Design-of-experiments-based development and in vitro evaluation of a cationic lipid-based, triple adjuvanted subunit pertussis vaccine

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BACKGROUND

One approach to explore in improving the efficacy of subunit pertussis vaccines is to use the intranasal route of administration, using adjuvants that enhance local mucosal immunity as well as generating a balanced systemic immune response. In this project, we are incorporating a triple adjuvant (TriAdj) into self-assembled cationic lipid nanoparticles (L-TriAdj) for co-formulation with pertussis antigens. The role of lipid composition is a particularly important parameter to study, because it affects not only the physical features of the particle such as size, but also the cellular processing in antigen presenting cells and subsequent immunogenicity of the vaccines.

OBJECTIVES

The aim of this study was to optimize the lipid composition of the nanoparticles using a "design-of-experiments" approach, and to elucidate the role of lipid composition on the physicochemical properties and in vitro behavior (cellular viability, uptake and cytokine expression) of L-TriAdj pertussis vaccine formulations applied to antigen presenting dendritic cells. The goal of the DoE analysis was to narrow down towards a lead candidate formulation with a hydrodynamic diameter less than 150 nm and PDI < 0.3.

METHODS

Lipid Nanoparticles: The chosen lipid composition was based on a cationic lipid [dimethyldioctadecyl ammonium bromide (DDAB)], fusogenic lipid [dioleoylphosphoethanolamine (DOPE)] and structural lipids [distearoylphosphatidylcholine (DSPC) or dipalmitoylphosphatidylcholine (DPPC) and cholesterol]. A lipid complex was prepared by self-assembly with TriAdj: poly(I:C), IDR-1002 innate defense regulator peptide, polyphosphazene (1:2:1). LTriAdj A – DDAB/DOPE/DSPC/Chol (20/20/40/20 mol); LTriAdj B – DDAB/DOPE/DSPC/Chol (20/40/20/20); LTriAdj C - DDAB/DOPE/DPPC/Chol (20/20/40/20); LTriAdj D – DDAB/DOPE/DPPC/Chol (20/40/20/20).

For whole vaccines, the total antigen concentration was 0.05 µg/µL comprised of equal parts of the three antigens: pertactin, pertussis toxin mutant and filamentous hemagglutinin.

Design-of-experiments optimization: The software package, DesignExpert® (version 13.0) was utilized to create the design space, for statistical analysis, generation of contour plots and for optimization of L-TriAdj nanoparticle lipid composition.(Table 1) A randomized I-optimal mixture design comprising of 20 experimental runs was built to determine the effect of L-TriAdj lipid molar composition (independent variable) on two responses: particle size and polydispersity.

In vitro assessments: Cellular viability was determined by MTT assay, whereby metabolism of (3-[4,5-dimethyliazol-2yl]-2,5-diphenyl-tetrazolium bromide) by live cells creates a change in color which is quantified by OD570 on a plate reader. Cellular uptake was determined by flow cytometry, incorporating 18:1 Cy7PE to label L-TriAdj. Data acquisition and analysis was conducted using the CytExpert® software package. The relative mean fluorescence intensity of JAWSII mouse dendritic cells treated with L-TriAdj for 24hrs was calculated relative to untreated cells. Qualitative fluorescence imaging was conducted using a Zeiss LSM700 laser scanning confocal microscope operated through the ZEN acquisition software program. Cells were fixed with paraformaldehyde and stained with Phalloidin iFluor 647 for F-actin (red), and Hoechst 33342 for nuclear staining (blue) and L-TriAdj was labeled by incorporation of NBD-PE lipid (green). Dentritic cell maturation and activation: The expression of surface markers (MHC-I, MHC-II, and CD86) and intracellular cytokines (TNF-α, IL-10, IL-12 and IFN-γ) by JAWS II dendritic cells after treatment with vaccine formulations (24hr) was evaluated using flow cytometry. Single color compensation was performed using untreated cells. Data analysis and acquisition from the Beckman Coulter CytoFlex was conducted using CytExpert®.

Statistical analysis: For the DoE studies, data transformations (square root and Logit) were applied for each response factor to obtain data normality for ANOVA analysis and to restrict data modelling within specific bounds. Factor-response data was fit to linear, quadratic and reduced (special) cubic models. ANOVA testing was conducted using the software package, DesignExpert® (13.0) to determine the statistical significance of mathematical models to the response data. For the non-DOE studies, the software program, OriginPro® 2021 (version 9.8) was used for one-way ANOVA testing with Tukey's post-hoc analysis; the significance level was set to $p < 0.05$. Graphical data is represented as mean ± standard deviation (n=3) unless indicated otherwise. .*p<0.05, **p<0.01, ***p<0.001.

RESULTS

Response	Predicted	Actual (n=3)
Hydrodynamic diameter (nm)	133	150 ± 10
Polydispersity	0.18	0.29 ± 0.1
Zeta potential (mV)	Range (38 – 62)	51 ± 3

Table 3: Differences in the actual and predicted values for L-TriAdj [DDAB/DOPE/DSPC/ cholesterol (20/40/20/20 mol)] formulations.

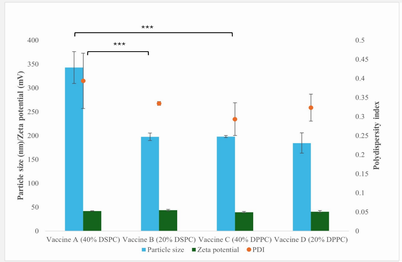

Figure 5: Effect of varying lipid composition on the particle size, polydispersity and zeta potential of L-TriAdj pertussis vaccine formulations.

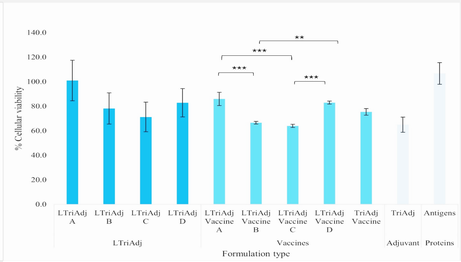

Figure 6: Effect of vaccine lipid composition on the cellular viability of JAWS II dendritic cells after treatment for 24 h

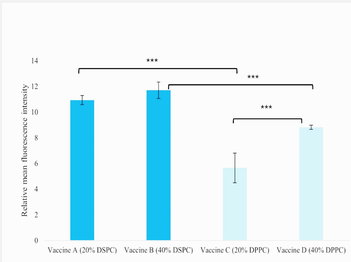

Figure 7: Effect of varying lipid composition on the cellular uptake of L-TriAdj vaccine formulations in JAWS II dendritic cells (24h)

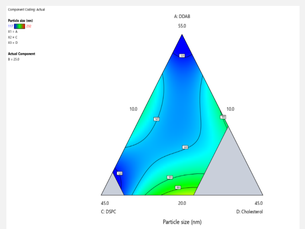

Figure 1: Effect of varying (A) DDAB (20 – 55%), (C) DSPC (10 – 40%) and (D) Cholesterol (10-30%) composition at a fixed DOPE amount (25%), on the particle size of L-TriAdj.

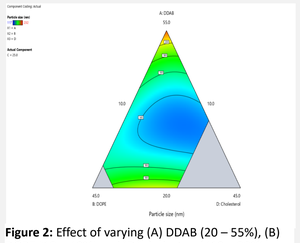

Figure 2: Effect of varying (A) DDAB (20 – 55%), (B) DOPE (10 – 40%) and (D) cholesterol (10 – 30%) composition at a fixed DSPC amount (25%), on the particle size of L-TriAdj.

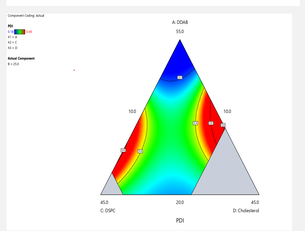

Figure 3: Effect of varying (A) DDAB (20 -55%), (C) DSPC (10-40%) and (D) cholesterol (10-30%) composition at a fixed DOPE amount (25%), on the polydispersity of L-TriAdj.

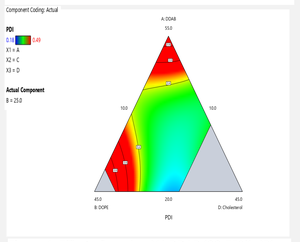

Figure 4: Effect of varying (A) DDAB (20-55%), (B) DOPE (10 – 40%) and (D) Cholesterol (10-30%) composition at a fixed DSPC amount (25%), on the polydispersity of L-TriAdj.

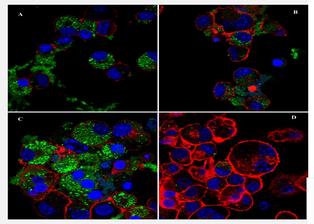

Figure 8: Confocal microscopy imaging of 18:1 NBD-PE labelled L-TriAdj formulations (green) after treatment to JAWS II dendritic cells. Red: F-actin; blue: nucleus

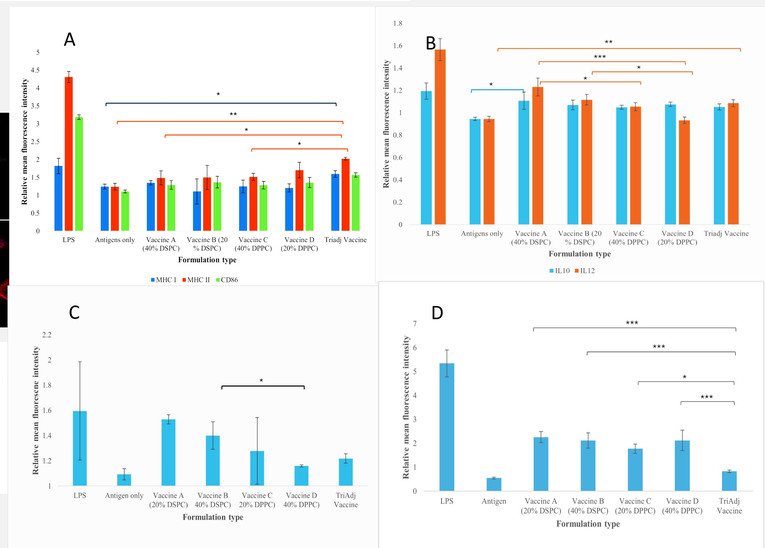

Figure 9: Effect of varying L-TriAdj pertussis vaccine lipid composition on the expression of: A) MHC I, MHC II and CD86 in JAWS-II dendritic cells after 24 hours of treatment, relative to untreated; B) IL-10 and IL-12; C) IFN-γ; D) TNF-α

Low Limit (%)		Constraint		High Limit (%)
20	≤	A: DDAB	≤	60
10	≤	B: DOPE	≤	40
10	≤	C: DSPC	≤	40
10	≤	D: Cholesterol	≤	30
		A+B+C+D	=	100

Table 1. Summary of the design constraints for lipid composition

Run	Component 1 A: DDAB	Component 2 B: DOPE	Component 3 C: DSPC	Component 4 D: Cholesterol	Response 1 Hydrodynamic Particle size (nm)	Response 2: PDI
1	20.0	30.2	32.0	17.8	193	0.49
2	50.0	10.0	10.0	30.0	232	0.38
3	43.1	27.8	10.0	19.1	145	0.29
4	31.8	40.0	18.2	10.0	139	0.34
5	28.3	21.4	20.2	30.0	144	0.39
6	33.2	16.8	40.0	10.0	137	0.37
7	22.5	18.7	40.0	18.8	117	0.18
8	20.0	40.0	10.0	30.0	175	0.38
9	60.0	14.3	15.7	10.0	120	0.24
10	30.5	10.0	29.5	30.0	129	0.3
11	20.0	10.0	40.0	30.0	150	0.41
12	34.1	26.3	24.1	15.5	137	0.33
13	42.6	10.0	27.4	20.0	139	0.35
14	47.4	19.1	23.5	10.0	167	0.39
15	28.3	21.4	20.2	30.0	143	0.32
16	20.0	40.0	20.3	19.7	192	0.46
17	43.1	27.8	10.0	19.1	133	0.30
18	20.0	30.2	32.0	17.8	160	0.40
19	42.6	10.0	27.4	20.0	168	0.43
20	43.1	27.8	10.0	19.1	143	0.35

Table 2: Summary of the lipid composition values for each experimental run and associated responses for hydrodynamic particle size and polydispersity. (n=3)

CONCLUSIONS

- The curvature in the 2D plots demonstrates the complex non-linear effects of lipid composition on the particle size of L-TriAdj.
- With 25% DOPE, a greater proportion of DDAB is associated with lower particle sizes; however, the opposite trend is observed with 25% DSPC. A common region of size optimality was at DDAB 30-40% when DOPE or DSPC were constant at 25%.
- Ultimately, varying the DOPE: PC lipid ratio does not significantly modulate the immunostimulatory properties of L-TriAdj pertussis vaccines.
- Varying the alkyl chain length of PC lipids in L-TriAdj pertussis vaccines induces significant changes in cellular viability and uptake, and expression of pro-inflammatory cytokines.
- Overall, Vaccine Formulation A (DDAB/DOPE/DSPC/Chol (20/20/40/20 mol) was characterized by the most optimal results with respect to cellular viability, cellular uptake and immune cell activation.

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