MÜSBED 2013;3(4):220-228
DOI: 10.5455/musbed.20131213100404

Derleme / Review

Medical Use of Squalene as a Natural Antioxidant

Fatma Esra Güneş

[1]Marmara Üniversitesi Sağlık Bilimleri Fakültesi, Beslenme ve Diyetetik Bölümü, İstanbul - Türkiye

Yazışma Adresi / Address reprint requests to: Fatma Esra Güneş,
Marmara Üniversitesi Sağlık Bilimleri Fakültesi Beslenme ve Diyetetik Bölümü, E-5 Yanyol Üzeri 34865 Cevizli, İstanbul - Türkiye
Elektronik posta adresi / E-mail address: fegunes@marmara.edu.tr
Kabul tarihi / Date of acceptance: 13 Aralık 2013 / December 13, 2013

ÖZET

Doğal bir antioksidan olarak skualenin medikal kullanımı

Bu derlemenin amacı, koruyucu ve tedavi edici tıp içerisinde skualenin yerini ve önemini vurgulamaktır. Skualen bir triterpen olup, hemoglobinden bağımsız olarak oksijeni hücreye doğrudan taşıyarak tüm vücuda ulaştırabilmektedir. Skualenin kanser oluşumunu önlediği ve yüksek anti-tümör aktivitesine sahip olduğu bildirilmiştir, bu nedenle, çeşitli kanserlerin tedavi sürecinde destekleyici olarak kullanılabilmektedir. Skualenin bu konu ile ilgili epidemiyolojik ve deneysel hayvan araştırmaları ilginç ve umut vericidir. Bununla birlikte, skualenin kimyasal özellikleri nedeniyle farklı hastalıkların tedavisinde kullanılması açısından, insanlarda daha geniş çalışmalarla desteklenmesi gerekmektedir.
Anahtar sözcükler: Skualen, triterpen, sitotoksik aktivite, anti-tümor aktivite, deri hastalıkları, kolesterol metabolizması

ABSTRACT

Medical use of squalene as a natural antioxidant

The aim of the present review is to emphasize the importance of squalene, a nutrient, in preventive and therapeutic medicine. Squalene is a triterpene that carries oxygen independent of the hemoglobin and it can carry oxygen directly to cell membranes throughout the body and reach the regions having low oxygen supply. Squalene has been reported to have an inhibitory effect on cancer promotion and a high anti-tumor activity; thus, it is primarily used as a supportive therapy in a variety of cancers. Epidemiological and experimental animal researches regarding squalene's anti-cancer properties are interesting and promising. However, these properties of squalene should be supported by further extensive trials in humans.
Key words: Squalene, triterpene, cytotoxic activity, anti-tumor activity, skin disease, cholesterol metabolism

INTRODUCTION

Squalene was discovered in 1906 by Mitsumaru Tsujimoto, a Japanese industrial engineer. In 1930, Dr. Keijiro Kogami studied beneficial effects of squalene on health, and in 1963, Paul Karrer, a Nobel Prize winner, discovered that squalene also existed in human body. Along with the article published in 1963 in Nature Journal mentioning that squalene was stimulating macrophages, investigations have been started on this issue (1). In 1982, detoxification function of squalene has been reported (2). In 1993, its protective effects against radiation have been exposed (3); in 1995, Japanese researchers demonstrated that squalene prevented UV-related oxidation of lipids in the skin (4).

Squalene (2,6,10,15,19,23-hexamethyl-6,6,10,14,18,20-tetracosahexane) is a polyunsaturated triterpene widely found in nature, includes six isoprene units, a biochemical precursor of cholesterol and other steroids, and can not only be synthesized at cellular level but also be taken as a dietary factor (5-7). The known richest source of squalene is the liver oil of sharks of Squadelidea family (8) and is found in different amounts in herbal oils; 1.28 g/kg in nut oil, 3.53 g/kg in squash, and 5.99 g/kg in olive oil (9). Biosynthesis of squalene occurs in the skin and liver in human and this synthesized squalene is carried by LDL and VLDL in circulating blood and largely secreted in sebaceous glands (10,11).

Biochemistry of Squalene

Squalene is a 30 carbon polyprenyl compound including 6 isoprenoids. Squalene, which is structurally similar to beta-carotene, is an intermediate product in cholesterol synthesis. Squalene and associated compounds, oxidosqualene and bis-oxidosqualene, are the precursors

of approximately 200 triterpenes (12). Endogenous synthesis of squalene begins with the synthesis of 3-hydroxy-3-methyl glutaryl coenzyme A (HMGCoA) (6). HMGCoA is synthesized from acetyl CoA (13). Reduction of HMGCoA via a niacin-dependent reaction results in mevalonate. Mevalonate is phosphorilised in three steps via magnesium-dependent catalase enzyme (13,14). These steps include 5-phosphomevalonic acid, 5-pirophosphomevalonic acid, and isopentenyl pyrophosphate (13). Finally, it is decarboxylated into 3 isopentenyl diphosphate, which is the donor of overall proprenyl compounds. Successive addition of prenyl groups results in formation of 15-carbon farnesyl diphosphate. Squalene occurs when two molecules of farnesyl diphosphate are combined via enzymatic way and are niacin-dependently reduced (13,14). Following the biosynthesis, squalene turns into cholesterol and other steroid metabolites (6).

Dietary squalene is absorbed well, transported by chylomicrons into circulation, and is rapidly taken up by the liver, where it is cyclized to sterols and bile acids. It is important to note that post-absorptive plasma from normal individuals has been found to contain squalene distributed among various lipoprotein fractions; 50.8% in VLDL, 25.6% in LDL, and 23.6% in HDL. Such efficient absorption of squalene obviously suggests an application as a carrier to deliver orally administered therapeutic molecules (15).

Pharmacokinetics of Squalene

A part of serum squalene is obtained from endogenous cholesterol synthesis, whereas the other part is obtained from squalene-rich diets. Of dietary squalene, 60-85% is absorbed (16,17). In blood circulation, squalene is carried by LDL and VLDL. Squalene is found in high concentrations in fat cells of human adipose tissue. Eighty percent of squalene in adipose tissue is found in central neutral lipid droplet and 20% is found being attached to the microsomal membranes (18,19). Only squalene attached to the microsomal membranes is metabolically active and while 90% of newly synthesized squalene is stored in lipid droplet and 10% is used in cholesterol synthesis (20). Squalene is strongly attached to the hydrophobic bound between two lipid layers in biomembrane, which has a risk of peroxidation, and acts independently. Kalvadova (21) observed that phagocytes stored lipids together with other compounds in lipid droplets when exposed to lipid emulsions including squalene. It has been determined that squalene is mainly found in lipid droplets in Schwann cells (22).

Application, Dose, Toxicity and Adverse Effects of Squalene

After squalene was given for three months to the rats and dogs, hepatic functional and biochemical tests showed no remarkable adverse effect or toxicity (6). Since squalene is a component of a lipid content of healthy diet, long-term use of fair amount of squalene has been reported to be safe in the absence of lipoid pneumonia secondary to shark oil ingestion (23). Besides, it has been reported that high amount of serum squalene is safe and has beneficial, chemopreventative and hypocholesterolemic features (6,24).

Change in dose of squalene depends on the application. When considered as a support for lipid lowering drugs, a dose of 500 mg/day may be beneficial; however, experiments have reported that a dose higher than 1 g/day does not show the same efficacy. The dose recommended for detoxification of xenobiotics would probably be higher. In animals, squalene is required to be minimum 5% of the diet. This amount corresponds to 11g/day in human. However, safety of this dose has not been studied in human yet. There is no information about dosing when used to support cancer therapy; however, results of animal experiments suggest a daily dose between 2-5 g (6).

There is an association between squalene synthesis and visceral obesity. Squalene/cholesterol ratio is highest in VLDL particles and thus there is a relationship between visceral obesity and high VLDL synthesis in the liver. Although insulin resistance is associated with high VLDL synthesis, it was not related with serum squalene. Additionally, squalene is widely synthesized in fat and can increase serum squalene levels in obese subjects (25).

Clinical Applications of Squalene

Squalene emulsions and vaccines

Squalene is widely used in emulsions either as the primary component or the secondary lipid (26,27). Although

squalene emulsions are produced for various purposes, they are frequently used to carry vaccines and drugs into the body (15,28,29). Squalene used for this purpose bears the role of immunological adjuvant and remains safe and non-toxic for the host while regulating or enhancing immune response against the adjuvant (30).

Squalene is emulsified with other different substances when used for antigen supply. It is used to form vaccines with highly potent transfection activity (31) as well as to form stable and viscous emulsions of adjuvants that have been made quite soluble with a small droplet (32).

Substances that are frequently used and reported to be safe include polysorbate 80 (33), lecithin+polysorbate 80 (34), glycerol+polysorbate 80 (35), polyoxyethylene sorbitan monooleate+sorbitan trioleate (MF59) (36), and poloxamer 105+Abil-Care (Bis-PEG/PPG-16/16 PEG/PPG 16/16 Dimethicone, Caprylic/Capric Triglyceride) (37,38). Different recombinant antigens, such as influenza virus, hepatitis B and C viruses, cytomegalovirus, herpes simplex virus, and HIV virus are used in the vaccines prepared with squalene emulsions and they have been demonstrated to be used safely in different age groups including neonates.

Tumor vaccines are the other therapy group including emulsions including squalene. Specific immune response has been obtained during immunization trials, in which antigens are used in combination with squalene emulsions in B-cell lymphoma patients (39). In a similar study, anti-idiotype vaccine in combination with squalene emulsion has been administered for immunotherapeutic purpose in metastatic melanoma patients and effective antibody response has been obtained (40).

Nevertheless, there are also controversies about the use of squalene in vaccines (41). Based on rashes, headache, arthralgia, loss of memory, increased allergy, sensitivity, and neurological abnormalities seen in the soldiers participated in Gulf War (42), squalene found in anthrax vaccines as an adjuvant was blamed (43). Moreover, anti-squalene antibodies were detected in Gulf War syndrome-like patients (44). Further studies have demonstrated that anti-squalene antibodies can be naturally found in human and may not be associated with anthrax vaccine (45). MF59 emulsion adjuvant in the vaccines has neither increased anti-squalene antibody levels nor altered preexisting antibody titers (36).

Anti-cancer Properties of Squalene

It is known that sharks are rich sources of squalene and more than 40% of shark liver contains squalene. It has been reported that absence of cancer in sharks is associated with such high squalene levels (5,46). Moreover, low incidence of cancer in Mediterranean people, whose diet content is rich in olive oil, is thought to be partially associated with squalene (47-49).

Recent pro-drug strategies concerning high transportation of nucleotide analogues into the cells has given rise to a new study field. Nucleotide analogues act as potent inhibitors of DNA synthesis and are used as antiviral and anti-cancer therapeutics (50,51). Intracellular access of nucleotide analogues is limited due to high hydrophobic feature and weak in vivo stability. Squalenilation of these compounds improves their slow diffusion. For example, biomembrane interaction of gemcitabin-squalene, which is a lipophilic drug, is extremely increased as compared to free gemcitabin (52,53).

In general, squalene is thought to inhibit carcinogenesis by following mechanisms: 1) Inhibiting farnesylation of Ras oncoprotein and restricting transformation of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) into mevalonate; 2) modulating biosynthesis and functions of xenobiotic-metabolizing enzymes, and 3) scavenging free radicals (24). It has been reported that squalene emulsions given simultaneously with anti-cancer drugs provide favorable effects either directly or indirectly by enhancing efficacy of anti-cancer drugs (54-56) Nakagawa (56) reported that squalene had increased cytotoxicity of adriamycin, 5-fluorouracil, bleomycin, and cisplatin.

Das (57) demonstrated that squalene had selective cytoprotective effect, protected normal cells against toxicity of chemotherapeutics, but that, same protective efficacy was not valid for tumor cells. In another study, Das (58) reported that squalene protected normal bone marrow cells, but not neuroblastoma cells, against the toxicity induced by cisplatin and other anti-cancer drugs. Similar studies conducted by Senthilkumar (59,60) demonstrated effective protection against cyclophosphamide-induced toxicity in heart, liver, and kidney provided by oral squalene given to the rats (0.4 mL/day/rat).

Oxygenation effect of squalene on the cells is considered beneficial because higher cellular oxygen levels provide a

more efficient metabolic process and thus enhance cell energy metabolism. By this way, host immunity is improved through the protection of the body against foreign agents which may lead to weakening or damage in natural defense systems (15).

Mammary epithelial cells and breast tumor cells can be differed by their responses to squalene treatment. Squalene reduces levels of reactive oxygen species in vitro and protects against oxidative DNA damage in human mammary epithelial cells but not in breast tumor cells (61).

Protective effects of squalene

It has been reported that shark liver oil, which is rich in squalene and alkylglycerol, is protective against bacterial and fungal infections, particularly in the patients with atopic dermatitis and xerosis-related skin lesions (62).

It is known that squalene protects the skin against UV-induced radiation damage due to its high secretion in sebum (~12%) (4,63). A study conducted by Hashim et al.(64) demonstrated radioprotective effects of squalene in animal models.

In addition, it has been reported that squalene and phenolic compounds in olive oil provide substantial protection against coronary heart diseases and aging (49). There are studies reporting that continuous squalene administration in rats shows cardio-protective effect. Sabeena (65) administered oral squalene to the rats for 45 days and proved its antioxidant effect against isoproterenol-mediated myocardial infarction by inhibiting lipid peroxidation.

In animal models, squalene plays an important role in retinal health in terms of rod outer segment (ROS) disc membranes, and rod photoceptor cells. The majority of in vivo and in vitro newly synthesized squalene is carried to ROS, where it is transformed in line with disc membranes (66).

In addition, it has been reported that squalene, as a component of olive oil, is protective against breast cancer (67,68) and reduces serum cholesterol concentration (69,70).

In a study, the role of DL-alpha-lipoic acid and squalene was evaluated in oxidative cardiac, testicular, and urotoxic damage which was induced by cyclophosphamide and the efficacy of lipoic acid and squalene as cytoprotectants was emphasized in cyclophosphamide-induced toxicity (71).

Squalene and cholesterol metabolism

When dietary squalene is absorbed, a certain amount turns into cholesterol. Nevertheless, such an increase in synthesis is not associated with increased serum cholesterol concentration, but probably with the increase in fecal excretion. A study conducted in human administered 900 mg/day squalene for 7-30 days reported no change in serum cholesterol and triglyceride concentrations despite 15 fold increase in serum squalene level (72,73).

Kritchevsky (74) added 3% squalene in a diet with high cholesterol content and fed the rabbits for 14 weeks; they found that atheroma development was not higher than the rabbits fed with diet including similarly high cholesterol. Chan (75) performed a double-blind controlled study and compared 10 mg pravastatin, an anti-cholesterol drug, with 860 mg squalene in terms of hypercholesterolemia. A total of 102 patients accepted to be either in the group treated for 20 weeks or in the placebo group. As the result, pravastatin decreased total cholesterol, LDL cholesterol, and triglyceride levels and increased HDL cholesterol levels as compared to squalene. However, using their combination was shown to provide higher reduction in LDL cholesterol and higher increment in HDL cholesterol levels.

There is a balance between biosynthesis and uptake pathways of internal and external sources of cholesterol, which is regulated by feedback mechanisms. The most important control mechanisms include HMGCoA reductase enzyme and LDL receptors. Feedback mechanisms in cholesterol synthesis control the activity of HMGCoA reductase enzyme to prevent excessive cholesterol deposition and might decrease by 90% when necessary; moreover, it may reduce the number of LDL receptors (76).

Total and LDL cholesterol levels can be decreased by 3.5 mg/day squalene supplementation in rats consuming a cholesterol rich diet due to increased cholesterol elimination and bile synthesis and cholesterol excretion into bile mediated by changes in gene expression of key enzymes (77).

In a study conducted on male albino rats, the cardioprotective effect of squalene against isoprenaline-induced myocardial infarction was evaluated and. troponin T, homocysteine, apolipoprotein Al, apolipoprotein B, lipoprotein (a), total cholesterol, lipid peroxides (in plasma

and heart tissue), and some endogenous non-enzymatic antioxidants (in heart tissue) were assessed. The isoprenaline-induced elevation in these diagnostic markers was significantly prevented by squalene supplementation (2%) with feed for 45 days. Squalene supplementation showed an antilipidemic action against isoprenaline-induced hypercholesterolemia by keeping cholesterol and lipoprotein levels at normal range. Moreover, by blocking lipid peroxidation induction, it showed antioxidant effect against isoprenaline-induced myocardial infarction. In the non-enzymatic antioxidants, squalene supplementation has a tendency to prevent the isoprenaline induced reduction. Cardioprotective effect of squalene might be attributed to its antilipidemic, antioxidant, and membrane stabilizing properties (78).

Although CVD treatments have not yet been tested against periodontal alterations, there is a relationship between cardiovascular alterations and periodontal disease. The effects of squalene, hydroxytyrosol, and coenzyme Q10 on gingival tissues were investigated in rabbits, of that forty were fed with an atherogenic diet for 50 days and eight (controls) were with standard chow for 80 days. After 50 days, eight rabbits on atherogenic diet were sacrificed and the remaining thirty-two rabbits were equally divided into four groups and fed with commercial chow alone or supplemented with coenzyme Q10, squalene or hydroxytyrosol for an extra 30 days. Fibrosis and endothelial activation was higher and cellularity in gingival mucosa was lower in the atherosclerotic rabbits compared to those in the controls. Squalene decreased fibrosis and hydroxytyrosol reduced endothelial activation. Thus, gingival vascular changes after the atherosclerotic diet was reversed by hydroxytyrosol and squalene (79).

Liu (80) evaluated the effect of high dose squalene on plasma leptin, glucose, testosterone, blood pressure, and body fat in rats and concluded that squalene decreased the body weight gain, blood pressure, and levels of plasma leptin, glucose, cholesterol and triglycerides.

In their study, Okada and Matsumoto (81) used an aqueous gel including squalene for hemodialysis patients with mild uremic pruritus and reported that skin dryness and itching were significantly improved after 2 weeks. Moreover, reappearing of itching and skin dryness 2 weeks after stopping treatment suggested the positive effect of gel treatment.

Squalene and skin diseases

Molecular oxygen turns into single oxygen when stimulated by certain chemical and physical influences at electron level. Single oxygen includes high energy and thus leads to many harmful reactions such as lipid peroxidation. Squalene collects thermodynamically active single oxygen and terminates its efficacy and effectively binds free oxygen molecules. In order to detect binding capacity of squalene, its oxidation products have been measured and weight of oxygenated squalene has been found to be 25%. Squalene is found in the skin as a protector against lipid peroxidation, which occurs due to exposure to UV radiation and other sources of oxidative damage. Development of chain reactions of lipid peroxidation is quite reduced depending on the amount of squalene on skin surface (4,6).

It has been demonstrated that high dose squalene (higher than daily 13.5 g) in vivo reduced the wrinkles on aging skin of human, enhanced type I procollagen, and decreased UV-induced DNA injury (82). Besides, there are papers about protective effect of squalene against skin cancers (83).

Squalene is one of the most important hydrating agents in nature (28). It rapidly and effectively penetrates into skin and provides healthy elasticity and regaining of flexibility without leaving a greasy residue. Experimental studies on recently developed cosmetic emulsions have investigated biomimetic molecules containing squalene (84). For this purpose, stability, centrifuge characteristics, viscosity, and pH of squalene have been assessed and microscopic analyses have been performed. According to the results, amount of squalene has come into prominence as the most important component of stability and viscosity of emulsions.

Photo-oxidized metabolites of squalene are signalling molecules for keratinocytes for mounting adaptive/protective metabolic, proliferative, and inflammatory responses to the UV component of solar light. However, photo-oxidized squalene may lead to persistent inflammation and overstimulated skin metabolism in the case of chronic exposure to UV and/or aged or pathological skin. These processes may be exaggerated in the oily skin as well. Photo-oxidized squalene can be considered as a molecular target for prevention and treatment of

UV-associated skin disorders (85).

Squalene peroxide formation by singlet oxygen has a key role in photo-induced skin damage. Under UV exposure, squalene was oxidized more efficiently by singlet oxygen derived from coproporphyrin than that of other skin surface lipids. Moreover, topical application of squalene peroxide has been reported to induce skin hyperpigmentation by increasing prostaglandin E2 (86).

A nanostructured lipid carrier based on squalene and precirol enhances skin permeability and controls delivery of the encapsulated psoralen, an anti-psoriatic medicine (87).

According to the results of in vitro studies performed on antioxidant properties of squalene, it has been considered as a notably effective oxygen-scavenging agent. Squalene protects the human skin against lipid peroxidation as free oxygen scavenger after an oxidative stress such as sunlight exposure (88). Kohno (4) reported that squalene up-took higher amounts of free oxygen as compared to the other lipids on human skin surface.

Detoxification of squalene and xenobiotics

Squalene shows tendency to attach to nonionized substances since it is nonpolar. It collects xenobiotics with high lipophilic property and helps them to be eliminated out of the body (6). In addition, squalene stimulates hepatic detoxification enzymes (p450 enzyme system). Dibenzofurans, hexachlorobifenyl, hexachlorobenzene, 12-o-tetradekanoilforbol-13-acetate, and 4(methylnitrozamino)-l-(3-pyridyl)-l-(butanone) are some of the toxins detoxified by squalene (83).

Richter (89) used squalene instead of paraffin, which was used for the elimination of hexachlorobenzene, an organochlorine xenobiotic, and found that dietary squalene supplementation by 8% excessively increased fecal elimination of hexachlorobenzene. Three-week treatment of animals with squalene has three times increased the fecal elimination of hexachlorobenzene and reduced half-life to 34-38 days from 110 days (2,89). Kamimura (90) suggested squalene as a good candidate for being an antidote that reduced toxicity of drugs taken arbitrarily. Animal studies have stressed on the fact that squalene enhances fecal elimination of theophylline and strychnine. Squalene becomes free when xenobiotics are accumulated in fat cells and accelerates the elimination of xenobiotics by stimulating bile secretion.

Squalene and immune system

Squalene is a strong antioxidant due to its large electron exchange capacity without being exposed to molecular disruption. Experimental studies have shown that dietary squalene supplementation enhances immune system performance and improves macrophage function. Studies put forward that squalene protects immune cell biomembranes against oxidative stress during phagocytosis (91).

Desai (92) reported that squalene and squalene-containing compound Roidex could prevent chemical-originated cancer development in the skin of mice and cause regression in the course of tumor development. Rao (68) demonstrated that 1% squalene diet reduced azoxymethane-induced abnormal cellular formation and cellular diversity in colon by 46% and stated that squalene might display chemical protective activity in colon cancers. Katdare (93) reported that squalene inhibited abnormal hyperproliferation. Studies have shown that squalene inhibits ornithine decarboxylase enzyme, which takes place in endogenous synthesis of polyamines (83). In an experimental model of sarcoma in mice, squalene has been reported to enhance reticuloendothelial system functions, increase amount of IgM in particular, and prolong survival (94).

Studies have revealed that squalene is an antioxidant and prevents development of free-radical induced oxidative injury particularly in the skin. In addition, it is known to be an important step in cholesterol synthesis. It shows effective physiological and biochemical activity in cancer treatment. Squalene has been reported that it is able to suppress development of tumor cells, prevents chemical-originated cancers, reduces tumor growth rate, stimulates reticuloendothelial system, and increases white blood cell count.

Under the light of all of these data, further human studies are needed to detect and evaluate potential benefits of squalene in the body.

REFERENCES

1. Heller JH, Pasternak VZ, Ransom JP, Heller MS, A new reticuloendothelial system stimulating agent ('Restim') from shark livers. Nature, 1963;199: 904-905.

2. Richter E & Schafer SG. Effect of squalane on hexachlorobenzene (HCB) concentrations in tissues of mice. J Environ Sci Health B. 1982; 17: 195-203.

3. Storm HM, Oh SY, Kimler BF, Norton, S. Radioprotection of mice by dietary squalene. Lipids. 1993; 28: 555-559.

4. Kohno Y, Egawa Y, Itoh S, Nagaoka S, Takahashi M, Mukai K. Kinetic study of quenching reaction of singlet oxygen and scavenging reaction of free radical by squalene in n-butanol. Biochim Biophys Acta. 1995; 1256: 52-56.

5. Liu GC, Ahrens EH Jr, Schreibman PH, Crouse JR. Measurement of squalene in human tissues and plasma: validation and application. J Lipid Res 1976; 17: 38-45.

6. Kelly GS. Squalene and its potential clinical uses. Altern Med Rev. 1999; 4: 29-36.

7. Spanova M, Daum G. Squalene – biochemistry, molecular biology, process biotechnology, and applications. Eur J Lipid Sci Technol. 2011; 113: 1299-1320.

8. Vazquez L, Fornari T, Senorans FJ, Reglero G, Torres CF. Supercritical carbon dioxide fractionation of nonesterified alkoxyglycerols obtained from shark liver oil. J Agric Food Chem. 2008; 56: 10781083.

9. Tuberoso CIG, Kowalczyk A, Sarritzu E, Cabras P. Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use. Food Chem. 2007; 103: 1494-1501.

10. Koivisto PV, Miettinen TA. Increased amounts of cholesterol precursors in lipoproteins after ileal exclusion. Lipids. 1988; 23: 993-996.

11. Stewart ME. Sebaceous gland lipids. Semin Dermatol. 1992; 11: 100-105.

12. Xu R, Fazio GC, Matsuda SP. () On the origins of triterpenoid skeletal diversity. Phytochemistry. 2004; 65: 261-291.

13. Slakey LL, Ness GC, Qureshi N, Porter JW. Occurrence of the enzymes effecting the conversion of acetyl CoA to squalene in homogenates of hog aorta. J Lipid Res. 1973; 14: 485-494.

14. Laaksonen R, Ojala JP, Tikkanen MJ, Himberg JJ. Serum ubiquinone concentrations after short- and long-term treatment with HMG-CoA reductase inhibitors. Eur J Clin Pharmacol. 1994; 46: 313-317.

15. Reddy LH, Couvreur P. Squalene: A natural triterpene for use in disease management and therapy. Adv Drug Deliv Rev. 2009; 61: 1412-1426.

16. Miettinen TA, Vanhanen H. Serum concentration and metabolism of cholesterol during rapeseed oil and squalene feeding. Am J Clin Nutr. 1994; 59: 356-363.

17. Gylling H, Miettinen TA. Postabsorptive metabolism of dietary squalene. Atherosclerosis. 1994; 106: 169-178.

18. Spanova M, Czabany T, Zellnig G, Leitner E, Hapala I, Daum G. Effect of lipid particle biogenesis on the subcellular distribution of squalene in the yeast Saccharomyces cerevisiae. J Biol Chem. 2010; 285: 6127-6133.

19. Milla P, Athenstaedt K, Viola F, Oliaro-Bosso S, Kohlwein SD, Daum G, Balliano G. Yeast oxidosqualene cyclase (Erg7p) is a major component of lipid particles. J Biol Chem. 2002; 277: 2406-2412.

20. Tilvis R, Kovanen PT, Miettinen TA. Metabolism of squalene in human fat cells. Demonstration of a two-pool system. J Biol Chem. 1982; 257: 10300-10305.

21. Kalvodova L. Squalene-based oil-in-water emulsion adjuvants perturb metabolism of neutral lipids and enhance lipid droplet formation. Biochem Biophys Res Commun. 2010; 393: 350-355.

22. Goodrum JF, Earnhardt TS, Goines ND, Bouldin TW. Lipid droplets in Schwann cells during tellurium neuropathy are derived from newly synthesized lipid. J Neurochem. 1990; 55: 1928-1932.

23. Asnis DS, Saltzman HP, Melchert A. Shark oil pneumonia. An overlooked entity. Chest. 1993; 103: 976-977.

24. Smith TJ. Squalene: potential chemopreventive agent. Expert Opin Invest. Drugs. 2000; 9: 1841-1848.

25. Peltola P, Pihlajamaki J, Koutnikova H, Ruotsalainen E, Salmenniemi U, Vauhkonen I, et al. Visceral obesity is associated with high levels of serum squalene. Obesity (Silver Spring). 2006; 14: 1155-1163.

26. Whittenton J, Harendra S, Pitchumani R, Mohanty K, Vipulanandan C, Thevananther S. Evaluation of asymmetric liposomal nanoparticles for encapsulation of polynucleotides. Langmuir. 2008; 24: 8533-8540.

27. Fox CB, Anderson RC, Dutill TS, GotoY, Reed SG, Vedvick, T. Monitoring the effects of component structure and source on formulation stability and adjuvant activity of oil-in-water emulsions. Colloids Surf B Biointerfaces. 2008; 65: 98-105.

28. Huang ZR, Lin YK, Fang JY. Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. Molecules. 2009; 14: 540-554.

29. Fox CB. Squalene emulsions for parenteral vaccine and drug delivery. Molecules.2009;14:3286-312.

30. Mesa C, Fernandez LE. Challenges facing adjuvants for cancer immunotherapy. Immunol Cell Biol. 2004; 82: 644-650.

31. Kim YJ, Kim TW, Chung H, Kwon IC, Sung HC, Jeong SY. The effects of serum on the stability and the transfection activity of the cationic lipid emulsion with various oils. Int J Pharm. 2003; 252: 241-252.

32. Wang JJ, Sung KC, Hu OY, Yeh CH, Fang JY. Submicron lipid emulsion as a drug delivery system for nalbuphine and its prodrugs. J Contr. Release. 2006; 115: 140-149.

33. Allison AC, Byars NE. An adjuvant formulation that selectively elicits the formation of antibodies of protective isotypes and of cell-mediated immunity. J Immunol Methods 1986; 95: 157-168.

34. Hjorth RN. Adjuvants for Viral Vaccines. 1997, US Patent No: 5690942; available at http://www.patentstorm.us/patents/5690942/fulltext.html.

35. Hjorth RN. Adjuvants for Viral Vaccines. 1998, US Patent No: 5718904; available at http://www.patentstorm.us/patents/5718904/fulltext.html.

36. Del Giudice G, Fragapane E, Bugarini R, Hora M, Henriksson T, Palla E, et al. Vaccines with the MF59 adjuvant do not stimulate antibody responses against squalene. Clin Vaccine Immunol. 2006; 13: 1010-1013.

37. Suli J, Benisek Z, Elias D, Svrcek S, Ondrejkova A, Ondrejka R, et al. Experimental squalene adjuvant. I. Preparation and testing of its effectiveness. Vaccine. 2004; 22: 3464-3469.

38. Benisek Z, Suli J, Elias D, Lenhardt L, Ondrejkova A, Ondrejka R, et al. Experimental squalene adjuvant. II. Harmlessness and local reactogenity. Vaccine. 2004; 22: 3470-3474.

39. Hsu FJ, Caspar CB, Czerwinski D, Kwak LW, Liles T, Syrengelas A, et al. Tumor-specific idiotype vaccines in the treatment of patients with B-cell lymphoma--long-term results of a clinical trial. Blood. 1997; 89: 3129-3135.

40. Quan WD Jr, Dean GE, Spears L, Spears CP, Groshen S, Merritt JA, et al. Active specific immunotherapy of metastatic melanoma with an antiidiotype vaccine: a phase I/II trial of I-Mel-2 plus SAF-m. J Clin Oncol. 1997; 15: 2103-2110.

41. Lippi G, Targher G, Franchini M. Vaccination, squalene and anti-squalene antibodies: facts or fiction? Eur J Intern Med. 2010; 21: 70-73.

42. Gronseth GS. Gulf war syndrome:a toxic exposure?A systematic review. Neur. Clin. 2005; 23: 523-540.

43. Asa PB, Cao Y, Garry RF. Antibodies to squalene in Gulf War syndrome. Exp Mol Pathol. 2000; 68: 55-64.

44. Asa PB, Wilson RB, Garry RF. Antibodies to squalene in recipients of anthrax vaccine. Exp Mol Pathol. 2002; 73: 19-27.

45. Matyas GR, Rao M, Pittman PR, Burge R, Robbins IE, Wassef NM, et al. Detection of antibodies to squalene: III. Naturally occurring antibodies to squalene in humans and mice. J Immunol Methods. 2004; 286: 47-67.

46. Mathews J. Sharks still intrigue cancer researchers. J Nat Cancer Inst. 1992; 84: 1000-1002.

47. Trichopoulou A, Toupadaki N, Tzonou A, Katsouyanni K, Manousos O, Kada E, Trichopoulos D. The macronutrient composition of the Greek diet: estimates derived from six case-control studies. Eur J Clin Nutr. 1993; 47: 549-558.

48. Willett WC. Diet and health: what should we eat? Science. 1994; 264: 532-537.

49. Owen RW, Giacosa A, Hull WE, Haubner R, Wurtele G, Spiegelhalder B, Bartsch H. Olive-oil consumption and health: the possible role of antioxidants. Lancet Oncol. 2000; 1: 107-112.

50. Sampath D, Rao VA, Plunkett W. Mechanisms of apoptosis induction by nucleoside analogs. Oncogene. 2003; 22: 9063-9074.

51. Galmarini CM, Mackey JR, Dumontet C. Nucleoside analogues and nucleobases in cancer treatment. Lancet Oncol. 2002; 3: 415-424.

52. Castelli F, Sarpietro MG, Micieli D, Stella B, Rocco F, Cattel L. Enhancement of gemcitabine affinity for biomembranes by conjugation with squalene: differential scanning calorimetry and Langmuir-Blodgett studies using biomembrane models. J Colloid Interface Sci. 2007; 316: 43-52.

53. Pili B, Bourgaux C, Amenitsch H, Keller G, Lepêtre-Mouelhi S, Desmaële D, et al. Interaction of a new anticancer prodrug, gemcitabine-squalene, with a model membrane: coupled DSC and XRD study. Biochim Biophys Acta. 2010; 1798: 1522-1532.

54. Yarkoni E, Rapp HJ. Tumor regression after intralesional injection of mycobacterial components emulsified in 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene (squalene), 2,6,10,15, 19,23-hexamethyltetracosane (squalane), peanut oil, or mineral oil. Cancer Res. 1979; 39: 1518-1520.

55. Pimm MV, Baldwin RW, Lederer E. Suppression of an ascitic rat hepatoma with cord factor and Nocardia cell wall skeleton in squalene emulsions. Eur J Cancer. 1980; 16: 1645-1647.

56. Nakagawa M, Yamaguchi T, Fukawa H, Ogata J, Komiyama S, Akiyama S, Kuwano, M. Potentiation by squalene of the cytotoxicity of anticancer agents against cultured mammalian cells and murine tumor. Jpn J Cancer Res. 1985; 76: 315-320.

57. Das B, Yeger H, Baruchel H, Freedman MH, Koren G, Baruchel S. In vitro cytoprotective activity of squalene on a bone marrow versus neuroblastoma model of cisplatin-induced toxicity. implications in cancer chemotherapy. Eur J Cancer. 2003; 39: 2556-2565.

58. Das B, Antoon R, Tsuchida R, Lotfi S, Morozova O, Farhat W, et al. Squalene selectively protects mouse bone marrow progenitors against cisplatin and carboplatin-induced cytotoxicity in vivo without protecting tumor growth. Neoplasia. 2008; 10: 1105-1119.

59. Senthilkumar S, Devaki T, Manohar BM, Babu MS. Effect of squalene on cyclophosphamide-induced toxicity. Clin Chim Acta. 2006; 364: 335-342.

60. Senthilkumar S, Yogeeta SK, Subashini R, Devaki, T. Attenuation of cyclophosphamide induced toxicity by squalene in experimental rats. Chem Biol Interact. 2006; 160: 252-260.

61. Warleta F, Campos M, Allouche Y, Sánchez-Quesada C, Ruiz-Mora J, Beltrán G. Squalene protects against oxidative DNA damage in MCF10A human mammary epithelial cells but not in MCF7 and MDA-MB-231 human breast cancer cells. Food Chem Toxicol. 2010; 48: 1092-1100.

62. Nowicki R, Baranska-Rybak W. Shark liver oil as a supporting therapy in atopic dermatitis. Pol Merkur Lekarski. 2007; 22: 312-313.

63. Newmark HL. Squalene, olive oil, and cancer risk: a review and hypothesis. Cancer Epidemiol Biomarkers Prev. 1997; 6: 1101-1103.

64. Hashim YZ, Eng M, Gill CI, McGlynn H, Rowland IR. Components of olive oil and chemoprevention of colorectal cancer. Nutr Rev. 2005; 63: 374-386.

65. Sabeena Farvin KH, Anandan R, Kumar SH, Shiny KS, Sankar TV, Thankappan TK. Effect of squalene on tissue defense system in isoproterenol-induced myocardial infarction in rats. Pharmacol Res. 2004; 50: 231-236.

66. Keller RK, Fliesler SJ. Incorporation of squalene into rod outer segments. J Biol Chem. 1990; 265: 13709-13712.

67. Newmark HL. Squalene, olive oil, and cancer risk. Review and hypothesis. Ann N Y Acad Sci. 1999; 889: 193-203.

68. Rao CV, Newmark HL, Reddy BS. Chemopreventive effect of squalene on colon cancer. Carcinogenesis. 1998; 19: 287-290.

69. Trichopoulou A, Lagiou P, Kuper H, Trichopoulos D. Cancer and Mediterranean dietary traditions. Cancer Epidemiol Biomarkers Prev. 2000; 9: 869-873.

70. Aguilera Y, Dorado ME, Prada FA, Martinez JJ, Quesada A, Ruiz-Gutierrez V. The protective role of squalene in alcohol damage in the chick embryo retina. Exp Eye Res. 2005; 80: 535-543.

71. Motawi TM, Sadik NA, Refaat A. Cytoprotective effects of DL-alpha-lipoic acid or squalene on cyclophosphamide-induced oxidative injury: An experimental study on rat myocardium, testicles and urinary bladder. Food Cheml Toxicol. 2010; 48: 2326-2336.

72. Strandberg TE, Tilvis RS, Miettinen TA. Metabolic variables of cholesterol during squalene feeding in humans: comparison with cholestyramine treatment. J Lipid Res.1990; 31:1637-1643.

73. Richter E, Schafer SG. The effect of squalane on the absorption of dietary cholesterol by the rat. Res Exp Med (Berl). 1982; 180: 189-191.

74. Kritchevsky D, Moyer AW, Tesar WC, Logan JB, Brown RA, Richmond G. Squalene feeding in experimental atherosclerosis. Circ Res. 1954; 2: 340-343.

75. Chan P, Tomlinson B, Lee CB, Lee YS. Effectiveness and safety of low-dose pravastatin and squalene, alone and in combination, in elderly patients with hypercholesterolemia. J Clin Pharmacol. 1996; 36: 422-427.

76. Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. Cell. 2006; 124: 35-46.

77. Janevski M, McGlynn M, Lewandowski P. Squalene supplementation alters genes associated with liver cholesterol metabolism. Asia Pac J Clin Nutr. 2006; 15:3, S105.

78. Farvin KHS, Anandan R, Kumar SHS, Shiny KS, Mathew S, Sankar TV, Nair PGV. Biochemical studies on thecardioprotective effect of squalene against isoprenaline-induced myocardial infarction in rats. Fishery Technology. 2009; 46: 139-151.

79. Bullon P, Quiles JL, Morillo JM, Rubini C, Goteri G, Granados-Principal S, Battino M, Ramirez-Tortosa M. Gingival vascular damage in atherosclerotic rabbits: hydroxytyrosol and squalene benefits. Food Chem Toxicol. 2009; 47: 2327-2331.

80. Liu Y, Xu X, Bi D, Wang X, Zhang X, Dai H. Influence of squalene feeding on plasma leptin, testosterone and blood pressure in rats. Indian J Med Res. 2009; 129: 150-153.

81. Okada K, Matsumoto K. Effect of skin care with an emollient containing a high water content on mild uremic pruritus. Ther Apher Dial. 2004; 8: 419-422.

82. Cho S, Choi CW, Lee DH, Won CH, Kim SM, Lee S, et al. High-dose squalene ingestion increases type I procollagen and decreases ultraviolet-induced DNA damage in human skin in vivo but is associated with transient adverse effects. Clin Exp Dermatol. 2009; 34: 500-508.

83. Murakoshi M, Nishino H, Tokuda H, Iwashima A, Okuzumi J, Kitano H, Iwasaki,R. Inhibition by squalene of the tumor-promoting activity of 12-O-tetradecanoylphorbol-13-acetate in mouse-skin carcinogenesis. Int J Cancer. 1992; 52: 950-952.

84. Blasco L, Duracher L, Forestier JP. Skin constituents as cosmetic ingredients. Part I: A study of bio-mimetic monoglycerides behavior at the squalene-water interface by the "pendant drop" method in a static mode. J Dispers Sci Technol. 2006; 27: 799-810.

85. Kostyuk V, Potapovich A, Stancato A, De Luca C, Lulli D, Pastore S, Korkina L Photo-Oxidation Products of Skin Surface Squalene Mediate Metabolic and Inflammatory Responses to Solar UV in Human Keratinocytes. PLOS One. 2012; 7: e44472.

86. Ryu A, Arakane K, Koide C, Arai H, Nagano T. Squalene as a target molecule in skin hyperpigmentation caused by singlet oxygen. Biol Pharm Bull. 2009; 32: 1504-1509.

87. Fang JY, Fang CL, Liu CH, Su YH. Lipid nanoparticles as vehicles for topical psoralen delivery: solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC). Eur J Pharm Biopharm. 2008; 70: 633-640.

88. Saint-Leger D, Bague A, Cohen E, Chivot M. A possible role for squalene in the pathogenesis of acne I. In vitro study of squalene oxidation. Br J Dermatol. 1986; 114: 535-542.

89. Richter E, Fichtl B, Schafer SG. Effects of dietary paraffin, squalane and sucrose polyester on residue disposition and elimination of hexachlorobenzene in rats.Chem Biol Interact.1982; 40: 335-344.

90. Kamimura H, Koga N, Oguri K, Yoshimura H. Enhanced elimination of theophylline, phenobarbital and strychnine from the bodies of rats and mice by squalane treatment. J Pharmacobiodyn. 1992; 15: 215-221.

91. Owen RW, Mier W, Giacosa A, Hull WE, Spiegelhalder B, Bartsch H. Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignansand squalene. Food Chem Toxicol. 2000; 38: 647-659.

92. Desai KN, Wei H, Lamartiniere CA. The preventive and therapeutic potential of the squalene-containing compound, Roidex, on tumor promotion and regression. Cancer Lett. 1996; 101: 93-96.

93. Katdare M, Singhal H, Newmark H, Osborne MP, Telang NT. Prevention of mammary preneoplastic transformation by naturally-occurring tumor inhibitors. Cancer Lett. 1997; 111: 141-147.

94. Ohkuma T, Otagiri K, Tanaka S, Ikekawa T. Intensification of host's immunity by squalene in sarcoma 180 bearing ICR mice. J Pharmacobiodyn. 1983; 6: 148-151.