Microbial Pathogenesis 122 (2018) 122–129

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Efficacy of whole-cell pneumococcal vaccine in mice: A systematic review and meta-analysis

Mona Mohammadzadeh[a], Babak Pourakbari[a], Shima Mahmoudi[a], Abbas Keshtkar[b], Mahdi Habibi-Anbouhi[c], Setareh Mamishi[a,d,*]

[a] Pediatric Infectious Diseases Research Center, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Health Sciences Education Development, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[c] National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran
[d] Department of Pediatric Infectious Disease, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Streptococcus pneumoniae
Vaccine
Efficacy

ABSTRACT

Background: Despite the fact that pneumococcal conjugate vaccines (PCVs) have significantly reduced the rate of invasive pneumococcal diseases through the use of vaccine serotypes, infection with *Streptococcus pneumoniae* remains a major public health hazard. Serotype-independent vaccines that are economically viable species of common protein antigens such as whole-cell vaccines (WCVs) are needed. Considering the ongoing debate about the effectiveness of WCVs, a systematic literature review and meta-analysis was carried out to determine the efficacy of WCVs against colonization in mice.

Material and methods: A systematic review was undertaken of published studies on the protection (colonized/uncolonized) of whole cell pneumococcal vaccine in mice. The search terms used were "whole cell vaccine" and "*Streptococcus pneumoniae*" in PubMed, Google Scholar, Embase, Web of Science and Scopus engines. Data was extracted from original publications and a meta-analysis was performed on studies divided into sub-groups by the number of inoculations, type of sample, type of adjuvant, time of sampling, design of study and quality of study.

Results: Ten eligible articles published from 2000 to 2016 were included in this review. The meta-analysis was performed on eight out of 10 studies and demonstrated that the estimated pooled risk ratios (RRs) for comparison of colonization between the vaccinated and unvaccinated mice for outcomes 1 and 2 were 0.18 and 0.24, respectively. Lower RRs were observed in sub-groups that were inoculated with vaccines three times, those using cholera toxin (CT) adjuvants and those obtained as tracheal specimens from the mice.

Conclusions: The best protocol for use of a WCV is its application with CT adjuvant administered intranasally in three inoculations at doses of 10^8 CFU. Further studies performed under similar conditions to obtain accurate results on the effectiveness of this vaccine are recommended.

1. Introduction

Streptococcus pneumoniae is a gram positive bacteria that causes otitis media, pneumonia, bacteremia and meningitis, particularly in children, the elderly and immunocompromised individuals [1]. Pneumococcal infection causes approximately 826,000 childhood deaths worldwide annually, of which 90% occur in developing countries [2]. *S. pneumoniae* and immunity to this infection have been studied extensively [3]. From 1920 to 1940, investigators at the Rockefeller Institute showed that *S. pneumoniae* expresses a variety of capsular polysaccharides (CPs) that are antigenic [4]. CPs are important virulence factors and are the basis of serotyping. Current vaccines are based on CPs, either alone or conjugated to carrier proteins [5].

Pneumococcal conjugate vaccines (PCVs) in which the CPs antigens are covalently linked to a protein have proven to be highly protective against invasive pneumococcal infection and have dramatically reduced the carriage of the vaccine type organism [6]. Although PCVs have significantly reduced the rate of invasive pneumococcal infection due to vaccine-serotypes, diseases caused by pneumococcus remain a major public health issue [7]. Although PCVs are highly effective in reducing vaccine-type carriage and infection, they have several disadvantages, such as limited coverage against known pneumococcal serotypes,

* Corresponding author. No. 62, Dr. Gharib St., Pediatric Infectious Disease Research Center, Children Medical Center, Hospital, Tehran, Iran.
E-mail addresses: monamohammadzadeh@yahoo.com (M. Mohammadzadeh), pourakbari@yahoo.com (B. Pourakbari), mahmoudi18033@gmail.com (S. Mahmoudi), abkeshtkar@gmail.com (A. Keshtkar), habibi.mha@gmail.com (M. Habibi-Anbouhi), smamishi@sina.tums.ac.ir (S. Mamishi).

https://doi.org/10.1016/j.micpath.2018.06.026
Received 19 November 2017; Received in revised form 14 January 2018; Accepted 13 June 2018
Available online 14 June 2018
0882-4010/

replacement in carriage and disease prevalence by non-vaccine serotypes and the high cost of production [8]. To achieve cost-effectiveness and broad and long-lasting protection, the best choice for vaccines are protein or whole bacterial formulations [9].

Several laboratories have investigated surface proteins common to all serotypes of pneumococcus with the goal of inducing serotype-independent protection and lowering the cost. More than 20 such proteins with a protective effect have been discovered [10]. Malley et al. studied killed unencapsulated bacteria which presented a number of such antigens in native configuration unoccluded by capsules [11]. In this context, researchers at the Boston children's Hospital proposed the use of an inactivated non-encapsulated strain of *S. pneumoniae* as a vaccine against pneumococcus [11]. This vaccine preparation induced both humoral and cellular immune responses against multiple antigens conserved among serotypes and protected mice in various challenge models against colonization, pulmonary pneumonia and sepsis [11–13].

TH17 cells play a vital role in protection against nasal colonization of mice elicited by whole cell pneumococcal vaccine [14]. Protection was observed in mice that were deficient in the production of antibodies [15]. It has been shown that there is a strong negative correlation between IL-17A levels in the blood and pneumococcal colonization density in the nasopharynx of mice vaccinated with whole cell pneumococcal vaccine [14]. Although a number of original articles have been published on protection conferred by whole cell pneumococcal vaccine in mice in recent years, there has been no systematic review or meta-analysis of the data. The present study was undertaken to summarize the results of original articles that have been published concerning protection (colonized/uncolonized) conferred by whole cell pneumococcal vaccine in mice.

Animal experimentation has an important role in research aimed at improving human health [16], but to avoid unnecessary duplication of animal studies, systematic reviews of such studies should be conducted routinely [17]. A systematic review offers new information that is not available by analyzing each study individually [16]. Such reviews can improve the methodological quality of animal experiments, suggest the best choice for an animal model, and examine the effectiveness of the use of the 3 R (replacement, reduction and refinement) principle [18]. A systematic review and meta-analysis of animal studies can provide more accurate results than those from individual animal studies [16]. The aim of this work was to obtain the best choice for type of vaccine inoculation, number of inoculations, type of adjuvant and dose of vaccine.

2. Material and methods

2.1. Literature search

The terms “whole cell vaccine” AND “*Streptococcus pneumoniae*” were searched in PubMed, Google Scholar, Embase, Web of Science and Scopus engines. The search strategies were followed from 1 Jan 2000 to 30 Dec 2016. The journal *Vaccine* (key journal) was searched by hand for this period. The references of the articles were checked for additional articles. The ProQuest database was also searched. The Web of Science and Scopus databases were searched for conference papers. The preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement was implemented in the searches [19].

2.2. Inclusion criteria

Any experimental article that investigated the protection conferred by the whole cell pneumococcal vaccine in an animal model was examined. The term of protection in this study was colonization. The whole cell pneumococcal vaccine is a killed unencapsulated strain of *S. pneumoniae* derived from RX1, in which the *lytA* gene has been deleted and the *ply* gene has been substituted for the *pdT* [11]. Strain RX1 is a capsule-negative mutant derived from *S. pneumoniae* serotype 2 [20]; thus, any original article that used this model of pneumococcal vaccine was incorporated.

2.3. Exclusion criteria

Studies with at least one of the following were excluded:

1) Studies that were not relevant.
2) Articles that were reviews.
3) Articles that did not use animal models.
4) Articles that used a different form of whole cell vaccine.
5) Articles that used animals with deficient in B-cells or T-cells.
6) Articles that measured antibodies only and did not examine protection.
7) Articles that measured survival only and did not examine protection.
8) Articles that used the live or attenuated form of the vaccine.

2.4. Data extraction

For all studies, the following data was extracted: author, year, animal model, type of inoculation, number of inoculations, intervals between inoculation, time of challenge, serotype for challenge, time of sampling, type of sample, adjuvant, dose of vaccine and design of study. The original publications are shown in Table 1. The number of animals that received WCV and did colonize, the number of animals that received WCV and did not colonize, the number of animals that received the adjuvant only and did colonize and the number of animals that received adjuvant only and did not colonize were recorded during the meta-analysis, but are not shown.

Literature identification and data extraction was performed by two researchers independently. Quality assessment of methodological sections and results of the articles included was performed by the use of the ARRIVE checklist [21]. The 3 R principle [22] was considered for all original articles regarding the use of animals in the study.

2.5. Statistical analysis

For meta-analysis, studies were categorized into two groups (outcomes 1 and 2). Outcome 1 comprised studies that utilized the serotype 6 B wild type pneumococci for the mice challenge and examined nasopharyngeal or tracheal specimens of mice using the cholera toxin (CT) adjuvant for immunization. Studies in outcome 2 adhered to the same conditions as in outcome 1, except that they used Al(OH)3 or CT as an adjuvant. Meta-analysis was performed for studies in both outcomes 1 and 2. For each study included, the unadjusted risk ratios (RRs) comparing colonization in vaccinated and unvaccinated mice were calculated at a 95% confidence interval (CI).

Meta-analysis was performed using a fixed-effects model and the combined RR was estimated using the Mantel-Haenszel statistical method. The results of the meta-analysis are shown as forest plot diagrams which represent the estimated RRs and their 95% CIs. An estimate of heterogeneity across studies was assessed using the Cochran's Q test and I^2 statistics; heterogeneity was considered significant at $p < 0.05$. I^2 values of 25%, 50% and 75% were considered to represent low, medium and high heterogeneity, respectively. Subgroup meta-analysis was utilized to compare the RRs of colonization in vaccinated and unvaccinated mice on the basis of the number of inoculations (1, 2 or, 3), type of sample (nasopharyngeal, tracheal), adjuvant (CT, CTB), design (2 groups, > 2 groups), and quality score (< 12, ≥12).

In addition to these subgroups, the time of sampling (7 or 10 days) was also investigated for outcome 2. The Q and I^2 statistics were calculated for each subgroup to determine the factors effecting the RRs of colonization and heterogeneity of the studies. The relationship between time of challenge (time of mice infection after the last immunization) and mice colonization was evaluated using the meta-regression model.

The publication bias was assessed using Egger's and Begg's