Shark versus virus

By Tracey Baas, Senior Editor

Over the past 18 years, the aminosterol squalamine has changed corporate hands twice and been tried in indications ranging from cancer to age-related macular degeneration. Now, the academic who originally isolated the compound from dogfish shark liver thinks he has found a new use for squalamine as a broad-spectrum antiviral.[1]

Michael Zasloff, dean of research and translational science at the **Georgetown University Medical Center**, identified squalamine in 1993. Magainin Pharmaceuticals Inc., a biotech cofounded by Zasloff, in-licensed the compound and positioned it as an angiogenesis inhibitor for cancer.

In 2001, Magainin changed its name to Genaera Corp. and repositioned squalamine as a therapeutic for age-related macular degeneration (AMD). However, the company put the molecule on hold after Phase II data showed an i.v. formulation was unlikely to improve vision with the speed or frequency necessary to compete with marketed treatments.

Genaera dissolved in 2009, at which point **Ohr Pharmaceutical Inc.** picked up squalamine and an unrelated asset for $200,000. Ohr reformulated the compound and has a topical version in preclinical development for AMD.

Meanwhile, Zasloff maintained an interest in squalamine as a component of the dogfish shark's primitive immune system that keeps the shark free from viral infections. Now, he has studied whether the compound can also ward off viruses in humans.

Squalamine is sequestered and cleared from circulation by the liver and excreted through the biliary system. Thus, Zasloff focused his efforts on testing viruses whose primary tissue location is liver.

In human cell culture, squalamine inhibited dengue virus, HBV and hepatitis-δ virus (HDV) infection compared with no treatment.

In hamsters infected with a lethal dose of yellow fever virus or eastern equine encephalitis virus, squalamine cured the animals of virus and increased survival compared with vehicle. In mice infected with a sublethal dose of murine cytomegalovirus (MCMV), squalamine decreased levels of virus in the liver and spleen compared with vehicle, although it did not have an effect on levels of virus in the lung and salivary gland.

Rodents treated with squalamine had normal serum hepatic enzyme levels and showed no signs of liver toxicity.

Results were published in the *Proceedings of the National Academy of Sciences*.

One molecule, many hats

Zasloff now hopes to advance squalamine into the clinic for viral infections and said he is most interested in HBV, HCV, HDV and CMV. **Zasloff Laboratories LLC**, a company he founded in 2008, holds IP covering the use of squalamine to treat and prevent viral infections and covering formulations and synthesis of the molecule.

The suggested mechanism of action for squalamine's antiviral effect is distinct from its previously reported mechanism of action on angiogenesis.[2,3] Zasloff thinks that the positively charged squalamine is attracted by and binds to the cellular membrane's anionic phospholipids on host cells, neutralizing the membrane without causing structural damage.

The charge neutralization causes displacement of membrane-anchored proteins, which Zasloff hypothesizes disrupts virus-host protein interactions and an efficient viral life cycle.

In support of his hypothesis, Zasloff pointed to squalamine's effects on dengue virus. During its early entry phase, dengue relies on a membrane-anchored protein called RAC1 (ras-related C3 botulinum toxin substrate 1; rho family, small GTP binding protein Rac1). In the paper, Zasloff's team reported that in model membranes, RAC1 was displaced by squalamine.

"By targeting host membranes, squalamine differs in mechanism" from conventional antivirals that target the virus, said Jean Michel Brunel, research associate at the **University of the Mediterranean Aix-Marseille II**. "If the proposed mechanism of action of squalamine is correct, development of viral resistance would be unlikely. Thus, squalamine or one of its derivatives could constitute the basis of a new class of anti-infectious agents."

Brunel's lab is studying squalamine as an antimicrobial.

Benhur Lee, a professor of clinical pathology and laboratory medicine at the **University of California, Los Angeles**, was less sure of squalamine's proposed new mechanism. "The *in vivo* data is encouraging, but the mechanism of antiviral action needs to be nailed down—not only for future pharmacokinetic optimization but also to inform the limits of squalamine's broad-spectrum activity," he said. "It is not clear if squalamine-mediated displacement of RAC1 from cellular membranes, while likely to be true, has anything to do with its viral inhibitory effect. In fact, it is also not clear where squalamine acts in the viral life cycle."

"Testing viruses with different replication times, and that spread with or without cell lysis, will go a long way in characterizing the extent of its broad-spectrum activity and may also reveal underlying mechanisms behind its antiviral activity," added Lee.

"If the antiviral mechanism of action of squalamine has to do with membrane charge neutralization, the agent could be applicable to the vast array of nonhepatic viruses that use membrane fusion as their mode of entry into the host cell as part of their replication cycle," said Ohr CEO Irach Taraporewala. The key, he said, will be finding out whether host tissues other than the liver "can sequester sufficiently high squalamine concentrations to effectively inhibit viral replication without causing systemic toxic effects or toxicity to those tissues."

He added: "If further research on the *in vivo* antiviral activity continues to bear out, it would potentially be of interest to us, especially considering our experience working with the molecule and expertise in squalamine formulations research and development, to expand the use of squalamine for infectious disease."

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COMPANIES AND INSTITUTIONS MENTIONED

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