Squalene And Its Potential Clinical Uses

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Abstract

Squalene, an isoprenoid compound structurally similar to beta-carotene, is an intermediate metabolite in the synthesis of cholesterol. In humans, about 60 percent of dietary squalene is absorbed. It is transported in serum generally in association with very low density lipoproteins and is distributed ubiquitously in human tissues, with the greatest concentration in the skin, where it is one of the major components of skin surface lipids. Squalene is not very susceptible to peroxidation and appears to function in the skin as a quencher of singlet oxygen, protecting human skin surface from lipid peroxidation due to exposure to UV and other sources of ionizing radiation. Supplementation of squalene to mice has resulted in marked increases in cellular and non-specific immune functions in a dose-dependent manner. Squalene may also act as a "sink" for highly lipophilic xenobiotics. Since it is a nonpolar substance, it has a higher affinity for un-ionized drugs. In animals, supplementation of the diet with squalene can reduce cholesterol and triglyceride levels. In humans, squalene might be a useful addition to potentiate the effects of some cholesterol-lowering drugs. The primary therapeutic use of squalene currently is as an adjunctive therapy in a variety of cancers. Although epidemiological, experimental and animal evidence suggests anti-cancer properties, to date no human trials have been conducted to verify the role this nutrient might have in cancer therapy regimens.

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Introduction

Squalene (see Figure 1) is a naturally occurring polyprenyl compound primarily known for its key role as an intermediate in cholesterol synthesis. It received its name because of its occurrence in shark liver oil (*Squalus spp.*), which contains large quantities and is considered the richest source of squalene. However, it is widely distributed in nature, with reasonable amounts found in olive oil, palm oil, wheat-germ oil, amaranth oil, and rice bran oil. Many other polyprenyl compounds structurally similar to squalene exist in nature and perform critical biological functions. For example, animals utilize prenyl groups to form the side chain of ubiquinone. The most common form of ubiquinone in the human body is coenzyme Q10 (CoQ10); the Q10 designation indicating the molecule has 10 prenyl groups in its side-chain. Other well-known substances requiring prenyl groups for their synthesis, and therefore having a structural similarity with squalene, include carotenes, vitamin A, vitamin K, vitamin D, vitamin E and

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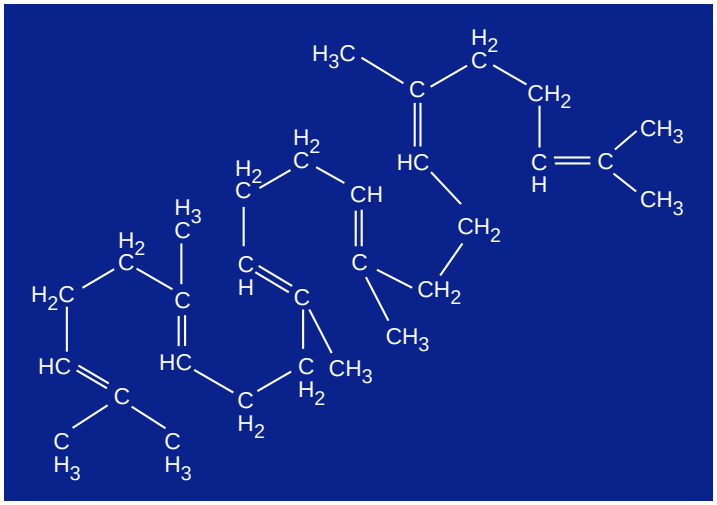
Figure 1. Chemical structure of squalene.



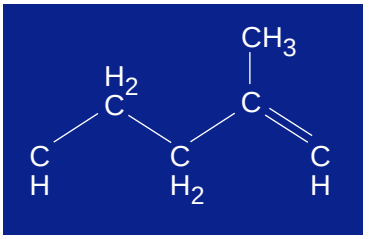
other tocopherols, tocotrienols, cyclic terpenoid compounds (such as camphor, pinene, and limonene), and dolichol.

Biochemistry

Squalene is a symmetrical 30-carbon polyprenyl compound containing six prenyl (also known as isoprenoid or isoprene) units. (See Figure 2 for structure of a prenyl group)

Structurally similar to beta-carotene, squalene is an intermediate metabolite in the synthesis of cholesterol. The basic skeletal structure of all steroids is formed by the condensation of six prenyl groups. The 30-carbon polyprenyl chain structure of squalene is enzymatically collapsed into a series of three interconnected, closed, six-carbon rings attached to a five-carbon ring with a prenyl side-chain.

Figure 2. Prenyl unit.



The endogenous synthesis of squalene (see Figure 3) begins with the production of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA). The initial reduction of HMG CoA (a niacin-dependent reaction) results in the formation of mevalonate. The enzyme involved in this reduction, HMG CoA reductase, has been the target of a class of cholesterol-lowering drugs; however, since these drugs reduce the formation of prenyl compounds, they also interfere with the synthesis of CoQ10.[1-3]

Mevalonate is then phosphorylated in three stages (via magnesium-dependent enzymes) and finally decarboxylated to form delta 3-isopentenyl diphosphate, the donor molecule for all polyprenyl compounds. Successive additions of prenyl groups result in the formation of the 15-carbon farnesyl diphosphate. Two molecules of farnesyl diphosphate are then enzymatically joined and reduced (niacin dependent) resulting in the formation of squalene.

After its biosynthesis, squalene can be transported to other areas of the body for incorporation into tissues or it can be further metabolized, resulting in the eventual formation of cholesterol and its steroid metabolites.

Pharmacokinetics

Serum squalene originates partly from endogenous cholesterol synthesis and partly from dietary sources, especially in populations consuming large amounts of olive oil or shark liver. Although its post-absorptive metabolism has not been studied in detail in humans, available evidence indicates somewhere between 60-85 percent of dietary squalene is absorbed from an oral dose.[3-6] Up to 90 percent of the post-absorptive dose is transported in serum, generally in association with very low density lipoproteins, until it is distributed ubiquitously in human tissue.[4] The greatest concentration of squalene occurs in the skin, where it is one of the major components of skin surface lipids.[6] Based on animal evidence, prolonged oral administration might result in a significant accumulation of squalene in the liver (3-6 percent of an oral dose).[7]

Metabolic studies of squalene in human adipose tissue indicate fat tissue contains very high concentrations of squalene, about 80 percent of which is located in the central neutral lipid droplet, while 20 percent is bound to the microsomal membranes. Experimental evidence also suggests only microsomal membrane-bound squalene is metabolically active and that approximately 90 percent of the newly formed squalene is stored in the lipid droplet and only 10 percent is used in cholesterol synthesis.[8]

Clinical Applications

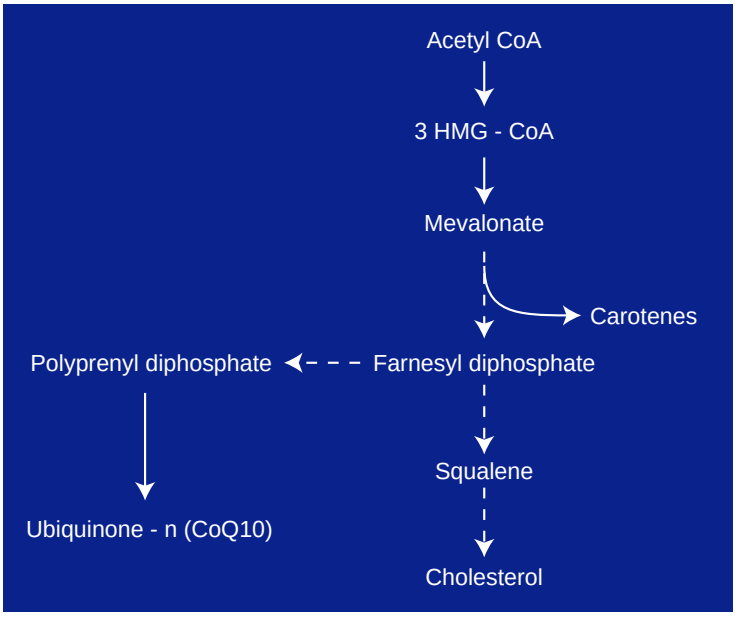
Squalene and Cholesterol Metabolism: Although oral administration of squalene appears to consistently decrease hepatic and serum levels of plant sterols,[9] its impact on cholesterol metabolism in humans is less clear. Available evidence suggests a substantial amount of dietary squalene is absorbed and converted to cholesterol in humans; however, this increase in synthesis is not associated with consistent increases of serum cholesterol levels, possibly as a result of a concomitant increase in fecal elimination.[4]

Dietary supplementation with one gram of squalene daily for nine weeks was reported to cause increases in serum total, VLDL-, IDL-, and LDL-cholesterol concentrations by 12, 34, 28, and 12 percent, respectively. However, a subsequent six-week period on a lower dose of squalene (0.5 g/day) normalized serum sterols.[5]

Strandberg et al supplemented humans with squalene (900 mg/day for 7-30 days). While serum squalene levels were increased 17-fold, no significant changes were reported in serum triglyceride and cholesterol contents. Squalene feeding produced a significant increase in fecal excretion of cholesterol, its nonpolar derivatives, and bile acids, suggesting that, although cholesterol synthesis probably increased by as much as 50 percent, fecal elimination was also upregulated, resulting in no net effect on serum cholesterol concentrations.[4]

Replacement of dietary triglyceride by squalene is reported to result in up to a 50-percent reduction in the absorption of cholesterol in rats.[10] However, two human reports indicate no change in cholesterol absorption while supplementing the diet with squalene, suggesting the elimination of triglycerides, not the addition of squalene, was responsible for the decline in cholesterol absorption.[4,5]

Figure 3. Squalene, carotene, cholesterol, and coenzyme Q10 synthesis.



Of particular interest is a study indicating squalene, added into a protocol with low-dose pravastatin, enhanced the efficacy of pravastatin as a hyperlipidemic drug, suggesting squalene might be a prudent dietary addition for individuals on similar cholesterol-lowering drugs. Chan et al conducted this

double-blind, placebo-controlled study to compare pravastatin (10 mg) and squalene (860 mg), either alone or in combination for the treatment of hypercholesterolemia. One hundred and two patients received either treatment or placebo for 20 weeks. Although pravastatin was more effective than squalene in reducing total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides and in increasing levels of high-density lipoprotein (HDL) cholesterol, the combination of both pravastatin and squalene was even more efficacious in reducing total and LDL cholesterol and increasing HDL cholesterol.[11]

Squalene as a Skin and Eye Antioxidant: Squalene is not very susceptible to peroxidation and appears to function in the skin as a quencher of singlet oxygen, protecting human skin surface from lipid peroxidation due to exposure to ultraviolet light and other sources of oxidative damage.[12]

In vitro experimental evidence indicates squalene is a highly effective oxygen-scavenging agent.[13] Subsequent to oxidative stress, such as sunlight exposure, squalene functions as an efficient quencher of singlet oxygen and prevents the corresponding lipid peroxidation in human skin surface. Kohno et al found the rate constant of quenching of singlet oxygen by squalene to be much larger than those of other lipids in human skin surface, and to be comparable to 3,5-di-t-butyl-4-hydroxytoluene (BHT). They also reported squalene is not particularly susceptible to peroxidation and is stable against attacks by peroxide radicals, causing them to suggest "the chain reaction of lipid peroxidation is unlikely to be propagated with adequate levels of squalene present in human skin surface."[12]

In animal models, squalene also appears to play an important role in the health of the retina, with particular regard to rod photoreceptor cells and the assembly of rod outer segment (ROS) disk membranes. Both *in vitro* and *in vivo*, the majority of newly synthesized squalene in the retina is transported to the ROS, where it turns over in parallel with the disk membranes.[14]

Squalene and Detoxification of Xenobiotics: Experimental evidence suggests squalene might act as a "sink" for highly lipophilic xenobiotics, assisting with their elimination from the body. Since it is a nonpolar substance, it appears to have the highest affinity for un-ionized drugs.[15]

Richter et al investigated squalene as an alternative to paraffin to enhance the elimination of [14C]hexachlorobenzene (HCB), an organochlorine xenobiotic. They found dietary treatment with squalene (as eight percent of the diet) was as effective as paraffin (as eight percent of the diet) in markedly enhancing fecal excretion of HCB. Following three weeks of dietary squalene treatment, the amount of HCB excreted with feces was about three times higher, and the half-life of HCB elimination from the body was markedly decreased (mean 34-38 days as compared to 110 days for controls) in treated animals.[16] Although Richter and Schafer have also indicated the effect of squalene upon HCB concentrations is strongly dose dependent in some tissues, no significant differences in liver and blood HCB concentrations were seen with animals fed 5.0 or 7.5 percent of their diet as squalene.[17]

Kamimura et al have suggested that squalene, by enhancing drug elimination from the body, might be a good candidate as an antidote to reduce the toxicity of accidentally-ingested drugs. In animal experiments, they observed increased fecal excretion of theophylline and strychnine in rats fed squalene.[15]

Squalene and Cancer: The primary therapeutic use of squalene currently is as an adjunctive therapy in a variety of cancers. Although epidemiological, experimental and animal evidence suggests anti-cancer properties, to date no human trials have been conducted to verify the role this nutrient might have in cancer therapy regimens.

Newmark has proposed, due to the relatively high squalene content of olive oil, squalene might be a contributing factor for the epidemiological observations of reduced risk for several cancers associated with olive oil intake.[18] Rao et al have joined Newmark in support of this hypothesis, also suggesting that squalene, as a constituent of olive oil, might be partially responsible for olive oil's chemopreventive effect.[19]

Squalene, as well as some of its related substances, vitamin A, vitamin E, vitamin K, beta-carotene, ubiquinone (CoQ10), and phytol, were examined in an animal experimental model to determine the existence of chemopreventive effects. Observations indicated squalene, as well as the related compounds, was capable of suppressing the growth of tumor cells.[20] Desai et al have reported both squalene and Roidex, a squalene-containing compound, are able to partially prevent the development of chemically-induced cancer and to cause regression of some already existing tumors in a mouse skin model.[21]

Rao et al assessed the chemopreventive efficacy of squalene on azoxymethane-induced colonic aberrant crypt foci. They reported diets containing one percent squalene inhibited aberrant crypt formation and crypt multiplicity by about 46 percent, suggesting chemopreventive activity against colon carcinogenesis.[19] Katdare et al have reported squalene is able to inhibit aberrant hyperproliferation, a cellular marker associated with pre-neoplastic transformation.[22] In animal models, squalene is capable of mitigating the sodium arsenite-induced sister chromatid exchanges and micronuclei proliferation in a dose-dependent manner.[23] Murakoshi et al have reported squalene is able to inhibit the effect of the tumor promoting agent 12-O-tetradecanoylphorbol-13-acetate in mouse-skin carcinogenesis. The authors noted an ability to inhibit the induction of ornithine decarboxylase (ODC), the enzyme considered to be the rate-limiting step in the endogenous biosynthesis of polyamines.[24]

Ohkuma et al investigated the effect of squalene on anti-tumor activity and host immune response in sarcoma 180-bearing female mice. Following intraperitoneal administration of squalene, enhanced reticuloendothelial system function, particularly IgM antibodies, and prolonged survival of the mice was observed.[25] Storm et al have reported administration of squalene as two percent of the diet to mice for 14 days pre- and 30 days postexposure to lethal whole-body gamma-irradiation, resulted in cellular and systemic radioprotection. White cell counts were consistently higher in animals treated with squalene. Squalene treatment was also able to significantly prolong survival time, as compared with control-fed mice, subsequent to exposure to lethal doses of radiation.[26]

While evidence supporting the use of squalene in combination with chemotherapeutic cancer agents in humans is currently lacking, evidence suggests squalene might potentiate the cytotoxic activity of some of these agents. Yamaguchi et al investigated the effect of squalene in conjunction with an antitumor agent (3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea (ACNU)) in a murine tumor system. At a dose of 4.2 g/kg, squalene demonstrated a significant ability to potentiate the effect of ACNU (10 mg/kg) against lymphocytic leukemia P388. The simultaneous administration of this combination resulted in long-term survival of some of the animals, without significant toxicity to the host.[27] Nakagawa et al investigated squalene and its interaction with anticancer agents. Survival of cultured cell lines indicated squalene enhanced the cytotoxicity and anti-tumor activity of Adriamycin, 5-fluorouracil, bleomycin, and cis-diamminedichloroplatinum. Among the tested agents, squalene had strongest potentiating effect on the anti-tumor activity of bleomycin.[28]

Toxicity, Side-Effects, and Dosage

Squalene has not been well studied in humans, so information on toxicity and side-effects is limited. In animal experiments (rats and dogs) conducted over a three-month interval, no appreciable side-effects and no appreciable toxic signs were observed in serum biochemical tests and hepatic functional tests for squalene-treated animals.[7] Since squalene is a naturally occurring lipid component present in healthy diets, it is likely that at reasonable supplemental levels, it is also safe for prolonged administration in humans. Of note, however, is a case of lipoid pneumonia secondary to ingestion of shark oil.[29]

Dosage recommendations vary considerably depending upon the application. As an adjunct to assist with cholesterol lowering, the dose studied in conjunction with pravastatin was 860 mg/day. A dose of 500 mg/day might also have some benefit in normalizing lipid values, but evidence suggests high doses (greater than 1 g/day) might be contraindicated for this application.

Extrapolating from animal evidence, the suggested dose needed for detoxification of xenobiotics would be relatively high. Animals were typically given eight percent of the diet as squalene, and although effects are dose dependent, a dose equal to five percent of the diet might produce similar results. This would represent a minimum dose in humans of about 11 g/day of squalene. Since the long-term safety at this dose of squalene has not been determined in humans, and since this dose significantly exceeds the amount of squalene found in normal diets, it would be prudent to limit high doses of squalene to short intervals.

As an adjuvant therapy for cancer treatment in humans, information on dosing is again unavailable. Extrapolating from the available animal data, a dose of between 2-5 g/day would appear to be the therapeutic window.

Conclusion

The future role of squalene supplementation in cholesterol-lowering regimes remains to be clearly elucidated; however, available evidence suggests a beneficial role in conjunction with pravastatin. Squalene might have similar benefits when given in combination with other HMG-CoA inhibitors. As a stand-alone intervention in hypercholesterolemia, squalene is unlikely to have a significant role. Evidence suggests a substantial amount of dietary squalene is absorbed and converted to cholesterol in humans; however, this increase in synthesis is not associated with consistent increases of serum cholesterol levels, possibly as a result of a concomitant increase in fecal elimination. The concern of low doses of squalene contributing to high cholesterol levels appears to be misplaced. At reasonable dietary levels (0.5 g/day) squalene appears to have no effect on increasing cholesterol and might actually have a normalizing effect on plasma sterol levels.

Squalene appears to be critical in reducing free radical oxidative damage to the skin and might play a similar role in the rod photoreceptor cells of the eye. A diet with an adequate intake of oils containing squalene (such as olive or rice bran oil) might be sufficient for these protective benefits, but supplementing the diet occasionally with a small amount of additional squalene (500 mg/d) might be prudent for individuals exposed to significant ultraviolet radiation.

Squalene's eventual role in detoxification of some xenobiotics largely remains unexplored. Available evidence suggests a promising role for the elimination of organochlorines, theophylline, and strychnine.

Squalene appears to influence several biochemical and physiological activities which are intriguing for the treatment of cancer. Observations indicate squalene can suppress the growth of tumor cells, partially prevent the development of chemically-induced cancer,

and cause regression of some already existing tumors. Supplementation stimulates the reticuloendothelial system, resulting in a marked increase in cellular and non-specific immune function in a dose-dependent manner. Evidence suggests squalene might assist in maintaining white cell counts during radiation treatment, and, in animal models, supplementation is associated with prolonged survival time subsequent to exposure to lethal doses of radiation. While evidence supporting the use of squalene in combination with chemotherapeutic cancer agents in humans is currently lacking, available experimental evidence suggests squalene potentiates the cytotoxic activity of some of these agents. Squalene's ability to inhibit ODC is also of significant interest in cancer prevention and treatment. Cancer cells are known to utilize polyamines as growth substrates, and since ODC is a rate-limiting enzyme in the generation of many of the polyamines, ongoing cancer research has been, and is currently, investigating agents with the ability to interfere with this enzyme's activity.

Although epidemiological, experimental and animal evidence regarding squalene's anti-cancer properties is intriguing and promising, it is important to remember that, to date, no human trials have been conducted to verify the role this nutrient might have in cancer therapy regimens.

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