Vaccine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Original article: Varicella vaccination elicits a humoral and cellular response in children with rheumatic diseases using immune suppressive treatment

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ARTICLE INFO

Article history:
Received 22 November 2016
Received in revised form 22 March 2017
Accepted 6 April 2017
Available online xxxx

Keywords:
Juvenile idiopathic arthritis
Juvenile systemic scleroderma
Juvenile dermatomyositis
Live-attenuated vaccination
Varicella
Immunogenicity

ABSTRACT

Objective: To assess humoral and cellular responses to live-attenuated varicella zoster virus (VZV) vaccination of patients with juvenile idiopathic arthritis (JIA), juvenile dermatomyositis (JDM) or juvenile scleroderma (JScle) compared to those of healthy controls (HC).
Methods: Before, 4–6 weeks and one year after VZV vaccination, blood samples of patients and HC were collected. VZV-specific antibody concentrations were measured by ELISA and multiplex immune-assay. IFN-γ ELISpot assays were performed to assess VZV-specific T-cell responses. Cytokine production upon VZV stimulation were measured with a Luminex-assay.
Results: 49 patients (39 JIA, 5 JDM, 5 JScle) and 18 HC were included. All patients used methotrexate (MTX), 16 also used corticosteroids, 3 patients used biologics. No disease flares were reported after vaccination. Antibody response to the vaccine was similar in patients and controls ($p = 0.139$). Use of immunosuppressive drugs did not affect the response ($p = 0.203$). A second vaccination ($n = 21$) increased VZV-specific antibody concentrations ($p = 0.02$). VZV-specific T-cells increased after vaccination ($p = 0.043$), with a cytokine profile suggesting a VZV-specific Th1 and cytotoxic T-cell response.
Conclusion: The humoral response to VZV vaccination in patients with pediatric rheumatic diseases (PRD) is similar to that of HC. Generally, patients are able to mount a VZV-specific cellular response.
This study has been registered in the Brazilian Clinical Trials Registry under number U1111-1189-9837.

1. Introduction

Immunocompromised patients have an increased risk of infections, which have a complicated course more often. Vaccinations can prevent these infections. However, both disease and treatment can affect vaccine response, resulting in reduced or absent protection [1,2].

Live-attenuated vaccines in immunocompromised patients pose a specific challenge in this population, due to a hypothetical risk of infections with the live-attenuated pathogen. By comparing vaccine responses of patients to those of healthy controls (HC), the benefits of vaccination can be assessed.

In 2014, we performed a randomized controlled trial of the immunogenicity of the live-attenuated measles, mumps and rubella (MMR) booster vaccine in 68 patients with Juvenile Idiopathic Arthritis (JIA). None of the patients had infections with the live-attenuated pathogens after vaccination. Twelve months after administration of the booster, all patients were protected against MMR [3].

Another possibly beneficial live-attenuated vaccine for patients with pediatric rheumatic diseases (PRD) like JIA is the varicella zoster virus (VZV) vaccine, to prevent chickenpox and herpes zoster (HZ). The incidence of HZ in patients with rheumatic diseases is higher than in the healthy population, and often has a complicated course [4,5]. In 2010, Pileggi et al. showed the VZV vaccine to be

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http://dx.doi.org/10.1016/j.vaccine.2017.04.015
0264-410X/

safe in 25 patients with PRD using methotrexate (MTX) as monotherapy or combined with corticosteroids (CS) [6].

Now, we focus on the immunogenicity of the VZV vaccine in HC and PRD patients using immunosuppressive drugs. Adequate cellular immunity is specifically important for effective protection against VZV [7]. Therefore, both humoral and cellular responses were investigated.

2. Patients and methods

Recruitment of patients and HC was performed as described by Pileggi et al. [6]. In summary, patients diagnosed with PRD and HC between the age of 1–20 years and no history of chickenpox were eligible to participate. All participants received at least a single dose (0.5 ml) of live-attenuated VZV vaccine containing >1000 plaque-forming units (PFU) of VZV (Oka strain). Patients who were included after 2008 received a second vaccine during clinical follow-up, within four months of the first vaccine due to adjusted regulation [8]. No HC were included after 2008, resulting in all HC receiving only one dose. Patients who received one dose were vaccinated with BIKEN Oka strain varicella vaccine. Patients who received two doses received Varilrix® $10^{3.3}$ PFU/0.5 ml as the second dose. Vaccines were stored according to the manufacturer's instructions. All patients were vaccinated subcutaneous, in their right arm. No other vaccines were administered during the study.

Serum samples and PBMCs were obtained immediately before, between 4 to 6 weeks and one year after (first) vaccination. Samples were frozen and stored in Brazil until analysis in the Netherlands. Vaccine adverse events, drug use and disease activity parameters (physician's global assessment (PGA) and parents' visual analog scale (VAS); JADAS-71 for JIA patients) were assessed on the day of the first vaccination and between 4 to 6 weeks after vaccination.

Serological testing: Serological testing for VZV-specific antibody concentrations was performed with a commercial enzyme-linked immunosorbent assay (ELISA) as described previously [6] and with a standardized fluorescent bead-based multiplex immunoassay (MIA, Luminex technology), performed at the National Institute of Public Health and the Environment (Bilthoven, the Netherlands) [9].

IFN-γ ELISpot: Multiscreen HTS plates (Millipore) were coated overnight with IFN-γ monoclonal capture antibody (mAb 1-D1 K, Mabtech), then blocked with FCS. $2x10^5$ PBMC/well were added, and were cultured for 24 h in culture medium (RPMI 1640 supplemented with 1% Penicillin/Streptavidin, 1% L-Glutamine and 10% FCS, all Gibco) together with either Gamma-irradiated (1×10^5 Gy) VZV vaccine (Sanofi Pasteur MSD) containing 2.5×10^3 PFU/ml, anti-CD3 or medium alone. After 24 h incubation, supernatant was stored for cytokine analysis. Biotinylated IFN-γ monoclonal detection antibody (7-B6-1-biotin, Mabtech) was added to the wells. Streptavidin-horseradish peroxidase (Mabtech) was subsequently added. Spots were developed by adding 3,3′,5,5′-Tetra methylbenzidin/2.4 mM hydrogen peroxide (Sanquin) and counted using the ELIScan plate reader system (Automated ELISA-Spot Assay Video Analysis Systems). Only samples with enough cells of acceptable viability were used for ELISpots. Of note, due to technical limitations the paired samples we were able to use were all from patients who showed an inadequate humoral vaccine response.

Cytokines: A panel of cytokines covering Th1, Th2, Th17, Treg and cytotoxic T-cell responses (IL-10, IL-12, IL-13, IL-17, TNFα, IP-10, Granzyme B) was measured in the supernatant of VZV-stimulated cells with a standardized Luminex assay [10].

FACS-analyses: Changes in T-cell and B-cell populations and T-cell and B-cell interaction markers before and after vaccination were assessed using flow cytometry. Cells were labelled for CD3, CD10, CD19, CD21, CD27, IgD and IgM to assess B-cell maturation and for CD3, CD4, CD8, CD45RA, CD45RO, CD25, FoxP3 and CD152 (CTLA4) to assess T-cell subsets and T-cell memory.

Statistics: Effects of disease type and medication use on antibody concentrations were compared using the Kruskall-Wallis (KW) test. Appropriate non-parametric tests (Mann-Whitney U for unpaired data, Wilcoxon signed-rank for paired data, Spearman's correlation to assess associations between two continuous variables) were used to investigate the effects of vaccination. All tests were performed with IBM SPSS statistics v21.

3. Results

3.1. Patient characteristics

Forty-nine patients and 18 HC were included (Table 1). VZV vaccine safety and humoral responses of 25 of these patients have

Table 1
Cohort characteristics.

	Patients who received 1 vaccine (n = 28)		Patients who received 2 vaccines (n = 21)		Healthy controls (n = 18)
% Female	50%		57%		50%
Age at vaccination *(median, range)*	5 (2–15)		3.5 (2–17)		8.5 (3–18)
Disease type					N/A
Systemic JIA	6 (21%)		4 (19%)		
Oligo JIA	3 (11%)		4 (19%)		
Poly JIA	14 (50%)		8 (38%)		
JScl	3 (11%)		2 (10%)		
JDM	2 (7%)		3 (14%)		
Medication use					N/A
MTX monotherapy	14 (50%)		11 (52%)		
MTX dose (mg/m^2/week, median, range)	18 (10–27)		15 (14–20)		
MTX + CS	8 (29%)		8 (38%)		
CS dose (mg/day, median, range)[b]	7.5 (5–20)		5 (3–10)		
MTX + Other IS (±CS)	5 (18%)		0 (0%)		
MTX + Biologic[a] (±CS)	1 (4%)		2 (10%)		
JADAS-71 (median, range; JIA only)	Pre	Post	Pre	Post	N/A
	5 (0–19)	2 (0–12)	7.5 (0–18)	5 (0–18)	
% Responder	57%		After 1 vaccine 30%	After 2 vaccines 95%	67%

JIA: Juvenile Idiopathic Arthritis, JScle: Juvenile Scleroderma, JDM: Juvenile Dermatomyositis, MTX: metothrexate, dose in mg/m^2/week, CS: corticosteroids, dose in mg/day, IS: immunosuppressive drug (2 ciclosporine, 2 leflunomide, 1 azathioprine, 1 penicillamine), JADAS: Juvenile Arthritis Disease Activity Score. Patients were defined as responders when their antibody concentration went from <50 mIU/mL to 100> mIU/mL (6).

[a] The patient who received one vaccine used adalimumab, the patients who received two vaccines used etanercept or abatacept.

[b] Information regarding the duration of CS use, and CS dose per kg was not available for this study.

Please cite this article in press as: Groot N et al. Original article: Varicella vaccination elicits a humoral and cellular response in children with rheumatic diseases using immune suppressive treatment. Vaccine (2017), http://dx.doi.org/10.1016/j.vaccine.2017.04.015