Effects of Orally Administered Pyrroloquinoline Quinone Disodium Salt on Dry Skin Conditions in Mice and Healthy Female Subjects

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Summary Pyrroloquinoline quinone (PQQ) is a coenzyme involved in the redox-cycling system. The supplemental use of PQQ has been examined based on its properties as an antioxidant and redox modulator. Although an animal study on deficiency of PQQ suggested that PQQ contributes to skin conditions, its efficacy in humans has not been reported. The present study aimed to investigate the effects of orally administered PQQ on skin moisture, viscoelasticity, and transepidermal water loss (TEWL) both in dry skin mouse models and in healthy female subjects with a subjective symptom of dry skin. In our dry skin mouse model study, oral intake of PQQ (0.0089%, w/w, in the diet for 6 wk) significantly decreased the number of mast cells in the dermis and the number of CD3⁺ T-cells in the epidermis. In our human study, oral intake of PQQ (20 mg/d for 8 wk) significantly inhibited the increase in TEWL on the forearm. Finally, subject questionnaires showed positive impressions for the improvement of skin conditions. These results suggest that oral intake of PQQ improves skin conditions both in female subjects with dry skin and in mice with a compromised skin barrier function.

Key Words pyrroloquinoline quinone, skin care, transepidermal water loss, skin moisturizing, clinical study

Pyrroloquinoline quinone (PQQ) was discovered as the third redox cofactor after nicotinamide and flavin in the redox-cycling system (1). Because PQQ-deprived mice show several abnormalities, such as poor development and fragile skin, PQQ has been considered as a candidate for dietary supplementation (2, 3). While the compound was first identified in 1964, its presence and function in animals has just been recognized in the last decade (4). Since its physiological functions are not yet well understood, investigations aimed at identifying its physiological roles and their underlying mechanisms are of high interest. PQQ is an antioxidant involved in mitochondrial functioning and has neuroprotective properties (5). PQQ has been reported to work in combination with coenzyme Q10, another nutrient critical for mitochondrial functioning, to improve learning in rats (6). Moreover, PQQ deficiency in mice results in fragile skin (7). However, the efficacy of oral PQQ intake on skin conditions in humans has not been reported. In the present study, we investigated the effects of oral PQQ intake on skin conditions in dry skin mouse models and female subjects with mildly dry skin.

MATERIALS AND METHODS

PQQ. PQQ was manufactured by Mitsubishi Gas Chemical Co., Inc. (Tokyo, Japan) in its disodium salt form (BioPQQ™).

Animal study design. Female hairless mice (HOS: HR-1; ~20 g, 4-wk-old) were obtained from Hoshino Laboratory Animals (Bando, Japan) and acclimated for 1 wk. Animals were randomly assigned to three groups (n=8 for each group) and fed standard chow (CE-2; CLEA Japan, Inc., Tokyo, Japan) or a low magnesium chow (HR-AD; Nosan Corporation, Yokohama, Japan) in which the magnesium content had been reduced (0.02%, compared to 0.34% in the normal chow CE-2). HR-AD-fed HOS:HR-1 mice show severe dry skin symptoms accompanied by a decrease in dermal water content and an increase in transepidermal water loss (TEWL) between days 14 and 28 following diet commencement (8). The normal control group received CE-2 alone. The non-PQQ control group was fed HR-AD alone. The PQQ group received HR-AD containing 0.0089% (w/w) PQQ. This dose was defined so
that the animals would ingest up to 20 mg PQQ per kg body weight per day. The actual amount of PQQ intake in this animal study was approximately 16 mg/kg body weight per day as calculated from the amount of food consumed. Of note, it was previously reported that PQQ intake at a dose of 0.2–0.3 mg/kg body weight effectively decreased parameters of inflammation in humans (7). TEWL (9, 10) and skin conductance represent the hydration state of skin (11), and these parameters were evaluated on the backs of mice for up to 6 wk by standard methods using a model TM210 Tewameter (Courage+Khazaka Electronic, Köln, Germany) and a SKICON-200EX (IBS Co., Ltd., Hamamatsu, Japan), respectively. Skin thickness, mast cell number in the dermis, and CD3⁺ T-cell number in the epidermis were measured after 6 wk from the start of the study.

Human study design. A randomized, double blind, placebo-controlled study was performed at a single location (Kaiseikai, Shin-Yokohama Skin Lab Center, Yokohama, Japan) under the supervision of the principle investigator, Dr. Mitsuhiko Fukagawa. Skin conductance was used for the screening test with a threshold value of <50 arbitrary units. Eligible subjects were women aged 20 to 49 y who had mildly dry skin and were free of skin diseases such as atopic dermatitis and psoriasis systemic diseases. The final study group consisted of 22 healthy female subjects with a subjective symptom of dry skin on their arms. All subjects had no relationship with Mitsubishi Gas Chemical Co., Inc. The study period was 8 wk, from April 15, 2013 to July 2, 2013. This study conformed to the principles in the Declaration of Helsinki and was approved by the clinical study review committee of the Aisei Hospital Ueno Clinic (Tokyo, Japan) based on the protocol and information on the test substances. The details of the study protocol were disclosed to the subjects before the start of the study, and the investigators obtained informed consent from each subject. After confirmation that the subjects fulfilled the defined eligibility criteria, they were randomly assigned to 2 groups (the PQQ group, n=11, and the placebo group, n=11). Each day, the PQQ group received 2 capsules that contained 10 mg of PQQ per capsule, and the placebo group ingested 2 capsules that contained no PQQ. The subjects ingested the two capsules once a day after breakfast for 8 wk. The dose of PQQ was determined based on a previous report (7).

Measurements of TEWL and skin conductance. The skin region of interest was cleansed with a cleansing agent (Unilever Japan, Tokyo, Japan) and rinsed with warm water twice. TEWL was measured using a Tewameter TM300 (Courage+Khazaka Electronic) after an acclimatization period of at least 15 min. The average value of 6 out of 8 test scores that had been calculated from a stable part of a 1-min measurement diagram was used and expressed in g/m²/h. The ambient room temperature was maintained at 20.0±1°C with a relative humidity of 50±5%. Skin hydration was assessed as skin conductance using a Corneometer CM825 (Courage+Khazaka Electronic), which measures the reactive capacitance of the skin, using the stratum corneum as a dielectric membrane. The point of intersection between left cheek and nose and 4.5 cm from the elbow on the left forearm were selected as the skin areas for measurement. The measurements were performed 8 times in the face-up position. The mid-6 values (expressed in arbitrary units) obtained were used to calculate the mean values.

Measurement of skin viscoelasticity. Skin viscoelasticity was measured with a Cutometer MPA580 (Courage+Khazaka Electronic). A 2-mm test probe was lightly pressed on the skin with a constant negative pressure. The skin was lifted, stretched, and then released. The resulting deflections were measured under the following conditions: off time, 2.0 s; on time, 2.0 s; repetition, 1; pressure, 300 mbar. The measured parameters included the overall elasticity, R2 (Uo/Uf); the net elasticity, R5 (Uo/Uf); the ratio of elastic recovery to the total deformation, R7 (Uf/Uo) (12). Measurement was repeated 8 times on the intersecting skin area between the right cheek and the nose of each subject in a sitting position. The mid-6 values obtained were used to calculate the mean values.

Evaluation of subjective recognition of skin conditions. We evaluated subjective overall skin conditions based on a questionnaire about the subjects’ skin conditions, including dryness, hydration, softness, viscoelasticity, and coarseness of the arm, face, and body. Data are expressed as an 11-grade score that represents the following subjective recognitions of wrinkles or pigmentation, such as macules and freckles: 5 points (good condition or no problem) to −5 point (bad condition or problematic) stepped by 1 point each (the point 0 thus represents the mean of these scores).

Statistical analysis. All of the measured values and changes are expressed as the mean±SE. In the animal study, statistical analyses of variance were performed using Bartlett’s test and with either one-way ANOVA employing a post hoc Tukey-Kramer test or Kruskal-Wallis test employing a post hoc Steel-Dwass test for data where variances were equal or unequal across groups, respectively. The same analysis procedure was done in the human study, except two-factor factorial ANOVA was performed instead of one-way ANOVA. The methods of statistical analysis are described in the legends of the figures and tables. p<0.05 was considered significant. Data analysis and statistical analysis were performed using Excel 2010 (Microsoft) with the add-in software Statcel3 (OMS Publishing Inc., Saitama, Japan).

RESULTS

Animal study

In HOS:HR-1 mice fed a diet with reduced magnesium content (HR-AD) for 6 wk, TEWL was significantly increased and skin conductance, which represents the skin hydration state, was decreased at the 6th week compared to mice fed normal chow (7.5±0.9 vs. 32.1±1.6 g/m²/h and 233±12 vs. 29±1 μS, respectively). There were significant differences in the TEWL score between the CE-2 group and HR-AD group or
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HR-AD+PQQ group compared within the week; however, there was no significant difference at 2nd, 4th, or 6th week between the HR-AD+PQQ group and HR-AD group. The TEWL score at the 6th week was 29.9±3.2 and 32.1±1.6 g/m²/h in the HR-AD+PQQ group and HR-AD group, respectively (Fig. 1A). With respect to skin conductance, the score in the HR-AD group was 13.4±1.4% of the initial value, while the score in the HR-AD+PQQ group was 18.8±2.5% of the initial value after 6 wk (Fig. 1B). The skin thickness was 64.0±4.0 and 71.9±3.7 μm in the HR-AD+PQQ and HR-AD groups, respectively (Fig. 1C). After 6 wk of the diet intake, a significant decrease in the number of mast cells in the dermis was observed in the PQQ group (p<0.05 compared to the non-PQQ group by the Tukey-Kramer test; Fig. 1D). The number of CD3⁺ T-cells in the epidermis was significantly decreased (p<0.05 compared to the non-PQQ group by the Steel-Dwass test; Fig. 1E). There was no significant difference in the amount of diet ingested among the groups (data not shown).

Human study

Subjects. Sixty-seven females aged from 29 to 49 y were enrolled, and 22 subjects were finally selected as participants in this study based on the study criteria. The subjects were randomly allocated to the PQQ group (20 mg PQQ per day, intake of two capsules containing 10 mg PQQ, starch, calcium stearate and starch hydrolysate each, n=11, 34.9±7.8 y old) and the placebo group (intake of two placebo capsules that contain no PQQ, n=11, 33.5±6.7 y old). At the start of this study, there was no statistically significant difference between the two groups with regard to body weight, height, body mass index, blood pressure, or skin measurement parameters. Three subjects in the placebo group resigned during the course of this study, reducing the number of subjects in the placebo group to 8 at the time

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Fig. 1. Effects of PQQ intake on TEWL (A), skin conductance (B), skin thickness (C), dermal mast cell number (D), and epidermal CD3⁺ T-cell number (E) in a dry skin mouse model. HOS-HR-1 mice were randomly allocated to 3 groups and fed diets as described in “Materials and Methods.” CE-2, normal chow group; HR-AD, low magnesium chow group; HR-AD+PQQ, PQQ-supplemented HR-AD chow group. Values with different superscripts are significantly different from each other at p<0.05 compared to the CE-2 group by the Steel-Dwass test (A: 0, 2nd, 4th, and 6th week, respectively, C: 6th week, and E: 6th week) or by the Tukey-Kramer test (D: 6th week). There was no statistical difference between the two groups in the experiment by the Mann-Whitney U test (B).

Fig. 2. Effects of PQQ intake on skin conductance in female subjects with a subjective symptom of dry skin. Subjects received oral PQQ (20 mg/d; n=11) or placebo (n=8) for up to 8 wk. The skin conductance on the left forearm (A) and the left cheek (B) was measured at the 4th and 8th week following the start of the study. The differences between the initial value (Week 0) and week 4 or 8 in individual subjects were calculated. The mean±SE value for each group is shown. The Steel-Dwass test (A) or Tukey-Kramer test (B) revealed no difference between the two groups for any parameter.
Effect of PQQ intake on skin hydration. The degree of skin hydration was evaluated based on skin conductance. There was no significant change in skin conductance on the left forearm or left cheek of either the PQQ or placebo group (Fig. 2A and B).

Effect of PQQ intake on TEWL. A decrease in TEWL represents improved skin barrier function, which protects skin from water loss at the skin surface (12). There was no significant difference in the TEWL score of the left forearm between the placebo and the PQQ group all through the study period (Fig. 3A). There was a statistically significant decrease in the change of TEWL on the left forearm after 4 wk in the PQQ group compared to that in the placebo group ($p < 0.05$ compared between the two groups by the Tukey-Kramer test; Fig. 3C). No significant differences in TEWL scores or changes in TEWL scores were observed in the left cheek of either the placebo group or the PQQ group after 4 or 8 wk (Fig. 3B and D).

Effect of PQQ intake on skin viscoelasticity. The skin viscoelasticity represents skin radiance and firmness (13). Higher skin viscoelasticity indicates better skin radiance/firmness. There was no significant difference in the overall elasticity (R2), the net elasticity (R5), or the ratio of elastic recovery to the total deformation (R7) between the two groups compared to the starting point or at the 4th or 8th week of the study period (Fig. 4A and B).

Effect of PQQ intake on skin conditions. Statistical analysis of questionnaire answers concerning the skin conditions of the arm, face, and body of each subject showed that subjective recognition of wrinkles and pigmentation, such as macules and freckles on the face, were significantly improved in the PQQ group at the 4th and 8th week of the study period (Table 1).

Adverse effect. Based on the judgment of the principal investigator of this clinical study (Dr. Mitsuhiko Fukagawa), PQQ intake caused no adverse effects.

Fig. 3. Effects of PQQ intake on TEWL in female subjects with a subjective symptom of dry skin. Subjects received oral PQQ (20 mg/d; $n=11$) or placebo ($n=8$) for up to 8 wk. The TEWL on the left forearm (A) and the left cheek (B) was measured at the 4th and 8th week following the start of the study. The changes between the initial value (Week 0) and week 4 or 8 in individual subjects were calculated (C, D). The mean ± SE value for each group is shown. Values with different superscripts are significantly different from each other at $p<0.05$ compared to Week 0 by the Tukey-Kramer test (C). There was no statistical difference between the two groups in the experiment by the Tukey-Kramer test (A, B) or Steel-Dwass test (D).

Fig. 4. Effects of PQQ intake on skin viscoelasticity in female subjects with a subjective symptom of dry skin. Subjects received oral PQQ (20 mg/d; $n=11$) or a placebo ($n=8$) for up to 8 wk. The following viscoelastic properties were measured on the left cheek at the 4th and 8th week following the start of the study: (A) the overall elasticity ($R_2$, $U_f/U_a$), (B) the net elasticity ($R_5$, $U_r/U_e$), and (C) the ratio of elastic recovery to the total deformation ($R_7$, $U_r/U_f$). The difference between the initial value (Week 0) and week 4 or 8 in individual subjects was calculated. The mean ± SE value for each group is shown. There was no statistical difference between the two groups in the experiment by the Tukey-Kramer test (A, B, and C).
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We found that there were significant decreases in the dermis and the number of CD3+ T-cells after PQQ intake. In the animal study, daily intake of PQQ significantly decreased the number of mast cells in the skin. HOS-HR-1 mice are generally used as an atopic dermatitis model that is induced by a low-magnesium diet (8). Since this model exhibits a severe dry skin condition with a marked increase in TEWL, it is widely used to evaluate the skin barrier function of those natural products expected to show a skin-moisturizing effect such as beet extract, which contains ceramide glucosides, or acacia extract, which contains polyphenols (14, 15). In atopic dermatitis and psoriasis, the skin barrier function is decreased, and T-cells play a major role in the development of dermatitis, which is associated with an increase in dermal mast cell number (16–18). These changes can be observed during the disruption of skin barrier function induced by dry skin. Therefore, in the present study, we measured the number of dermal mast cells and epidermal CD3+ T-cells. We found that there were significant decreases in the number of CD3+ T-cells and dermal mast cells after PQQ intake. Thus, it is likely that PQQ prevents the disruption of skin barrier function in this model.

In the double blind, placebo-controlled study in humans, the TEWL score in the left forearm was significantly improved in the PQQ group after 4 wk (Fig. 3C). Skin conductance and TEWL values in the left cheek were not significantly changed during this study either in the PQQ or placebo group.

Since PQQ has an anti-inflammatory and anti-oxidative effect (7), PQQ may be effective in depleting lipid peroxides on the skin surface (2, 19). The antioxidative effect of PQQ is considered to be mediated by the change in mitochondrial function (7). These effects suggest that PQQ may improve skin condition. In addition, natural antioxidants other than PQQ, including coenzyme Q10 and vitamin E, have been shown to exhibit skin-moisturizing effects in addition to their antioxidative effects (20). Addressing whether the mechanism of skin improvement by PQQ is the same as its antioxidative effect is an important area of future research.

The subjective recognition of wrinkles and pigmentation was significantly improved in the PQQ group. The biological process of wrinkle development largely depends on an age-related increase in TEWL and a decrease in sebum secretion (21). It is suggested that both an increase in stratum corneum hydration and a reduction of TEWL contribute to these effects. There was no adverse effect or abnormal change in any physiological parameters examined during the course of PQQ intake. Therefore, orally administered PQQ at 20 mg/d is most likely to be safe in humans.

Aging often leads to dry skin accompanied by a reduced ability of the skin to perspire and secrete sebum and other skin-protecting factors (22). Moreover, aged skin cells replicate more slowly than younger cells, and aged skin becomes thick and takes a longer time to repair (23). As a result, the hydration status of skin is reduced in the elderly. This age-related dry skin is seen more often in females than in males (24). The present study has demonstrated that orally ingested PQQ improves skin conditions because less water is lost from the skin’s surface, and the appearance of wrinkles and pigmentation is reduced in humans. Taken together, our results demonstrate the physiological benefits of PQQ as an oral agent to improve skin condition.

REFERENCES


**DISCUSSION**

The present study was conducted to investigate whether oral intake of PQQ effectively improves skin conditions in animal models of dry skin and in human subjects. In the animal study, daily intake of PQQ for 6 wk significantly decreased the number of mast cells in the dermis and the number of CD3+ T-cells in the epidermis (Fig. 1D and E). HOS-HR-1 mice are generally used as an atopic dermatitis model that is induced by a low-magnesium diet (8). Since this model exhibits a severe dry skin condition with a marked increase in TEWL, it is widely used to evaluate the skin barrier function of those natural products expected to show a skin-moisturizing effect such as beet extract, which contains ceramide glucosides, or acacia extract, which contains polyphenols (14, 15). In atopic dermatitis and psoriasis, the skin barrier function is decreased, and T-cells play a major role in the development of dermatitis, which is associated with an increase in dermal mast cell number (16–18). These changes can be observed during the disruption of skin barrier function induced by dry skin. Therefore, in the present study, we measured the number of dermal mast cells and epidermal CD3+ T-cells. We found that there were significant decreases in the number of CD3+ T-cells and dermal mast cells after PQQ intake. Thus, it is likely that PQQ prevents the disruption of skin barrier function in this model.

In the double blind, placebo-controlled study in humans, the TEWL score in the left forearm was significantly improved in the PQQ group after 4 wk (Fig. 3C). Skin conductance and TEWL values in the left cheek were not significantly changed during this study either in the PQQ or placebo group.

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The subjective recognition of wrinkles and pigmentation was significantly improved in the PQQ group. The biological process of wrinkle development largely depends on an age-related increase in TEWL and a decrease in sebum secretion (21). It is suggested that both an increase in stratum corneum hydration and a reduction of TEWL contribute to these effects. There was no adverse effect or abnormal change in any physiological parameters examined during the course of PQQ intake. Therefore, orally administered PQQ at 20 mg/d is most likely to be safe in humans.

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Table 1. Effect of PQQ intake on the subjective recognition of facial skin conditions.

<table>
<thead>
<tr>
<th>Item</th>
<th>Group</th>
<th>Week 0</th>
<th>Week 4</th>
<th>Week 8</th>
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</thead>
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<tr>
<td>Wrinkles</td>
<td>Placebo</td>
<td>−0.9±0.7</td>
<td>−0.1±0.2</td>
<td>0.5±0.5</td>
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<td></td>
<td>PQQ</td>
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<td>0.5±0.6**</td>
<td>0.6±0.7*</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>Placebo</td>
<td>−2.3±0.6</td>
<td>−1.4±0.4</td>
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</tr>
<tr>
<td></td>
<td>PQQ</td>
<td>−3.6±0.5</td>
<td>−1.3±0.6*</td>
<td>−0.7±0.6**</td>
</tr>
</tbody>
</table>

Each value represents the score described in “Materials and Methods.” *p<0.05; **p<0.01 by the Wilcoxon signed-rank test compared to the start of the study (Week 0).


