



Research note

Are Squalene - Containing Vaccines Potentially Harmful?

Robert C. Sizemore¹ and M.S. Zaman^{1,2*}

¹Department of Biological Sciences, Alcorn State University, Lorman, MS 39096, USA

²Department of Biology, South Texas College, McAllen, TX 78501, USA

(Submitted: October 2, 2018; Accepted: October 25, 2018)

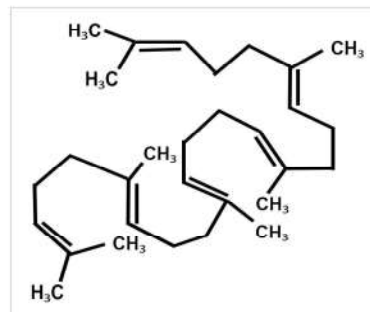
Abstract

Squalene is a natural 30-carbon molecule normally found in higher organisms. In humans, it is used in the synthesis of sterols such as cholesterol and for the production of vitamin D. The use of squalene as an adjuvant in vaccines to enhance immune responses has generated controversy due to previous reports that anti-squalene antibodies were produced as a result. A 2000 report suggested that Gulf War Syndrome (GWS) was due to the presence of these antibodies in soldiers that had been immunized against anthrax, some of whom were never deployed to the Middle East. The Department of Defense conducted their own studies and did not find a correlation between anti-squalene antibodies and GWS. They also indicated that squalene was never added to the vaccine for US troops. Currently, a flu vaccine (Fluad) for patients over 60 years of age contains MF59, which has squalene in oil as an adjuvant. This review summarizes the current data and evidence as to whether or not there should be a concern with squalene-containing vaccines.

Keywords: Squalene, adjuvant, Fluad, Gulf War Syndrome, MF59A, anti-squalene antibody

1.0 Introduction

Squalene is a 30-carbon molecule ($C_{30}H_{50}$) produced by numerous plant and animal species (Giudice *et al.*, 2006). Humans normally produce this hydrocarbon oil in the liver and it is often found in the bloodstream. Squalene is a precursor of sterols and is an intermediate byproduct in the production of cholesterol. Although found throughout human tissue, its highest concentration is in the skin where it is thought to help protect against UV and other radiation (Kelly, 1999). Squalene is also found in various foods, cosmetics and supplements for health (WHO, 2006). It is a common component of certain fish oils, notably shark liver oil (Kim and Karadeniz, 2012). In fact, the name squalene is derived from the Italian word for shark, i.e. *squalo*, which in turn is derived from the Latin *squalus* (Panatto *et al.*, 2017). Shark liver oil squalene is often the source for pharmaceutical products and adjuvants (WHO, 2006). Although some experimental data has suggested anti-cancer effects, the main clinical use of squalene has been as an adjuvant in vaccines (Kelly, 1999).



Squalene

2.0 Squalene as an Adjuvant

Adjuvants are substances, which when injected with an antigen, nonspecifically increase the immune response against that antigen. Adjuvants have been used for decades and various different molecules have been involved often utilizing bacterial-based components. Although potent, adverse reactions often occur with bacteria-containing adjuvants and their use is precluded for human vaccinations. Squalene alone does not act as an adjuvant; it must be combined and emulsified with surfactants in order

*Corresponding Author's E-mail Address: zaman@alcorn.edu

to bring about its effect (WHO, 2006). Squalene-based adjuvants have been considered non-hazardous for humans and have been used for many years, in particular with vaccines against influenza (Panatto *et al.*, 2017). Squalene is also used in several experimental vaccines including those being developed for malaria and HIV (WHO, 2006; McGuire *et al.*, 2017). In short, squalene is used to enhance the efficacy of a vaccine.

3.0 Gulf War Syndrome Controversy

Gulf War Syndrome (GWS) is a condition with various different symptoms and sequelae that appeared in veterans and civilian workers that participated in the 1990-91 Gulf War (Wikipedia, 2018; Hogg, 2018). The multi-symptomatic disorder included fatigue, chronic headaches, pain and tingling in muscles and limbs, cognitive dysfunctions and respiratory problems among many others (Hogg, 2018). One report stated that 250,000 of the 697,000 U.S. veterans who served in the war were afflicted (Research Advisory Committee on Gulf War Veterans' Illnesses, 2008). Various causes have been proposed including nerve gas, pyridostigmine bromide pills (for protection against nerve gas), depleted uranium in foreign stockpiles, oil and smoke from burning oil wells, and pesticides (Wikipedia, 2018). In 2002, scientists at Tulane University School of Medicine noted that several soldiers who were never deployed to the Middle East showed the same exact symptoms as those who were sent overseas. The United States Department of Defense started the Anthrax Vaccine Immunization Program in 1997 and immunized 2.4 million troops. Some of the adverse reactions to the vaccine were similar to those presented in GWS. They stated that certain lots of the vaccine contained squalene as an adjuvant, so they tested the veterans for the presence of anti-squalene antibodies (ASA). Besides finding ASA in many of the veterans, they concluded that the presence of the antibodies correlated with specific lots of the vaccine. They concluded that GWS was linked to the presence of squalene in those lots of the anthrax vaccine (Asa *et al.*, 2002).

In 2003, researchers at the Walter Reed Army Institute of Research and the U.S. Army Medical

Research Institute of Infectious Disease conducted their own studies and concluded that anti-squalene antibodies occur naturally in humans and were not associated with immunization with the anthrax vaccine (Matyas *et al.*, 2003). The U.S. Army also stated that squalene was never added to the vaccines administered to Gulf War soldiers (WHO, 2006). One researcher not associated with the military has suggested that the massive cocktail of vaccinations (anthrax and several others) given soldiers resulted in over-activation of the humoral (antibody-mediated) branch of the immune system, which could have led to allergic problems and autoimmune disorders similar to those associated with GWS (Hogg, 2018). Regardless, a 2004 report by researchers at George Washington University reported that there was no evidence for any serious adverse events or other medically important adverse events with the licensed anthrax vaccine given U.S. military personnel (Sever *et al.*, 2004).

4.0 MF59, a squalene-containing adjuvant

MF59 is an oil-in-water emulsion containing squalene. This adjuvant was developed by Chiron and is manufactured by Novartis Vaccines (Giudice *et al.*, 2006). Researchers at Novartis noted in 2006 that over 22 million doses of an influenza vaccine containing MF59 have been administered all without any notable safety issues. Furthermore, MF59 had been used with several other vaccines all without incident. The Novartis researchers then conducted a study to show that humans normally produce anti-squalene antibodies and that vaccination with MF59 “neither induced anti-squalene antibodies nor enhanced preexisting anti-squalene antibody titers” (Giudice *et al.*, 2006; WHO, 2006).

5.0 MF59-adjuvanted trivalent influenza vaccine (FLUAD)

A current tri-valent influenza vaccine (FLUAD) contains MF59. A report in 2017 indicated that in a Phase III pediatric trial, the MF59 containing vaccine produced much higher responses than a non-adjuvanted influenza vaccine (Cruz-Valdez *et al.*, 2018). FLUAD contains about 10 mg of squalene per dose and to date, no adverse effects due to the presence of squalene have been noted. FLUAD

was licensed in 2015 and it is now recommended that FLUAD (as opposed to the non-adjuvanted version) be given to those considered at risk for influenza, in particular those 65 years of age and older (WHO, 2006; CDC, 2019).

6.0 Discussion and Conclusion

Squalene is a 30-carbon molecule ($C_{30}H_{60}$) produced by numerous plant and animal species. Squalene has been used for years in vaccines as an adjuvant to enhance the efficacy of the vaccine. Although squalene is a normal component in the human body, a report in 2002 (Asa *et al.*, 2002) suggested that Gulf War Syndrome was due to the presence of anti-squalene antibodies in soldiers, some of who were never deployed to the Middle East. It was further suggested that squalene used in the vaccine to protect soldiers from potential biological warfare using anthrax, generated the autoimmune antibodies. However, several other reports including those by the U.S. Army suggested that squalene was not present in the vaccine or otherwise involved and that humans normally produce anti-squalene antibodies (WHO, 2006).

MF59 is an oil-in-water emulsion containing squalene and is currently used in FLUAD, a trivalent influenza vaccine. The World Health Organization and several other reports suggests that MF59 and squalene-containing vaccines are safe and without any significant risk other than standard side effects associated with any vaccine (Giudice *et al.*, 2006; WHO, 2006; O'Hogan, 2014; CDC, 2019).

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