

## Squalenylation platform for novel therapeutic nanoassemblies

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Squalene (SQ) is an acyclic isoprenoid compound of 30 carbon atoms, precursor in the biosynthesis of cholesterol, which is widely distributed in nature. This molecule is particularly interesting because it is strongly influenced by solvent polarity, showing a highly coiled, compact conformation in polar solvents, such as water. We have exploited this physico-chemical property of squalene by developing the concept of "squalenylation", which involves the chemical linkage of squalene with various nucleosides analogues, which surprisingly self-organized in water as spherical nanoassemblies of 100–300 nm, irrespective of the nucleoside analogue used. Moreover, the peculiar structure of squalene prodrug allowed particles to be obtained without using any polymeric material and surface-active agent, which represents, from a toxicological point of view, a major advantage for intravenous administration. This novel strategy, which preserves squalene properties in its prodrugs, was first applied to gemcitabine, an anticancer nucleoside analogue of deoxycytidine active against solid tumors, including colon, lung, pancreatic, breast, bladder and ovarian cancers. Gemcitabine is rapidly metabolized to inactive uracil derivative. Thus, when intravenously administered, gemcitabine has a very short plasma half-life, representing a relevant limitation. In order to protect the 4-amino group of gemcitabine from the rapid plasmatic deamination, this nucleosidic analogue has been linked via an amide bond to squalenic acid obtaining the 4-(*N*-squalenyl)gemcitabine [1,2] (SQ-Gem). This new compound showed higher plasma stability than the parent drug. Moreover, this squalenic amphiphilic prodrug was able to form nanoparticles in water by the nanoprecipitation technique [3]. Cryogenic transmission electron microscopy examination and X-ray diffraction analysis allowed investigating the SQ-Gem nanoparticles structure.

SQ-Gem cytotoxicity was measured on different cancer cell lines and compared to that of gemcitabine; results showed a higher cytotoxicity for the squalenic prodrug. Moreover, these squalenic nanoparticles have favorable pharmacokinetic properties and higher *in vivo* activity towards leukemia.

Squalenylation has been also applied to other hydrophilic antitumoral drugs, such as cytarabine (AraC) to obtain SQAraC, and to antiviral compounds, such as acyclovir (ACV), obtaining SQACV. SQACV nanoparticles, after ocular administration in albino rabbits, showed increased drug ocular bioavailability compared to the aqueous solution of free ACV due to a higher lipophilicity and, at the same time, an increased drug concentration in water of the new prodrug.

Moreover, squalenylation allowed us obtaining nonpolymeric nanoparticles even with some lipophilic antitumoral drugs, such as paclitaxel and doxorubicin free base. Several prodrugs of paclitaxel or doxorubicin, containing different spacer linkers, were synthesized and fully characterized [4]. All the hydrophobic conjugates resulted in the effective formation of stable nano-ranged self-assemblies. Among the produced compounds, doxorubicin-Squalene presented a strongly reduced toxicity, allowing, in *in vivo* tumor models, highest activity.

Other applications of squalenylation were focused on the preparation of actively targeted nanoparticles able to specifically address drugs to cancer cells. For this purpose we prepared SQ-Gem nanoparticles, which carry on their surface derivatizable functional groups to allow a covalent linkage with a targeting agent. For this purpose, a spacer was added between the nanoparticle surface and the targeting agent, which was a linear peptide formed by 6 aminoacids, CKAAKN, able to selectively bind the tumor vasculature in a model of pancreatic islets carcinoma [4]. After a complete physico-chemical characterization, the antitumoral activity of targeted nanoparticles was evaluated by *in vivo* tests on Rip1-Tag2 mice, a transgenic model of pancreatic islets tumor. The results showed that after *iv* injection, CKAAKN-targeted Gem-SQ nanoparticles cause a strong reduction of tumor volume in comparison both to native gemcitabine and also with nontargeted squalenic nanoparticles. Hystopathological evaluations confirmed the strong reduction of neovasculature.

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