CHAPTER 14

Biological Importance and Applications of Squalene and Squalane

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Contents

Abstract

Squalene is a polyunsaturated hydrocarbon with a formula of $C_{30}H_{50}$. Squalene can be found in certain fish oils, especially shark liver oil, in high amounts and some vegetable oils in relatively smaller amounts. Human sebum also contains 13% squalene as one of its major constituents. Squalane is a saturated derivative of squalene and also found in these sources. Interest in squalene has been raised after its characterization in shark liver oil which is used as a traditional medicine for decades. Several studies exhibited results that prove certain bioactivities for squalene and squalane. Up to date, anticancer, antioxidant, drug carrier, detoxifier, skin hydrating, and emollient activities of these substances have been

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reported both in animal models and *in vitro* environments. According to promising results from recent studies, squalene and squalane are considered important substances in practical and clinical uses with a huge potential in nutraceutical and pharmaceutical industries.

I. BACKGROUND

Squalene is a triterpene which can be found widely in organism varying from humans to plants (Fig. 14.1). It is a polyunsaturated hydrocarbon with a formula of $C_{30}H_{50}$ and formed by six isoprene units. Squalene has gained massive amount of attraction after being identified in the shark liver extract discovered by Dr. Tsujimoto in 1903, hence it received its name (*Squaluss* spp.). Following this discovery, the shark liver oil has been considered as the main source of squalene along olive oil which also includes squalene as a major hydrocarbon (Gershbein and Singh, 1969). Up to date, squalene has been investigated extensively and reported to play crucial roles in steroid (especially dietary cholesterol) synthesis in human. Under the light of its importance in marine animal, deep-sea sharks, squalene also has been tested for its beneficial bioactivities including antioxidant, antitumor, and cytoprotective effects (Auffray, 2007; Passi *et al*., 2002; Rao *et al*., 1998). It has been promoted as a singlet oxygen receiver and an important lead for future cosmeceutical researches. The cosmeceutical potential of squalene has been extensively raised after the studies which showed that squalene existed widely in human sebum, hair fat, and other surface lipids (Passi *et al*., 2002). In addition to cosmeceutical studies, squalene is regarded as an important compound for chemoprotective activities as well as nutraceutical for maintaining health under toxic exposure (Das *et al*., 2003). Fully natural occurrence and studied

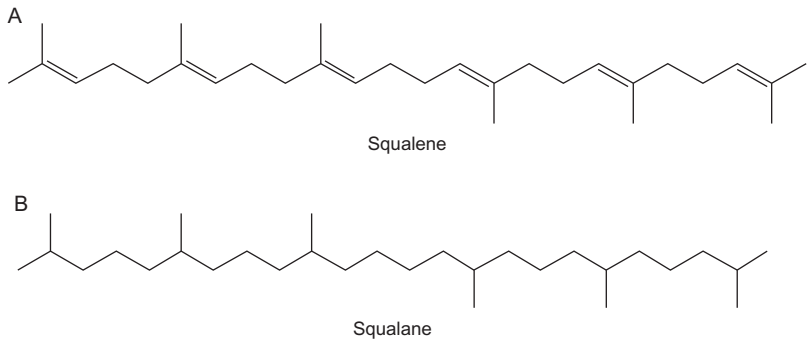

FIGURE 14.1 Chemical structures of squalene (A) and squalane (B).

beneficial properties of squalene promote this aliphatic hydrocarbon for leading significant improvements on the way to develop nutraceuticals which can help people to maintain a quality and healthy life in today's nonnatural trended life.

On the other hand, squalane is a saturated derivative of squalene which also was identified in human sebum. Its inert properties and suggested low toxicity compared to its analog squalene gained attraction from researchers, especially in the area of cosmetics (Allison, 1999). Proved emollient and moisturizing properties of squalane paved its way into protective nutraceutical and cosmeceutical area along its unsaturated relative, squalene.

These two components in shark liver oil have been used as traditional medicine for ages in order to keep people healthy. After their characterization, and highly intensified researches for their bioactivities, they are still important factors of supplementary diet for a healthier living. Up to date, several researches brought novel insights to their properties and proved various beneficial uses of these compounds.

II. BIOLOGICAL ROLES OF SQUALENE AND SQUALANE

Human skin is continuously exposed to environmental factors including ultraviolet (UV) light radiation through sunlight. Skin includes small glands named sebaceous glands which secrete sebum, a matter rich in lipids through hair follicles in order to lubricate and protect the skin and hair. It might be considered that due to high lipid content of human skin, it should be quite susceptible to UV radiation-induced oxidative stress. However, according to several studies, natural skin protection against this high exposure is suggested to be carried out by squalene which is one of the major components of human skin (Kohno *et al.*, 1995).

Squalene takes part in metabolism as precursor for synthesis of steroids and structurally quite similar to β-carotene, coenzyme q10, vitamins K_1, E, and D. The squalene in skin and fat tissue comes from endogenous cholesterol synthesis as well as dietary resources in people who consume high amounts of olive and fish oil especially shark liver (Gershbein and Singh, 1969). Squalene is synthesized by squalene synthase which converts two units of farnesyl pyrophosphate, direct precursor for terpenes and steroids, into squalene. As a secosteroid, vitamin D biosynthesis is also regulated by squalene. Moreover, being precursor for each steroid family makes squalene a crucial component of the body.

Further, squalane also exists in sebum composition. Additionally, due to its unique properties and more stable nature as industrial product than squalene, squalane is highly preferable in cosmeceutical and nutraceutical

approaches. Squalane is mainly synthesized by hydrogenation of squalene derived from variety of natural sources with the increasing demand for squalane in industrial area.

A. Antioxidant

One of the main causes of cell damage, especially on skin is oxidation. In case of human skin, exposure to sunlight induces a lipid peroxidation process in human skin layer which leads to deterioration. In this matter, squalene has been reported to protect human skin from lipid peroxidation caused by either UV exposure or any other oxidative stress.

Squalene has also been reported to possess a resistant to peroxidation and acts as singlet oxygen scavenger which explains its antioxidant properties (Kohno *et al*., 1995). Moreover, several *in vivo* and *in vitro* studies suggested the similar antioxidant efficiency results. Quenching singlet oxygen rate of squalene is found to be reasonably higher than that of all other human skin lipids and even in a similar manner with 3,5-di-*t*-butyl-4-hydroxytoluene. Same study suggests that stability of squalene against free radicals makes human skin surface able to resist lipid peroxidation propagation with an adequate amount of squalene in composition. In another study, Warleta *et al*. (2010) showed that squalene treatment successfully protected human mammary epithelial cells against oxidative DNA damage. Antiradical activities of squalene have been measured by 2,2-diphenyl-1-picrylhydrazyl, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), and oxygen radical absorbance capacity assays. According to assay results, radical scavenging capacity of squalene has been reported to be significantly important to be an efficient free radical scavenger. Following radical scavenging capacity assays, *in vitro* tests have been carried out in human mammary epithelial cells and human breast cancer cells which have been damaged by H_2O_2. As expected, squalene treatment relieved the oxidative stress in epithelial cells but not in tumor cells, suggesting a possible role of antitumor activity of squalene through oxidative stress protection. Lower cases of cancer in populations consuming high amount of squalene including nutrients such as olive oil and shark liver oil might be related to these results with being in awe of promising results that shows squalene protects human cells with tumor susceptibility against high oxidative stress.

In vivo experimental evidences support the *in vitro* antioxidant effect of squalene. Senthilkumar *et al*. (2006) suggested that dietary-supplied squalene to experimental rats normalized the enzymic and nonenzymic antioxidants after cyclophosphamide-induced toxicity. The rats were induced to have normal cell toxicity in their heart by being fed by 150 mg cyclophosphamide/kg of rat. In experimental design, squalene treatment at 0.4 ml/day/rat among various concentrations (0.2–1.0 ml/day/rat) has

been found to attenuate the oxidative damage at the maximum efficiency with minimum dose. In addition to protection of *in vivo* models' heart and red blood cells by squalene treatment, underlying *in vivo* mechanism of squalene efficiency is yet to be revealed.

Alcohol has been known to cause lipid peroxidation. Moreover, squalene has been suggested to protect cells from lipid protection. In this context, Aguilera *et al*. (2005) tested squalene efficiency against alcohol-induced toxicity in the chick embryo in order to have ideas about protection of embryos from high alcohol consumption during pregnancy. Chick embryos were injected with 10 µl of absolute ethanol and 10 µl of squalene in test groups. Later, retina was assayed for lipid composition and structure by lipid and optical microscope analysis, respectively. Results showed that squalene could reduce the damaging effects of alcohol in lipid composition and structure of retina and hence act as a preventive agent for alcohol ingestion during pregnancy.

B. Anticancer

As shark liver oil contains more than 40% squalene while the liver of shark accounts for 20–25% of its total body weight, sharks are thought to be the richest source of squalene (Mathews, 1992). Accordingly, the correlation between high amounts of squalene and absence of cancer in shark species ought to be suggested. There are reports which suggest partial and possible relationship between low incidence of cancer and consuming high olive oil product which also includes high amounts of squalene (Owen *et al*., 2000, 2004).

During recent years, squalene was found to be active for protection against various carcinogens. Therefore, an adjunctive squalene therapy in some kind of cancers happened to be the most primary use of squalene as a nutraceutical. Although there are no human trials to prove squalene role in cancer treatments, *in vitro* and animal experimental evidences suggest an anticancer role for this nutrient.

When squalene treated in conjunction with antitumor drugs, tumor growth has been observed to be arrested or prevented in various experimental models, although squalene itself has been known to be less effective on tumor inhibition. It has been reported that squalene-affiliated oleic acid treatment inhibits benzo(a)pyrene-induced skin carcinogenicity (Van Duuren and Goldschmidt, 1976). Similarly, in CD-1 mice with 7,12-dimethylbenz[a]anthracene-induced and 12-*O*-tetradecanoylphorbol-13-acetate-promoted skin tumors, 26.67% reduction in the incidence of tumors were observed after 5% squalene treatment during the prevention study (Murakoshi *et al*., 1992). Yamaguchi *et al*. (1985) suggested that squalene increased the effect of 3-[(4-amino-2-methyl-5-pyrimidinyl)-methyl]-1-(2-chloroethyl)-1-nitrosourea (ACNU), an antitumor agent.

Squalene showed potential at a concentration of 4.2 g/kg along 10 mg/kg ACNU in animal models. The adjunctive treatment resulted in prolonged survival for lymphocyctic leukemia P388 with an absence of notable toxicity to host. In a study by Nakagawa *et al*. (1985), interaction between squalene and anticancer agents was assayed. A potentiating effect of squalene was observed for Adriamycin, 5-fluorouracil, bleomycin, and *cis*-diamminedichloroplatinum. Squalene addition enhanced the cytotoxicity and antitumor activity. In similar studies, it has been shown that squalene treatment before and/or during anticancer treatment effectively enhanced the inhibition of chemically induced skin tumorigenesis and regression of some already existing tumors in animal models.

Squalene is suggested to show not only antitumor but also chemoprotective effects. Dietary-supplied 1% squalene is reported to be considerably effective against aberrant cryptic foci (early preneoplastic lesions in colon carcinogenesis; Rao *et al*., 1998). Mice with azoxymethane-induced colonic aberrant crypt foci were fed by 1% dietary squalene for 10 weeks. At the end of experimental procedure, it was observed that squalene treatment successfully resulted in more than 45% inhibition of the cryptic foci in colon. In a similar manner, nontoxic levels of squalene inhibited the mammary preneoplastic transformation which also shows its efficiency in chemoprotection. For instance, when squalene was initiated orally to mice along injection of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, mammary tumorigenesis causing agent, lung hyperplasia, and tumor multiplicity were decreased by 70% and 58%, respectively, and mammary carcinogenesis was significantly inhibited (Smith *et al*., 1998).

In a related research, Storm *et al*. (1993) have reported a radioprotective effect of squalene. Mice were administrated by 2% squalene for 14 days before and 30 days after a lethal whole-body γ-irradiation. Results showed that white cell counts in squalene-treated groups were considerably high when compared to nontreated control groups. Also, white cell protection was observed along a prolonged survival. Squalene treatment was suggested to exhibit cellular and systemic radioprotection as a result of this research.

All results indicate that squalene makes a big contribution to enhance tumor growth inhibition and tumor suppression by anticancer treatments as well as by acting as a successful chemoprotective agent.

C. Skin care

Squalene is preferred for skin care due to its notably high emollient properties. It can be easily absorbed deep by the skin acting as a normalizer for flexibility and suppleness of the skin without presenting an oily residue. Blasco *et al.* (2006) studied emulsions for skin constituents in

respect to squalene amount. The stability, centrifugation, viscosity, and pH of squalene were measured along a microscopic analysis in order to determine the optimal conditions for oil–water skin emulsions. Results suggested that the stability and viscosity of the emulsions are greatly influenced by the percentage of squalene.

As an emollient, squalene is expected to increase skin hydration due to skin surface occlusions. In addition, squalene is a substance believed to maintain moisture in the stratum corneum. Novel substitutes were researched for vernix caseosa which is a reported highly efficient barrier cream for facilitating stratum corneum hydration for barrier-deficient skins. For this purpose, various lipid fractions were mixed with squalene, triglycerides, cholesterol, ceramides, and fatty acids to produce a mixture that can generate similar compositions of vernix caseosa (Rissmann *et al*., 2008). According to results, squalene-including mixtures were able to increase the barrier to maintain hydration in a comparable manner to vernix caseosa. Five percent sodium lauryl sulfate-treated rat and human skin showed increased transepidermal water loss and riboflavin penetration. However, squalene treatment reverts the effects of sodium lauryl sulfate.

Okada and Matsumoto (2004) tested an aqueous gel for hemodialysis patients with mild uremic pruritus to replace their usual treatment with antihistamine and urea-containing ointments. A high water containing gel which includes squalane among other naturally derived ingredients has been applied to patients with mild uremic pruritus for 2 weeks. Results showed that skin dryness and itching were significantly improved after 2 weeks of gel treatment. Following 2 weeks after stopping the gel treatment, itching scale raised by 1.2 ± 0.5 and skin dryness reappeared in 40% which suggests the positive effect of gel treatment.

D. Drug delivery agent

Drug delivery compounds have been of much interest recently in order to achieve fully effective treatment and therapies. Drug carriers are needed to transport the effective agent to a targeted location or to keep the effective agent unharmed in different conditions of the body. In this manner, lipids are mostly preferable due to their nontoxic nature and ability to form a protective vesicle and fuse the cell membrane easily. In the light of this approach, squalene has been studied widely for drug delivery either by emulsions or by conjugates.

There are several reports for successfully prolonged release of short half-life drugs with squalene emulsions. Wang *et al*. (2008) reported that a squalene emulsion stabilized by phosphatidylethanolamine or pluronic F68 prolonged *in vitro* release of a morphine prodrug. Intravenous

administration of these emulsions exhibited longer analgesic activity in animal models. In similar studies, squalene-based emulsions were successfully applied to deliver lipophilic ester prodrugs of nalbuphine and increase skin permeability and delivery of the encapsulated psoralen (Fang *et al*., 2008; Wang *et al*., 2006).

Besides emulsions, lipid–drug conjugates gained attention in recent years. The conjugates usually are obtained by covalent coupling of the drug to biocompatible lipid moieties. Lipid–drug conjugates are shown to possess ability to improve pharmacological effect and decrease toxicity besides carrying the applied drug safely. Squalenoyl nanomedicine is one fine example for squalene-based lipid–drug conjugates. In this technology, squalene's natural folding conformation was used to attach hydrocarbon to analogues of anticancer and antiviral drugs which resulted in formation of nanoassemblies in water with a diameter of 100–300nm in the absence of any surfactants. Nucleoside anticancer agent gemcitabine (2,2′-difluorodeoxycytidine) was the first example of this attachment. Gemcitabine's several holdbacks as a drug was eliminated by a successful conjugation with squalene (Reddy *et al*., 2008). Gemcitabine has a rapid metabolism which results in a short biological half-life after administration. Therefore, higher doses are needed to express the required activity. However, squalene–gemcitabine nanoassemblies showed significantly improved cytotoxicity in KB-3 (human nasopharyngeal carcinoma) and MCF7 (human breast carcinoma) cells (Couvreur *et al*., 2006). When squalene is administrated as a dietary supplement, it has been reported that it is absorbed efficiently, reaching the liver rapidly and converted to sterol and bile acids and distributed with diverse amounts along various lipid fractions such as in very low-density lipoprotein, low-density lipoprotein, and high-density lipoprotein which contain 50.8%, 25.6%, and 23.6% squalene, respectively (Saudek *et al*., 1978). Therefore, high absorption rate of squalene enhances its potential use as delivery agent for orally administrated therapeutic compounds. Related studies exhibit highly promising results for oral delivery of therapeutic agents by squalene conjugation.

E. Detoxifier

Squalene is suggested to enhance elimination of lipophilic xenobiotics by several experimental evidences. Its nonpolar structure promotes a promising affinity for unionized drugs. Richter and Schäfer (1982) studied squalene for elimination of [14C]hexachlorobenzene (HCB) as an alternative method to paraffin treatment. Animal models have been fed by squalene and paraffin as 8% of the diet. Results indicated that squalene supplementation was as effective as paraffin on fecal excretion of HCB.

After 3 weeks of dietary squalene supplementation, three times higher HCB excretion was observed through feces as well as notably decreased HCB elimination half-life. In addition, it has been reported that this detoxifying effect of squalene was dose dependent and, as the percentage lowered to 5.0 of the diet, no significant effect was observed in liver on blood HCB concentrations. In a similar research, Kamimura *et al*. (1992) suggested squalene as a good agent for drug elimination from the body. Rats fed by squalene exerted increased amounts of fecal excretion of theophylline and strychnine.

F. Anti-infectant

Nowicki and Bara ska-Rybak (2007) studied the protective effect of shark liver oil. They observed a significant protection against bacterial and fungal infections by shark liver oil treatment which contains mostly squalene and alkylglycerol. Further, this treatment showed improved effects on xerosis and skin lesion-induced atopic dermatitis. This antibacterial and antifungal effect could be accounted for the high-squalene-including composition of the shark liver oil; however, detailed studies are needed to be carried out for reputed activity of squalene as an anti-infectant.

III. CONCLUSION

On the basis of the presented reports, squalene and squalane are two naturally derived compounds with bright future in nutraceutical and pharmaceutical area. Squalene, which can be obtained by natural sources, exists readily in human body and yet to be examined in detail for its biological role.

Squalene supplementation is suggested to be accounted for tumor growth inhibition and prevention of normal cells to turn into tumor cells under oxidative stress. Although there is lack of evidence for human trials to show anticancer and antioxidant effects of squalene, animal models and *in vitro* experiments highlight a significant activity which urges for further exploration.

Detoxifying and anti-infectant effects of squalene itself have not been reported yet. However, substances including squalene or squalene supplementation showed notable improvements. Further researches to elucidate the underlying mechanisms of shark liver oil and squalene application are expected to report more results that promote squalene and squalane as effective candidates for practical and clinical use.

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