Potential physiological importance of PQQ

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Review Article

Potential Physiological Importance of Pyrroloquinoline Quinone

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Abstract

Pyrroloquinoline quinone (PQQ) is a novel biofactor for which a proposition can be made for physiological importance. PQQ was first recognized as an enzyme cofactor in bacteria. It has recently been tentatively identified as a component of interstellar dust. Thus, PQQ may have been present throughout early biological conception and evolution. PQQ is also a potent plant growth factor. Consequently, for animals and humans, there has been constant exposure to PQQ. In animals, PQQ is reported to participate in a range of biological functions with apparent survival benefits (e.g., improved neonatal growth and reproductive performance). There are also benefits from PQQ supplementation related to cognitive, immune, and antioxidant functions, as well as protection from cardiac and neurological ischemic events. Although PQQ is not currently viewed as a vitamin, its involvement in cell signaling pathways, particularly those important to mitochondriogenesis in experimental animal models, may eventually provide a rationale for defining PQQ as vital to life. For humans, such evidence suggests there may be similar parallels or benefits from improving PQQ status. (*Altern Med Rev* 2009;14(3):268-277)

Introduction

Pyrroloquinoline quinone (PQQ) was first recognized as a bacterial cofactor by Hauge,[1] and later by Anthony,[2-4] Salisbury,[5,6] Duine,[7] and their co-workers. PQQ, also known as methoxatin (Figure 1), is water soluble and heat stable. Under appropriate conditions, PQQ is capable of catalyzing continuous redox cycling (the ability to catalyze repeated oxidation and reduction reactions), as well as oxidative deaminations.[8]

These chemical properties are novel in many respects. For example, in chemical assays, PQQ's stability renders it capable of carrying out thousands of redox catalytic cycles; whereas, other bioactive quinones capable of redox cycling (e.g., epicatechin) tend to self oxidize and/or form polymers (e.g., tannins). Table 1 contains data that in part demonstrates the effectiveness of PQQ as a redox cycling agent.[8-13] There is also a range of papers that describe PQQ's use in an analytical setting.[14-17] PQQ molecules can be immobilized and fixed at the surface of analytical electrodes. When coupled to appropriate enzyme systems, highly specific and sensitive assays have been developed to assay compounds ranging from glucose to common narcotics.[14-17] As will be highlighted in subsequent sections, the novel chemical attributes of PQQ help explain many of its metabolic and health-related features.

Biochemical Roles and Mechanisms

Evolution and Its Functions in Bacteria and Plants

To make the case for physiological and biomedical importance in humans, it is important to note that many of PQQ's functions are universal. For example, for many bacterial species, PQQ stimulates growth and serves as a cofactor for a special class of dehydrogenases/

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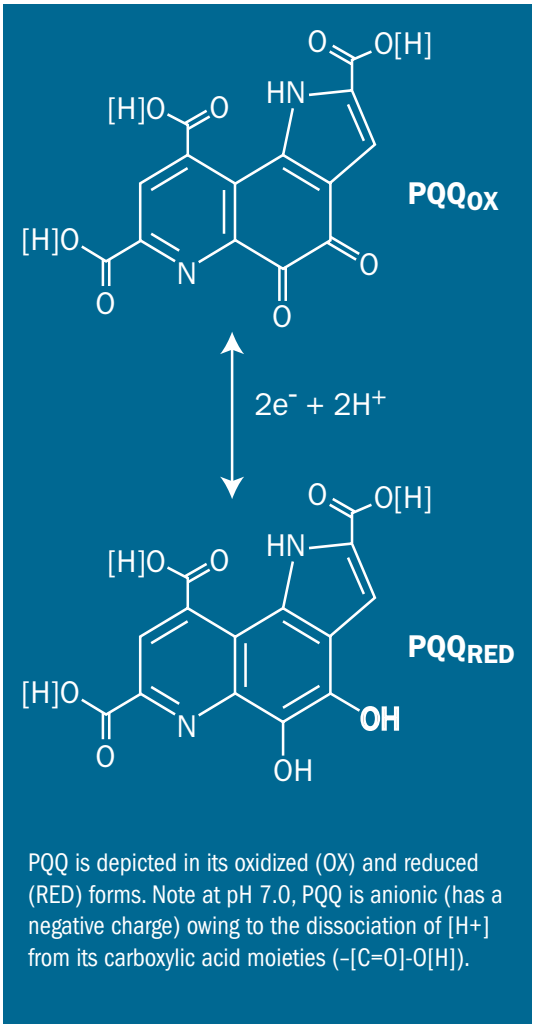
Figure 1. Structure of PQQ



PQQ is depicted in its oxidized (OX) and reduced (RED) forms. Note at pH 7.0, PQQ is anionic (has a negative charge) owing to the dissociation of [H+] from its carboxylic acid moieties (-[C=O]-O[H]).

oxidoreductases. Enzymes containing PQQ are sometimes designated quinoproteins.[8] Although the "quinoproteins" include many types of quinone-containing proteins and enzymes, the PQQ-requiring glucose and alcohol dehydrogenases are distinguished, because the PQQ associated with these proteins is dissociable and synthesized in metabolic pathways that can be separately controlled from those pathways important for the generation of the eventual targeted protein.[18-20] In addition to a cofactor role, PQQ can also be thought of as a trophic factor important to the growth and metabolism of bacteria, particularly methylotrophic bacteria (bacteria capable of growing on simple carbon sources).

From an evolutionary perspective, current evidence suggests PQQ is a component of interstellar dust as analyzed by particle impact time-of-flight mass spectrometry.[21,22] Cometary grains are considered to be the precursors of organic materials in early life on the earth. It can also be argued that strong redox catalysts would be required to trigger the earliest chemical evolutionary steps. The presence of PQQ in stellar dust raises the question of PQQ's evolutionary importance to simpler life forms, given its wide range of chemical properties, such as redox catalysis and the ability to carry out useful amino acid modifications (e.g., oxidative deamination reactions).

At the next level it is important to highlight the symbiotic relationship between plants and soil bacteria, such as rhizobacterium.[23-25] Plants cultivated in hydroponic culture systems with rhizobacterium have significantly increased height, flower number, fruit number, and total fruit weight; whereas, this does not occur with genetically modified rhizobacteria unable to produce PQQ.[23] PQQ added directly to hydroponic culture systems also confers a significant increase in the fresh weight of seedling plants. In part, the role of PQQ is related to phosphate uptake by plants, because PQQ, as a cofactor for rhizobacteria dehydrogenases, facilitates making soil and the local environment more acidic.[24] As a consequence, phosphate is made more available to plant roots. In addition, independent roles have been proposed for PQQ related to plant growth via activation of cell signaling, antioxidant defense, and viral protection.[25]

For humans and animals, the ubiquitous presence of PQQ in common types of bacteria, soil, and plants suggests constant exposure to PQQ. PQQ has been found in all plant foods analyzed to date.[8,26-28] In this regard, it is interesting to note that although many bacteria make PQQ, this is not the case for common intestinal bacteria, such as *Escherichia coli*. *Escherichia coli* can synthesize PQQ-dependent enzymes capable of utilizing PQQ under certain nutrient limiting conditions;[29] however, the enzymes only become functional when PQQ is present. Hence, an external source of PQQ may be important in sustaining human and animal tissue levels of PQQ, as well as maintaining an optimal enteric environment.

Table 1. PQQ as a Redox Cycling Agent

Compound	Potential Number of Catalytic Cycles
PQQ	20,000
Quercetin	800
Catechin	75
Epicatechin	700
Norepinephrine	200
Epinephrine	100
DOPA	20
6-OH-DOPA	20
Ascorbic Acid	4

Redox cycling systems result in repeated chemical reactions in which molecules that act as catalysts are repeatedly oxidized and/or reduced. The potential number of catalytic cycles (number of repeated reactions) depends in part on chemical stability. PQQ is relatively stable; whereas, self-oxidation, polymerization, and/or changes in chemical structure are factors that compromise the chemical stability of many bioactive quinones or enediols (e.g., ascorbic acid). Details of the redox cycling system and basis for defining the relative number of catalytic cycles may be found in references 8, 13 and 67.

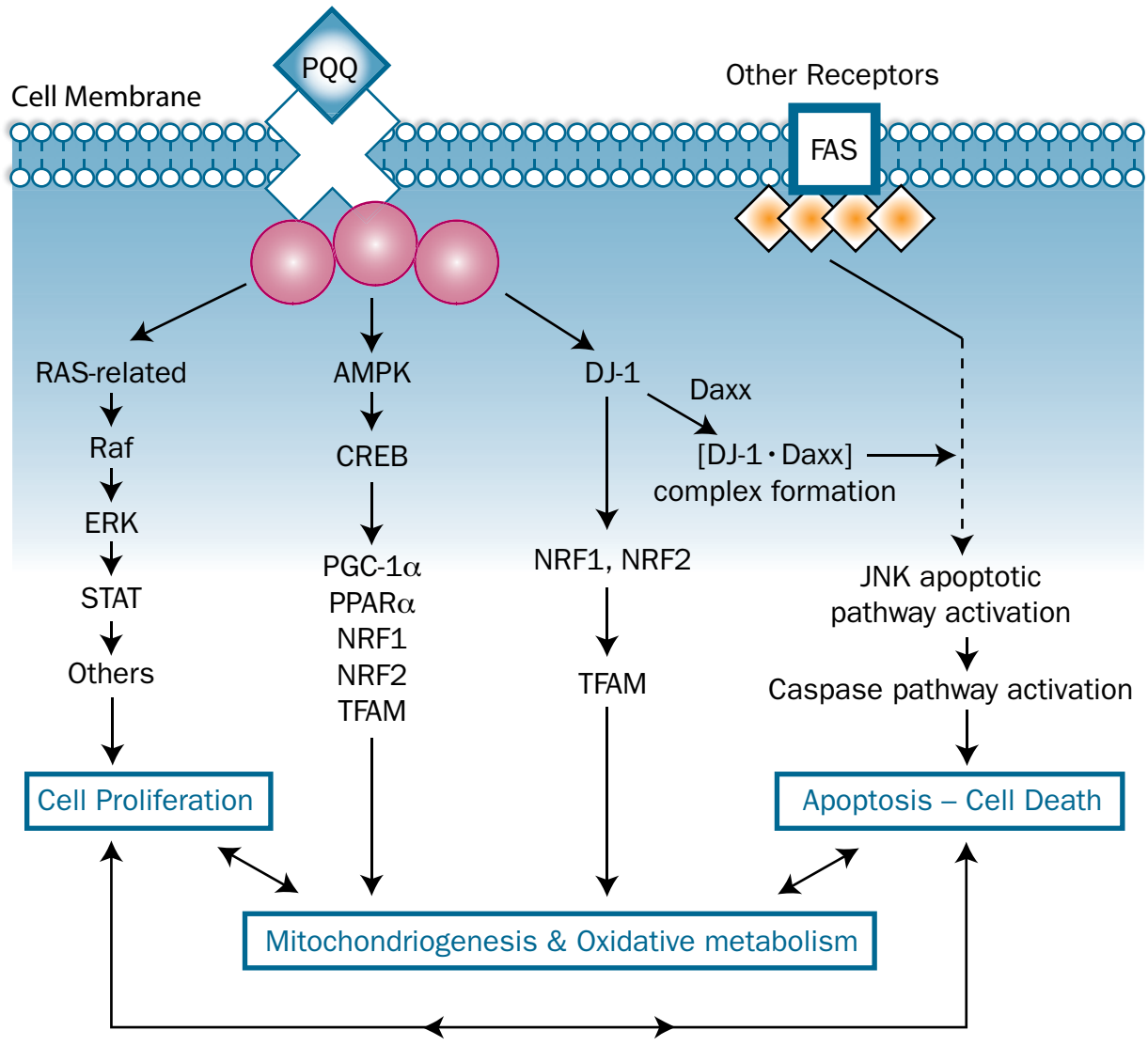
Mechanisms and Proposed Functions in Humans and Animals

A number of physiological properties have been attributed to PQQ, ranging from classical water-soluble vitamin/cofactor functions to those important to antioxidant potential.[8,30-39] While a role as a vitamin in animal or human nutrition seems unlikely at this time, similar to other polyphenolic biofactors, there is strong evidence PQQ may play an important role in pathways important to cell signaling.[35-39] PQQ can also serve as an antioxidant.[40] The importance of PQQ to mammalian health is evident when it is omitted from chemically defined diets, resulting in a wide range of systemic responses, including growth impairment, compromised immune responsiveness, and abnormal reproductive performance in mouse and rat experimental models.[8,25,26,41,42] Furthermore, varying PQQ in highly refined diets causes modulation in mitochondrial content, alters lipid metabolism, and reverses inhibition elicited by classical complex I inhibitors.[39,41-43]

Improvements in mitochondrial respiratory control are potentially important to a number of health issues, ranging from increased longevity to improved energy utilization and protection from reactive oxygen species. Mitochondrial DNA depletion and mutations are associated with cardiomyopathy, developmental delays, and impaired neurological and mitochondrial function,[44] which further highlights the importance of optimal mitochondrial function for health and well-being. Regarding possible mechanisms of PQQ action (Figure 2), given that many mitochondrial-related events are regulated by peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1α) and nuclear respiratory factors, an interaction between PQQ and a PGC-1α-related pathway seems a logical possibility. Indeed, such interactions have recently been reported.[44-46]

PGC-1α is a transcriptional coactivator that regulates genes involved in energy metabolism.[45,46] An interaction with this protein and its association with multiple transcription factors can provide a direct link between an external physiological stimulus and the regulation of mitochondriogenesis. PGC-1α is also a major factor that regulates muscle fiber type determination and appears to be involved in controlling blood pressure, regulating cellular cholesterol homeostasis, and the development of obesity. Moreover, PGC-1α is associated with a reduction in reactive oxygen species and protection against various mitochondrial toxins.[45]

Figure 2. Cell Signaling and PQQ



In addition to interacting with PGC-1α, PQQ can also affect the activity of ras,[38] an oncogene important to signal transduction processes involved in growth and development. PQQ can stimulate activated c-Ha-ras-transformed NIH3T3 mouse fibroblasts,[33] resulting in increased cell proliferation. With regard to ras, activation usually results in changes in cell growth, differentiation, and survival. In addition to ras, Kumazawa et al[33] have also observed that activation of ERK occurs in response to adding PQQ to cultured fibroblasts. ERK is one of the many protein kinases that functions in the ras-signaling pathway to activate other components of transcription (e.g., activators, co-activators, and transcription factors). Similarly, raf is depicted in Figure 2, because like ERK it is also part of the chain of events that progress from ras to eventual

activation of signal transducers and activators of transcription (STAT) factors that are essential to control of cell growth, survival, and differentiation. Regarding PGC-1α-related cell signaling,[8,39,41-43] activation starts with signals from 5' AMP-activated protein kinase (AMPK) or one of the many mitogen-activated protein (MAP) kinases that are linked to various cell surface receptors.[44,45] Such kinases activate cAMP response element binding protein (CREB), which is a transcription factor that binds to certain DNA sequences called cAMP response elements. The authors recently reported[39] that CREB activation in combination with other transcription factors, such as nuclear respiratory factors 1 and 2 (NRF) and mitochondrial transcription factors (e.g., Tfam), leads to increased mitochondriogenesis observed with PQQ administration.[41-43]

PQQ has been reported to influence the activity of DJ-1[37]. DJ-1 is involved in cellular oxidative stress responses, and autosomal-recessive mutations in DJ-1 lead to Parkinson's disease. A possible role of an interaction between DJ-1 and PQQ may be to facilitate and fine-tune overall cellular regulation.[37] The cell signals for growth and energy utilization via mitochondria are in communication with apoptotic (programmed cell death) signals. DJ-1, most likely by associating with Daxx (a multifunctional protein associated with apoptotic events), is capable of modulating apoptosis by inactivating yet another cell signaling pathway, the so-called Janus kinase (JNK) cell-signaling pathway. An important feature of this interaction is potential "crosstalk" with STAT components and control of caspase activation/deactivation (cysteine-aspartic acid proteases). Caspases are associated with apoptosis or in pathological situations with cellular necrosis and inflammation. Accordingly, when all is taken together, an important feature of the interactions depicted in Figure 2 is the crosstalk between the signals, such ras, DJ-1, and numerous kinases, that control proliferation, apoptosis, and mitochondriogenesis.

Regarding PQQ's role as an antioxidant, recent studies on the aroxyl radical-scavenging action of reduced $PQQH_2$ have shown PQQ exists in cells in a reduced form.[40] Like vitamin C, glutathione, vitamin E, and uric acid, PQQ can act as an antioxidant.[8] As examples, Tsuchida et al[47] and Urakami et al[48] reported PQQ protects against acute liver damage induced by agents such as carbon tetrachloride or endotoxin. Hamagishi et al[49] observed that PQQ administered (i.p.) at 10 or 30 mg/kg body weight causes a decrease in carrageenan -induced edema by 39- and 76 percent, respectively. It is also noteworthy that on a molar basis, PQQ is a better inhibitor of tissue oxidation in peritoneal cells than α-tocopherol and ascorbic acid, following initiation by zymosan, carrageenan, or N-formyl-methionyl-leucyl-phenylalanine, all of which provoke inflammatory responses.[44] From a mechanistic perspective, in addition to serving as an antioxidant, the effects of PQQ on genes, such as PGC-1α, DJ-1, and genes in the ras family help explain many of the physiological and clinical functions ascribed to PQQ.

Clinical Implications

The following subsections briefly describe clinical implications of PQQ use. Although much of this work was conducted in animal models, current efforts in humans and human cell lines demonstrate important parallels.

Improvements in Reproduction, Early Development, Growth, and Immune Function

Nutritional studies indicate PQQ can serve as a growth factor and improves neonatal survival.[8,31,41,42] In human fibroblast cultures, PQQ enhances cell growth and proliferation when added to cell cultures.[38,50] Signs of PQQ deprivation include friable skin, evidence of hemorrhage and diverticuli, and reduction in general fitness. The growth-related observations are novel in that adding 100-200 μg PQQ/kg to purified diets improves growth, development, and reproductive parameters in rodent models.[8,30] For perspective, the animal requirements for folic acid or for biotin range from 200-500 μg/kg diet, respectively. These effects are similar to the improvements when more complex diets are fed (i.e., made of less refined ingredients).[30,31] Moreover, in female mice and rats fed PQQ-deficient diets, fertility is decreased (fewer successful pregnancies and smaller litter size)[30,31] compared to mice or rats fed PQQ-supplemented diets.

PQQ deprivation also results in defects in immune function and reduction in interleukin-2 (IL-2) levels. There is loss of B- and T-cell sensitivity to mitogens. The body normally produces IL-2 during an

immune response. IL-2 is necessary for the development of T-cell immunologic memory, one of the unique characteristics of the immune system. Maximizing sensitivity of B- and T-cells to mitogens is achieved in mice when as little as 1 nmol PQQ is added per gram of diet, about 100-400 µg PQQ per day in human equivalents.[30,31]

PQQ and Neuroprotection

Neuronal cell death in experimental models of stroke and spinal cord injury is attenuated by PQQ.[31,32,51-54] PQQ has been demonstrated to protect the redox modulatory site of N-methyl-d-aspartic acid (NMDA) receptors.[36,51-59] Agents that protect NMDA-receptor function are often neuroprotective in experimental stroke and spinal cord injury models. In this regard, intraperitoneal administration of PQQ effectively promotes the functional recovery of spinal cord injury in rats after hemi-transection.[36] Protection is preceded by a decrease in inducible nitric oxide synthase (iNOs) mRNA. Nitric oxide is implicated in NMDA receptor-mediated neurotoxicity. Administration of PQQ decreased lesion size and increased axon density associated with the lesion area. Furthermore, recent studies suggest PQQ protects against secondary damage by reducing iNOS expression following a primary physical injury to the spinal cord. Peroxynitrite is a potential byproduct of abnormally high nitric oxide (NO) or cellular hydrogen peroxide levels. The demonstration that iNOS expression is reduced is in part a validation of previous work showing that PQQ treatment suppresses peroxynitrite formation.[56] Moreover, PQQ's ability to affect the oxidative status of DJ-1 adds an additional dimension.[37] As has been noted previously, the expression level and oxidation status of DJ-1 have been shown to play a role in antioxidative stress reactions important to neurological function. These findings add to the initial observations by Jensen et al[54] that PQQ effectively reduces infarct size in an experimental model of cerebral hypoxia/ischemia. PQQ administered i.p. at 10-15 mg/kg body weight in rats was effective in reducing cerebral infarct volumes measured 72 hours or more after a neurovascular insult. Three hours after ischemia a dose of 3 mg/kg significantly reduces infarct volume compared to vehicle-treated animals. These data indicate PQQ may be a useful neuroprotectant in stroke therapy.

Even at a more subtle level, PQQ exposure can affect learning ability and memory function in rats.[60] Rats fed a PQQ-supplemented diet demonstrate improved learning using the Morris water maze test as an index. Rats were fed 20 mg PQQ, 300 mg coenzyme Q10 (CoQ10), 200 mg R,R,R-α-tocopherol, or 20 mg PQQ + 300 mg CoQ10/kg body weight/day for nine weeks (from age four weeks). Each rat was subjected to hyperoxia as the oxidative stress (using a 100% oxygen chamber) for 48 hours. Those fed PQQ-supplemented diets were protected from a memory deficit that was apparent in controls not fed PQQ. As a novel control, rats fed vitamin E-supplemented and -deficient diets were tested. Vitamin E-deficient rats fed PQQ and/or CoQ10 demonstrated improved learning function. In addition, longer-term memory function was maintained independently by PQQ, but not by CoQ10 supplementation. Thus, PQQ seems potentially effective in sustaining learning functions during oxidative stress, independent of and in a manner different from that of vitamin E.

PQQ and Cardiac Function

PQQ is useful in models of cardiac ischemia.[43,61,62] PQQ confers resistance to acute oxidative stress in freshly isolated cardiomyocytes.[61] Both oxidative damage and mitochondrial membrane potential depolarization (induced by hydrogen peroxide) are significantly reduced by preincubation with PQQ. Moreover, in whole animal models of damage due to cardiac ischemia and reperfusion, PQQ results in less cardiac damage, higher left ventricle pressures, and fewer ventricular fibrillation episodes, if given i.p. 30 minutes before occlusion.[61] In rodent models of cardiac ischemia, PQQ at doses ranging from 5-20 mg/kg administered i.p. was inversely related to infarct size. In the same tests, PQQ was superior to metoprolol in protecting mitochondria from ischemia/reperfusion oxidative damage.[43]

Side Effects and Toxicity

Safety studies for PQQ in humans have been conducted in preparation for several human use patents.[63,64] PQQ was administered at 20 or 60 mg/day for four weeks to two groups (10 each) of healthy adults given either a PQQ supplement or a placebo. These studies were double-blinded and conducted at two different

commercial drug-testing facilities: the New Drug Clinical Center, Fukuhara Clinic, Eniwa, Hokkaido, Japan and Cronova Co., Ltd., Suminoeku, Osaka, Japan. No adverse effects were observed in standard clinical tests at either dose (e.g., glucose, triglycerides, and various lipoprotein fractions). Functional tests for liver toxicity were also normal (e.g., aspartate aminotransferase and serum glutamic oxaloacetic transaminase). At 60 mg PQQ daily, the amounts of urinary N-acetyl-β-(D)-glucosaminidase activity were also within the normal range. N-acetyl-glucosaminidase is a renal hydrolytic enzyme located primarily in the lysosomal fraction of the renal tubular cell. Abnormal changes in renal tubular function or damage results in its elevation in urine.[65]

Single-dose oral toxicity tests in rats were performed in compliance with Good Laboratory Practice (GLP). The single-dose oral toxicity tests indicated the approximate lethal dose of PQQ is less than 1,000 mg/kg body weight of rats, but higher than 500 mg/kg.

Post-mortem pathological examinations of test rats suggest the kidney as the principal target organ for acute effects of PQQ. In part, this is a validation of an earlier published toxicology study[66] in which PQQ was administered intraperitoneally to rats at a dose of 11-12 mg/kg body weight. Signs of renal tubular damage and inflammation were observed. When lower doses were used, however, no treatment effects or obvious pathological signs were observed. Likewise, in a 90-day repeated dose study in which PQQ was administered to rats by oral gavage (3, 20, or 100 mg PQQ/kg body weight) no adverse effects were observed. Moreover, at oral dosage levels from 250-2,000 mg PQQ/kg in mice, an examination for micronucleus induction in red blood cells showed no effects. Lastly, the results from a battery of genotoxicity tests *in vitro* (the Ames, micronucleus, and chromosomal aberration tests) were negative, i.e., PQQ did not cause clastogenic toxicity (chromosome breaks, rearrangements and changes in chromosomal number).

In summary, these observations taken together suggest there is no evidence of acute side effects or overt toxicity from consuming PQQ in amounts up to 60 mg per day for humans or several hundred mg per kg of diet fed to animals.

Dosage

Regarding typical exposures of free PQQ, as noted above, the amount for humans is estimated to vary from 100-400 μg daily,[11,25,28,67] about the same as the daily nutritional recommendations for biotin and folic acid, respectively. However, PQQ easily forms condensation products upon interaction with amino acids,[26] complicating the precision of such estimates. The primary condensation products are imidazolopyrroloquinoline (IPQ) and imidazolopyrroloquinoline derivatives with attached amino acid side chains as part of the chemical structure. For example, only about 15 percent of the PQQ is present in free form in biological fluids such as human milk, while 85 percent is present as IPQ and derivatives.[26] Thus, it is not unreasonable to assume that for humans the total exposure to PQQ derivatives may be as much as 1-2 mg per day. This amount is in the range that clearly influences optimization of growth and health in animal models.[8] In the case of human milk, PQQ amounts to 1-2 μg PQQ/g of milk solid, which is also similar to the PQQ concentrations reported for bovine milk.[26] It is important to note that PQQ appears readily absorbed. Smidt et al[68] determined that the apparent absorption of an oral dose of ^{14}C-PQQ ranges from 20-80 percent when administered to adult mice in the fed state. The percentages were estimated from the amount of radioactivity present in urine and tissues 24 hours after administration.

Conclusions

The observation that increased mitochondriogenesis and antioxidant functions may be healthful features of PQQ supplementation opens the doors for both therapeutic applications and possible use as an ergogenic aid. Having normal mitochondrial function is essential to a broad range of health and disease relationships; thus, the need for continuing research that examines the efficacy and use of PQQ is compelling. PQQ derivatives are widely distributed in tissues and biological fluids at concentrations that may be sustained by typical dietary exposures. Given the range of functions and apparent survival benefits (e.g., improved reproductive performance), it is reasonable to suggest that PQQ may play a fundamental role in metabolism.

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Corrections

Altern Med Rev 2009;14(2):143.

Figure 2. Meriva should read Meriva curcuminoids, curcumin should read curcuminoids.

Paragraph 3: "One small unpublished...

Should read: "One small unpublished, single-dose trial demonstrated 450 mg of Meriva curcuminoids complexed with phosphatidylcholine was absorbed as efficiently as 4 g unbound *Curcuma longa* (95% curcumin), reflecting a significant increase in bioavailability for Meriva complex (Figure 2).15