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Review

Squalenoylation: A generic platform for nanoparticular drug delivery

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ABSTRACT

Squalene is a triterpene widely distributed in nature that is an intermediate in the cholesterol biosynthesis pathway. The remarkable dynamic folded conformation of squalene has been used to chemically conjugate this lipid with various therapeutic molecules to construct nanoassemblies of 100–300 nm. In this review, we discuss the new concept of "squalenoylation" through application to anticancer (i.e. gemcitabine, paclitaxel, cisplatin etc.…) or antiviral (ddI, ddC) compounds. In a lego-type approach, it is also possible to construct multifunctional nanoparticles endowed with additional imaging functionalities (i.e. "Nanotheragnostics"). This new nanotechnology platform is expected to have important applications in pharmacology.

Contents

1. Introduction

The concept of lipid–drug conjugates has gained considerable attention in recent years since in some cases, these bioconjugates have been shown to improve pharmacokinetics, decrease toxicity, and increase the therapeutic index of the associated drugs. Surprisingly, although squalene is a versatile biocompatible biopolymer known for its dietary benefits, biocompatibility and inertness, extensively used as

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excipient in numerous pharmaceutical formulations for oral and parenteral administration it was never used as covalent partner for drug delivery and targeting purposes. Recently, Couvreur et al. have shown that the covalent linkage of squalene to a drug belonging to various therapeutic classes produced a bioconjugate that self assembled as nanoparticles in aqueous media. In most case, the resulting nanoassemblies displayed an improved pharmaceutical profile compared to the parent compound. The purpose of this review article is to provide an overview of the recent advances in drug delivery of anticancer and antiviral agents using squalene-drug conjugates.

Squalene (1, Fig. 1) is an acyclic triterpene of formula $C_{30}H_{50}$ so named because of its occurrence in shark liver oil. However, it is widely distributed in nature with significant amounts found in olive oil and olive leaves. Squalene contains two farnesol moieties joined in a tail-to-tail fashion rather than in the head-to-tail fashion observed for all of the smaller terpenoids. Thus, the 6 double bonds, 10 methylenes and 8 methyl groups are symmetrically distributed in respect of the C12–C13 bonding (Fig. 1A). Squalene is the corner stone in the biosynthesis of most triterpenes including lanosterol and cycloartenol which in turn are the precursors of steroids. It has been postulated that one central role of polyterpenoids, in archaebacteria and prokaryote organisms, is to participate in the formation and reinforcement of biomembranes as surrogates of sterols [1]. Along the evolution, this role has been progressively devoted to sterols, the end products of the terpenoids biosynthesis. In eukaryotes, squalene and other terpenoids are precursors in the biosynthesis of steroids and are involved in other biological functions such as alleviating the oxidative stress [2]. In humans, squalene is transported in a serum generally in association with very low density lipoproteins and is distributed ubiquitously in tissues, with greatest concentration in the skin [3]. Squalene is well tolerated whether injected intravenously or consumed orally and is distributed to various tissues [4,5]. Because it is of biological origin and frequently used as a dietary supplement, squalene is favourably predisposed from a toxicological standpoint; thus, it has been extensively used as a carrier/adjuvant in therapeutic applications.

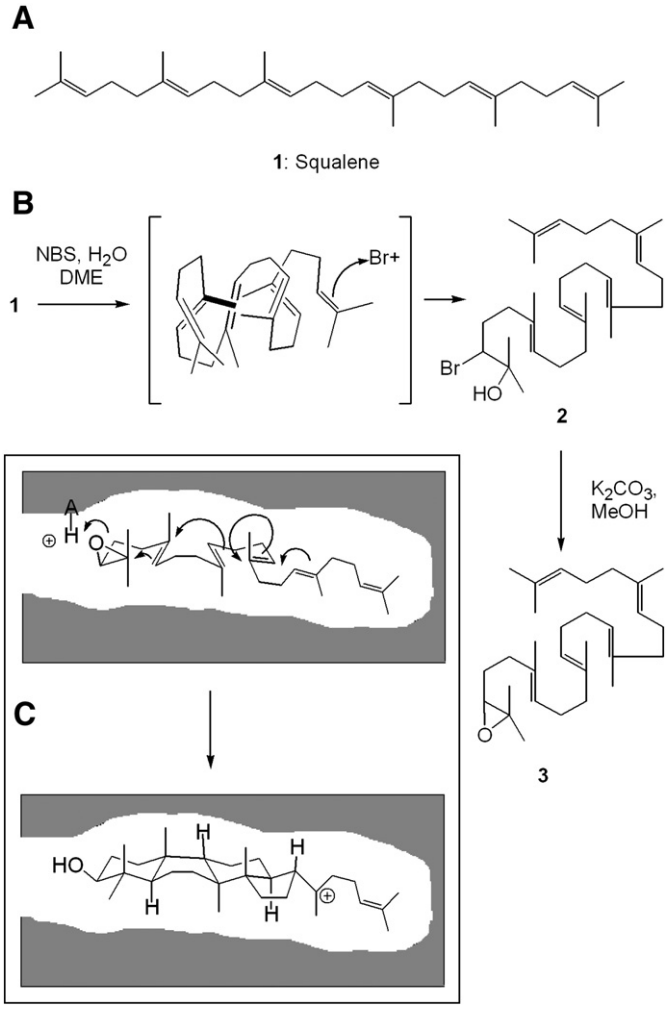

Fig. 1. A) Extended conformation of squalene. B) Schematic representation of the oxidation of the terminal double bond of squalene using *N*-bromosuccinimide in DME (dimethoxy-1,2-ethane) water mixture in a coiled compact conformation as proposed by van Tamelen [7]. C) Cyclization of 2,3-epoxysqualene in a chair-boat-chair-boat conformation into protosterol cation inside of the oxydosqualene cyclase catalytic pocket.

Though squalene is a free-flowing oil at room temperature, crystals have been obtained at low temperature and subjected to X-ray diffraction analysis [6]. The molecular structure displayed a C_i symmetry and a stretched conformation. This result contrasted with the highly coiled conformation proposed to explain the terminal selectivity observed in the course of oxidation of squalene by NBS in 1,2-dimethoxyethane (DME) or THF containing water (Fig. 1B) [7]. The flexibility of the squalene chain and the possibility of assuming a chair–boat–chair–boat conformation in low polarity environment are crucial to the interaction of (3*S*)-2,3-oxidosqualene within the oxydosqualene cyclase catalytic pocket (Fig. 1C) [8,9]. Based on NMR and molecular calculation studies it has been proposed that in a solvent of low polarity squalene would exist for the most part in an uncoiled fully extended state, whereas when squalene is embedded in polar solvent molecules, after an initial folding phase, the folded conformation becomes prevalent with respect to extended ones, because the formation of lipophilic intramolecular interactions is favoured. The folding maximizes the intramolecular lipophilic interactions and minimizes the contacts with the solvent, which is less lipophilic that the squalene itself (oil drop effect) [10,11]. On the other hand, 13C NMR spin-lattice relaxation times (T_1) study of a THF-d_8 solution of squalene in the presence of increasing water content did not evidence substantial changes making implausible that squalene abruptly changes conformations on transfer from organic to water-solvent mixtures. Alternative explanation of the selectivity based on the aggregation or micellisation are equally incompatible with this experimental T_1 results, making unlikely that there are significant changes in conformation on going from the rod-like structure in organic solvent to more aqueous media [12].

Whatever the structural origin of the colloidal behaviour of squalene, it is frequently used in the preparation of stable emulsions as either the main ingredient or secondary oil [13–15]. The high stability of squalene containing emulsion has been attributed to the fact that squalene which is essentially a hydrocarbon devoid of amphilic properties, is almost insoluble in water, making unlikely the diffusion from a small oil droplet to a larger through the aqueous medium (Ostwald ripening). Indeed, squalene can be used in combination with other oils to reduce their tendency for Ostwald ripening and increase emulsion stability [16]. For instance, a squalene emulsion stabilized by phosphatidylethanolamine or poloxamer 188 was shown to prolong the in vitro release of a morphine prodrug [17]. Administered intravenously in vivo, these prodrug-loaded squalene emulsion formulations exhibited prolonged analgesic activity in rats. Similar emulsions have been successfully employed to deliver lipophilic ester prodrugs of nalbuphine (a κ-opioid receptor agonist with a short half-life) [18]. In another report, a nanostructured lipid carrier based on squalene and precirol was shown to increase the skin permeability and to control the delivery of the encapsulated psoralen (an anti-psoriatic medicine) [19].

On the other hand, lipid–drug conjugates have shown interesting applications in therapeutics [20–25] and some of them have now