Journal of Pharmaceutical Sciences xxx (2018) 1-5

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Rapid Communication

Stable Nanoemulsions for the Delivery of Small Molecule Immune Potentiators

Rushit N. Lodaya [1], Luis A. Brito [1], Tom Y.H. Wu [2], Andrew T. Miller [2], Gillis R. Otten [1], Manmohan Singh [1], Derek T. O'Hagan [3,*]

[1] Novartis Vaccines and Diagnostics, Cambridge, Massachusetts 02139
[2] Genomics Institute of Novartis Research Foundation (GNF), San Diego, California 92121
[3] GSK Vaccines, Rockville, Maryland 20850

ARTICLE INFO

Article history:
Received 26 March 2018
Revised 4 May 2018
Accepted 17 May 2018

Keywords:
vaccine adjuvants
vaccine delivery
immune response(s)
nanoparticle(s)
formulation

ABSTRACT

Adjuvants are required to enhance immune responses to typically poorly immunogenic recombinant antigens. Toll-like receptor agonists (TLRa) have been widely evaluated as adjuvants because they activate the innate immune system. Currently, licensed vaccines adjuvanted with TLRa include the TLR4 agonist monophosphoryl lipid, while additional TLRa are in clinical development. Unfortunately, naturally derived TLRa are often complex and heterogeneous entities, which brings formulation challenges. Consequently, the use of synthetic small-molecule TLRa has significant advantages because they are well-defined discrete molecules, which can be chemically modified to modulate their physicochemical properties. We previously described the discovery of a family of TLR7 agonists based on a benzonaphthyridine scaffold. In addition, we described how Alum could be used to deliver these synthetic TLRa. An alternative adjuvant approach with enhanced potency over Alum are squalene containing oil-in-water emulsions, which have been included in licensed influenza vaccines, including Fluad (MF59 adjuvanted) and Pandemrix (AS03 adjuvanted). Here, we describe how to enable the co-delivery of a TLR7 agonist in a squalene-based oil-in-water emulsion, for adjuvant evaluation.

Introduction

Recombinant DNA technology has revolutionized the field of vaccines providing subunit antigens with improved safety, tolerability, and lower cost of production. However, these antigens have reduced potency because of the lack of immunostimulatory components, including pathogen-associated molecular patterns. Adjuvants play a vital role in enhancing immunogenicity of these antigens, so need to be added to vaccine formulations to enhance antigen potency.[1,2]

The discovery of the role of toll-like receptors (TLRs)[3,4] and their activation through pathogen-associated molecular patterns (TLR agonists [TLRa]), which are typically present in older vaccines has allowed a better understanding of how adjuvants work and how preferred immune responses can be induced.[5] Unfortunately, natural TLRa like monophosphoryl lipid (MPL) from gram-negative bacteria,[6] are typically large and complex molecules, which bring significant formulation challenges.[4] However, recognition of the receptor systems involved in adjuvant mechanisms has allowed the discovery of small-molecule immune potentiators (SMIPs).[7,8] Encouragingly, SMIPs have been shown to be more potent than the large biologic molecules[9,10] although they also have potential for inducing unwanted systemic inflammatory responses if they are allowed to diffuse away from the site of injection. Hence, a formulation approach is necessary to efficiently deliver the SMIP to local immune cells, while restricting the ability of the SMIP to diffuse from the site of injection.[11]

Conflicts of interest: All authors have declared the following interests: R.N.L., L.A.B., G.R.O., M.S. and D.T.O. were employees of Novartis Vaccines and T.Y.H.W. and A.T.M. were employees of the Genomics Institute of the Novartis Research Foundation (GNF) at the time of the study; in March 2015 the Novartis non-influenza Vaccines business was acquired by the GSK group of companies. D.O. is an employee of the GSK group of companies and reports ownership of GSK shares and/or restricted GSK shares. R.N.L. is a student at Northeastern University and is currently in PhD fellowship program at GSK. L.A.B. is now an employee of Moderna Therapeutics, T.Y.H.W. and A.T.M. are employees of GNF, G.R.O. is an employee of Seqirus and M.S. is an employee of Takeda.

Current addresses for Lodaya: Northeastern University, Boston, MA, 02115; for Brito: Moderna Therapeutics, Cambridge, MA, 02139; Wu and Miller: Genomics Institute of Novartis Research Foundation (GNF), San Diego, CA, 92121; Otten: Seqirus, Cambridge, MA, 02139; Singh: Takeda Vaccines, Cambridge, MA, 02139.

* *Correspondence to:* Derek T. O'Hagan (Telephone: +1-240-314-4400).
E-mail address: derek.t.o'hagan@gsk.com (D.T. O'Hagan).

https://doi.org/10.1016/j.xphs.2018.05.012
0022-3549/

Several technologies have been used to deliver TLRa to enhance immune responses. For example, MPL is delivered by adsorption on Alum (Cervarix, GSK). Alum is also used to deliver a novel TL7 SMIP agonist.[12] TLRa entrapment in nanoparticles or liposomes is an alternative delivery technique for TLRa.[13-15] Addition of TLRa to emulsion adjuvants has also been previously evaluated with lipid-like TLR4 agonists (E6020, GLA) or mixtures of TLR agonists such as MPL and saponins.[16-19] Emulsions have been used as vaccine adjuvants to improve immune responses since the 1930s and the first vaccine containing a squalene oil-in-water (o/w) emulsion adjuvant (MF59) was approved in 1997.[20,21] Previous studies with squalene o/w emulsions have shown that they work in a TLR-independent manner[22]; therefore, it is rational to improve their performance by the addition of a TLRa. In fact, it has been shown that a TLRa, within the emulsion droplet of an o/w emulsion, shows a synergistic effect in improving the breadth and depth of the immune response.[18]

We have previously described the discovery of a series of TLR7-specific agonists based on a benzonaphthyridine scaffold with a range of physicochemical properties.[23] Unfortunately, many of the compounds that we discovered were poorly water soluble because of a high log *p*. Here, we describe the formulation of one typical agonist, SMIP.7-9, within a squalene o/w adjuvant nanoemulsion (ANE), which is same in composition to MF59 (Table 1). We limited the oil phase of the o/w emulsion to squalene because it has an extensive safety record as a component in several licensed vaccine adjuvants.[24-26] Unfortunately, despite having a high log *p* and low aqueous solubility, SMIP.7-9 was very poorly soluble in squalene (<4 mg/mL). Therefore, it was evident that a higher solubility of SMIP was required to enable a suitable adjuvant dose to be administered in squalene oil.[23]

To improve the solubility of SMIP.7-9 in squalene and prevent precipitation, a series of excipients were evaluated. From the excipients evaluated, we determined that 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) improved SMIP solubility, whereas poly-vinyl pyrrolidine (PVP) prevented precipitation and maintained the stability of the emulsion. We also performed pharmacokinetic studies to show that the SMIP did not reach high plasma levels to prevent potential side effects.[27,28] Finally, we evaluated the potency of these formulations in a mouse model to determine bactericidal antibody titers against *Neisseria meningitidis* serotype b.

Materials and Methods

Materials

Meningitis B (MenB) antigens, namely GNA33, GNA1162, GNA1220, and GNA1946 were developed as previously described.[23,29] The discovery of SMIP.7-9 has also been previously described.[23] Squalene, Span 85, Polysorbate 80, and PVP (K-15) were purchased from Sigma-Aldrich (St. Louis, MO). DSPC was purchased from Genzyme (Cambridge, MA).

Formulation

Emulsions were prepared as previously described[17,18] with the following changes. DSPC was dissolved in 1:3 (v/v) methanol: dichloromethane; PVP and SMIP.7-9 were dissolved in dichloromethane (DCM). All organic solutions were combined and added directly into a container containing span 85 and squalene. An aqueous solution of tween 80 and citrate buffer was added directly to the organic phase; a coarse emulsion was made using an IKA bench top mixer. The resulting emulsion was placed on a stir plate at room temperature (RT) for 2 h to facilitate evaporation of organic solvents and then passed through a Microfluidizer M110S multiple times. Samples were kept at 4°C unless otherwise mentioned. Micronized SMIP was formulated in the microfluidizer to obtain a stable suspension.

Particle Size Distribution

Emulsion particle size was analyzed with a Malvern Nanosizer 3000 (Worcestershire, UK) and a Horiba LA930 (Kyoto, Japan). The emulsions were diluted in water prior to analysis.

Microscopy

Qualitative images of emulsions were taken on a Zeiss Axioscope (Thornwood, NY) inverted microscope. Emulsions were placed directly on a microscope slide with a coverslip on top. Images were taken with Axiovision software at 10× and 40× objectives.

SMIP.7-9 Loading of Emulsions

Emulsions were analyzed for SMIP.7-9 loading by reverse phase ultra high-performance liquid chromatography (UPLC). Emulsions were centrifuged using low shear airfuge to allow free SMIP precipitates, if any, to settle before sampling. Adequate amounts were taken and diluted 10 times in a 1:1 (v/v) mixture of isopropyl alcohol and dimethyl sulfoxide and vortex after dilution. Samples were then placed on a Waters UPLC with a UV detector. Percent loading was calculated by taking the amount of SMIP.7-9 found divided by the expected loading.

Pharmacokinetic Analysis

On day 0, female BALB/c mice (Charles River Laboratories) were injected intramuscularly with a total of 100 μL of a given compound. Mice were bled through their retroorbital vein at 0.5, 1, 3, 7, and 24 h postinjection. Blood was processed into serum by centrifugation at 12,000 rpm for 5 min and analyzed for compound pharmacokinetics (PK) activity. After the final blood collection at 24 h, animals were sacrificed, and muscle, inguinal lymph nodes, and liver were removed and flash-frozen in liquid nitrogen for PK analysis.

In Vivo *Evaluation*

Adjuvant potency was evaluated in CD-1 female mice, 7- to 14-week-old, from Harlan Laboratories (now Envigo) as previously published.[23] Briefly, emulsions were mixed with MenB antigens (10 μg each) at an adjuvant dose of 25 μg/mouse. Mice received 50 μL per leg bilateral injections in the quadriceps muscle. Two immunizations were given 2 weeks apart. Serum was taken 2 weeks after the second immunization. Samples were analyzed for bactericidal titers using a modified serum bactericidal assay against the NZ98 strain of MenB as previously described.[23]

All animal work was approved by the GNF's Institutional Animal Care and Use Committee and was carried out according to the NIH guidelines. All mice were maintained in a specific pathogen-free facility at the GNF.

Results and Discussion

SMIP.7-9 was found to crystallize in oil phase and also showed low solubility (<4 mg/mL). We screened a series of excipients (data not shown) to improve SMIP solubility and prevent its precipitation in the oil phase. DSPC and PVP were found to improve the stability