Vaccine 33 (2015) 451–458

Contents lists available at ScienceDirect

Vaccine

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

Specific and cross-reactive immune response to oral *Salmonella* Typhi Ty21a and parenteral Vi capsular polysaccharide typhoid vaccines administered concomitantly

Sari H. Pakkanen[a,b], Jussi M. Kantele[c], Laura E. Savolainen[a], Lars Rombo[d,e], Anu Kantele[a,b,f,g,*]

[a] Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, POB 21, 00014 Helsinki, Finland
[b] Department of Medicine, Division of Infectious Diseases, Helsinki University Hospital POB 348, 00029 Helsinki, Finland
[c] Department of Medical Microbiology and Immunology, University of Turku, Kiinamyllynkatu 13, 20520 Turku, Finland
[d] Karolinska Institutet, Department of Medicine/Solna, Unit for Infectious Diseases, Stockholm, Sweden
[e] Centre for Clinical Research, Sörmland County Council, Eskilstuna, Sweden
[f] Department of Medicine, University of Helsinki, POB 20, 00014 Helsinki, Finland
[g] Aava Travel Clinic, Medical Centre Aava, Helsinki, Finland

ARTICLE INFO

Article history:
Received 30 June 2014
Received in revised form 5 November 2014
Accepted 17 November 2014
Available online 26 November 2014

Keywords:
Typhoid fever
Paratyphoid fever
Salmonella gastroenteritis
Salmonella Typhi
S. Paratyphi
Non-typhoid *Salmonella*
Typherix
Typhim Vi
Vivotif
Salmonella Typhi Ty21a
Vi polysaccharide
Antibody-secreting cell
Cross-reactive
ELISPOT
HomingClinicalTrials.gov NCT02121145

ABSTRACT

Background: Since protective efficacy of the current typhoid vaccines—oral whole-cell *Salmonella* Typhi Ty21a and parenteral Vi-capsular polysaccharide preparation—is not optimal, and no vaccines are available against paratyphoid or non-typhoidal *Salmonella* (NTS) serotypes, new approaches deserve to be explored. The immunological mechanisms elicited by the two typhoid vaccines are mainly targeted against different structures. We studied whether these vaccines would enhance *S.* Typhi-specific immune response and cross-reactivity against other *Salmonellae*, if administered concomitantly.

Materials and methods: Volunteers were immunized simultaneously with Ty21a and Vi vaccines (Ty21a + Vi group) or with either of the two singly (Ty21a and Vi groups). All volunteers were investigated for circulating specific and cross-reactive plasmablasts, identified by ELISPOT as IgA, IgG or IgM antibody-secreting cells (ASC) reactive with *S.* Typhi, *S.* Paratyphi A/B/C, or selected NTS serotypes (*S.* Enteritidis, *S.* Typhimurium).

Results: In the Ty21a + Vi group, no specific or cross-reactive plasmablasts were detected before vaccination. After vaccination, the number of *S.* Typhi-specific plasmablasts (878 ASC/10^6 PBMC, 95%CI 554–1201) proved higher than in the Ty21a (339 ASC/10^6 PBMC; $p < 0.001$) and Vi (149 ASC/10^6 PBMC; $p < 0.001$) groups. Likewise, cross-reactive responses in the Ty21a + Vi group were higher than in the Ty21a and Vi groups (Ty21a + Vi vs Ty21a: ASC against *S.* Paratyphi A/B, *S.* Enteritidis and *S.* Typhimurium $p < 0.05$, against *S.* Paratyphi C $p < 0.01$; Ty21a + Vi vs Vi: against *S.* Paratyphi C not significant, others $p < 0.0001$). A gut-directed homing profile was seen among O antigen-specific and a systemic one among Vi antigen-specific plasmablasts.

Conclusions: Concomitant administration of Ty21a and Vi vaccines is well tolerated and induces an additive immune response to the two vaccines. Thus it enhances the magnitude of both typhoid-specific plasmablast responses and those cross-reacting with paratyphoid and most important NTS serotypes. The data encourage concomitant use of Ty21 and Vi vaccines for those at risk.

Abbreviations: ASC, antibody-secreting cell; CLA, cutaneous lymphocyte antigen; HR, homing receptor; iNTS, invasive non-typhoid *Salmonella*; NTS, non-typhoid *Salmonella*; PBMC, peripheral blood mononuclear cell; PBS, phosphate-buffered saline.

* Corresponding author at: Division of Infectious Diseases, Department of Medicine, Helsinki University Central Hospital, POB 348, FIN-00029 HUS, Helsinki, Finland. Tel.: +358 50 309 7640; fax: +358 9 471 75900.
E-mail address: anu.kantele@hus.fi (A. Kantele).

http://dx.doi.org/10.1016/j.vaccine.2014.11.030

1. Introduction

Diseases caused by *Salmonella* species constitute a serious health problem worldwide. *Salmonella enterica* subspecies *enterica* serotype Typhi (*S.* Typhi) causes typhoid fever, while *S. enterica* subspecies *enterica* serotype Paratyphi A/B/C (*S.* Paratyphi A/B/C) are the etiological agents of paratyphoid fever. Together known as enteric fever, typhoid and paratyphoid mostly prevail in developing countries, where they pose a health risk also to visitors. Non-typhoidal *Salmonellae* (NTS) cause food-borne gastroenteritis and invasive non-typhoidal salmonellosis (iNTS); they are encountered both in the developing and developed world. Globally, the annual incidence of typhoid fever is estimated at 22 million [1], paratyphoid fever at 5.4 million [1], and NTS diseases at 94 million cases [2]. Because of increasing antimicrobial resistance, the need for preventive measures such as vaccination has become more and more urgent.

Currently, two types of vaccines—oral live attenuated *Salmonella* Typhi Ty21a (Vivotif®) and the parenteral capsular Vi polysaccharide preparations (Typherix® or Typhim Vi®)—are available against typhoid fever, while none are licensed against paratyphoid or NTS serotypes. Interestingly, Ty21a and, to a lesser extent, the Vi vaccine have been shown to elicit cross-reactive immune responses against paratyphoid serotypes [3–9] and the most common NTS *Salmonellae* [10,11]. The main underlying cause of cross-reactivity are the O-antigenic structures these strains share with *S.* Typhi: while *S.* Typhi expresses O-9,12, *S.* Paratyphi A and B carry the O-12, and many NTS *Salmonellae* express either O-9,12 (e.g. *S.* Enteritidis) or O-12 (e.g. *S.* Typhimurium). Specific and cross-reactive humoural immune responses can be explored at single-cell level with the help of plasmablasts appearing transiently in the circulation after vaccination [5,10,16–18]. Indeed, plasmablasts are of special interest when studying typhoid vaccines: they have been suggested as surrogate markers of protection against the disease [19]. Plasmablasts represent recently activated B cells trafficking, via lymphatics and blood, from the site of antigen encounter to their final effector sites [20]. Before homing to tissues, these cells can be caught from the circulation for a period of approximately one week, their magnitude peaking on day 7 [16,17]. The homing process is known to be tissue-specific: sc. homing receptors (HR) and chemokine receptors (CCR) on lymphocyte surface recognize their ligands in target tissues [20]. Analysis of HR and CCR on circulating plasmablasts can be used to explore the expected localization of the immune response [21–25]. The cells are guided by the HR $\alpha_4\beta_7$-integrin to the intestine [26], L-selectin mainly to systemic sites [27], and cutaneous lymphocyte antigen (CLA) to the skin [28].

Even if the two typhoid vaccines, Ty21a and Vi, have in field trials exhibited a similar protection rate of 60–70% against typhoid fever [29,30], their underlying protective mechanisms differ. Ty21a is a live Vi-negative whole-cell vaccine shown both to elicit humoral [19] and cell-mediated immune responses [19], while the Vi preparation consists of purified capsular Vi polysaccharide eliciting a humoural response mainly to the Vi antigen [18,19]. We hypothesized that, given at the same time, the benefits of both vaccines could be exploited. This is the first study to explore immune response to the two vaccines administered concomitantly.

2. Materials and methods

2.1. Study design

Three groups were immunized: one receiving both Ty21a and Vi vaccines simultaneously (Ty21a + Vi group), the other two either Ty21a (Ty21a group) or Vi vaccine (Vi group). Circulating specific and cross-reactive plasmablasts were identified by enzyme-linked immunospot assay (ELISPOT) as IgA, IgG and IgM antibody-secreting cells (ASC) with given specificity. The plasmablasts' homing potentials were characterized by combining immunomagnetic cell sorting with the ELISPOT. Results of the Ty21a and Vi groups have been reported earlier [9,11,18].

The study protocol was approved by the ethics committee of the Helsinki University Central Hospital and the Finnish Medicines Agency (EudraCT 2009-012949-33), and registered in the databases required (ClinicalTrials.gov NCT02121145). Written informed consent was obtained from all subjects. The investigation was conducted at the Travel clinic of the Medical Centre Aava, Helsinki University Central Hospital and the Haartman Institute, University of Helsinki.

2.2. Volunteers, vaccinations and samples

The Ty21a and Vi groups, each comprised 25 vaccinees, 17 females and 8 males, aged 22–62, mean age 32, as presented earlier [9,11,18]. The Ty21a + Vi group included twenty-four Finnish-born volunteers (11 females, 13 males, aged 22–29, mean 25 years) with no history of enteric fever or typhoid vaccination. They received both the oral *Salmonella* Typhi Ty21a vaccine (Vivotif®, Crucell AB, Leiden, The Netherlands, lot 3000620) and the parenteral Vi capsular polysaccharide vaccine (Typherix®, GlaxoSmithKline Biologicals s.a., Rixensart, Belgium, lot ATYPB096AF with endotoxin contents of 13.30EU) at the same time. The oral vaccine, containing at least 2×10^9 live bacteria/capsule, was administered as one capsule on days 0, 2, and 4, and the Vi vaccine as one 0.5 ml dose intramuscularly (25-mm needle) on day 0. Blood samples were drawn on days 0 and 7. The Ty21a and Vi groups were immunized 2010–2011 and the Ty21a + Vi group 2013. The ELISPOT assay was validated before the study, and proved to be highly repetitive (data not shown).

Combined use of the two vaccines was well tolerated: 7/24 vaccinees reported mild adverse effects (loose stools 2, one each: stomachache, nausea, flatulence, dizziness after injection, aphtha in the mouth) resembling those seen in the Ty21a (stomachache 1, tiredness 1) and Vi (one each: fever, loose stools, pain at the injection site, constipation) group.

2.3. Isolation of peripheral blood mononuclear cells (PBMC)

PBMC were separated using Ficoll-paque centrifugation of heparinized venous blood, as described before [16].

2.4. Separation of HR-negative and -positive cell populations

Because of limited numbers of PBMC, HR analyses could not be carried out for all volunteers. Four in the Ty21a + Vi group were subjected to an analysis of HR expressions on O-9,12-specific ASC and one volunteer to HR expressions on Vi antigen-specific ASC as described earlier [10,18,22]. Briefly, PBMCs (3.4×10^6 PBMC per HR) were incubated with monoclonal antibodies against $\alpha_4\beta_7$ (ACT-1, Millennium Pharmaceuticals, Cambridge, MA), L-selectin (Leu 8, Becton Dickinson, Erenbodegem-Aalst, Belgium), or CLA (HECA-452, a gift from Dr. Sirpa Jalkanen, Finland). Next, the cells were incubated with Dynal® M-450 magnetic beads coated with sheep anti-mouse IgG (Dynabeads, Dynal Biotech, Oslo), followed by magnetic separation and ELISPOT assay.

2.5. ELISPOT assay for specific and cross-reactive ASC

PBMCs and, for HR analyses, the receptor-positive and -negative cell populations, were assayed for ASC with ELISPOT as described earlier [10,16]. In brief, 96-well microtitre plates (Maxisorp, Nunc, Roskilde, Denmark) were coated with whole-cell bacteria (Table 1)