.27</td>
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<td>HGB (g/L)</td>
<td>1448.5 ± 5.4</td>
<td>1535.8 ± 6.6</td>
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<tr>
<td>ALT (U/L)</td>
<td>71.4 ± 0.28</td>
<td>714.8 ± 48.6</td>
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<td>AST (U/L)</td>
<td>35.4 ± 2.9</td>
<td>622.8 ± 84.6</td>
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<tr>
<td>ALP (U/L)</td>
<td>1513 ± 8.4</td>
<td>1561.3 ± 4.5</td>
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Table 1

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<tr>
<th>Parameters</th>
<th>Females</th>
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<tr>
<td>Hb (g/L)</td>
<td>151.3 ± 13.9</td>
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<td>Mo (%)</td>
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<td>Pt (%)</td>
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<tr>
<td>Lym (%)</td>
<td>74.4 ± 3.2</td>
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<td>El (%)</td>
<td>39.3 ± 3.6</td>
<td>18.4 ± 2.3</td>
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Table 2

* P < 0.05 as compared with the control group.
gland, lung, lymph node, mammary gland, nasal turbinates, pancreas, pituitary gland, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle (thigh), skin, spinal cord, spleen, sternum with bone marrow, thymus, trachea, urinary bladder, vagina, ovary and adrenals.

4. Discussion

Previous studies have shown that PQQ has various benefits on physiological and biochemical processes and plays an important nutritional role in rodents, such as improvements in growth, reproduction performance, immune function, cardioprotection and neuroprotection (Steinberg et al., 1994, 2003; Tao et al., 2007; Zhang et al., 2006). However, PQQ cannot be synthesized in higher organisms, and so has a potential to be used as a dietary supplement. In our present study, no PQQ disodium salt-related deaths or abnormalities in clinical signs were noted. No significant changes in body weight, food consumption, necropsy findings were observed in relation to the administration of PQQ disodium salt by gavage at concentrations as high as 400 mg/kg bw/day.

In the histopathological examination, some microscopic changes in the liver, heart, kidneys and testes were noted. However, all of those pathological changes were within the range of normal background lesions and sporadically detected in control and treatment groups; therefore, these effects could be considered incidental and reflected the usual individual variability without any relationship to the treatment.

In conclusion, the results of our present study demonstrate that the NOAEL for PQQ disodium salt was 400 mg/kg bw/day in male and female rats, the highest dose tested.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

References


