Peer reviewed article

Clinical and immune response to undiluted and diluted smallpox vaccine

A prospective randomised, triple-blinded clinical trial, Switzerland

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Summary

Question under study: To assess clinical reactions, immune responses and adverse events to undiluted, three- and sixfold diluted Lister strain vaccine stockpiled in Switzerland.

Methods: A prospective, triple-blinded, randomised, parallel group clinical trial was performed.

Results: From 2001 to 2007 104 persons with an indication for vaccinia vaccination were recruited. They had a median age of 33 years (range 18–65), 56 (53.8%) were re-vaccinees and 48 (46.2%) primary vaccinees. There was no statistically significant variation in the proportion of re-vaccinees between diluted and undiluted vaccine groups (75% *vs* 51%, p = 0.118). With an overall clinical take rate (major reaction) of 97.1% the majority of the vaccinia-naïve participants exhibited an at least fourfold increase of neutralising antibody titres (32/38, 84.2%) post-vaccination. Interestingly this proportion was lower among re-vaccinees (29/46, 63.0%, p = 0.048). No significant difference was observed in the take rate or at least fourfold seroconversion rate between the threefold and sixfold diluted vaccine doses. Adverse events were reported by 98 (94.2%) participants, not accounting for itching at the vaccination site.

Conclusion: Subjects requiring immunisation were successfully (re-) vaccinated with undiluted as well as with three- or sixfold diluted vaccinia vaccine. Our findings complement previous studies with respect to the clinical take rate and immune response. The rate of adverse events was substantial but not unexpected and no severe adverse events occurred. In conclusion, the existing smallpox vaccine stockpile might be expanded by administering three- or sixfold diluted vaccine doses combined with a careful pre-vaccination screening and extensive instructions to vaccinees.

Key words: smallpox vaccine; vaccinia; take rate; neutralizing antibodies; adverse events

Competing interests:
R Steffen has accepted a fee for speaking, organising, and chairing education, consulting, and/or serving on advisory boards for and also reimbursement for attending meetings and funds for research from Berna Biotech, GlaxoSmithKline, Novartis Vaccine, Roche, Salix Pharmaceuticals, Sanofi Pasteur and SBL Vaccine.
R. Gassmann, M. Mutsch, O. Engler, M. Alex, C.P. Czerny: nothing to declare.

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Introduction

In Switzerland widespread vaccination against smallpox ceased in 1974 with few differences between cantons. Vaccination was maintained for risk groups only, especially for travellers. The last naturally infected case was registered 1977 in Somalia. In May 1980, WHO declared that a world free of smallpox illustrated the success of a comprehensive global vaccination strategy [1].

In the past few years, smallpox has been rated among the top four microbial agents that are "likely to be used" in a bioterrorist attack [2–4]. Although no longer licensed in Switzerland, smallpox vaccine consisting of live vaccinia virus strains offers cross-protection against variola viruses and monkeypox virus infections.

Smallpox vaccine provides a unique opportunity for investigating immune response characteristics after primary or re-vaccination decades apart, as well as for studying the duration of detectable immune responses in the absence of exposure history to variola virus [5]. According to previous studies mainly performed with the U.S. Dryvax vaccine, a long-lasting humoral and cell-mediated immune memory to vaccinia exists in previously vaccinated individuals. Further it was shown, that undiluted as well as diluted vaccine formulations would lead to successful clinical and

immune responses to vaccination [6–10]. However, up to now there have only been three studies providing data on the Lister strain vaccinia vaccine stored in Switzerland [11–13].

Our aims were:

– to assess descriptively clinical and immune responses and adverse events to use of the smallpox vaccine stockpiled in Switzerland.
– to evaluate clinical, epidemiological and adverse event parameters for three- and sixfold diluted smallpox vaccine doses. The rationale behind this approach is the fact that the Swiss supply of three million vaccine doses may need to be stretched in case of an emergency to make it available for all ~7.2 million residents.

Methods

Vaccine and administration

The vaccine and diluent were obtained from the Swiss Armed Forces Pharmacy, Ittigen, Switzerland. The vaccine was produced in 1982 or earlier and consists of the vaccinia virus Lister strain, Lister Institute, Elstree, UK. Having been kept lyophilised at -20 °C the vaccine was tested to assess its potency and stability following more than two decades [2]. The reconstituted undiluted vaccine lots contained a measured vaccinia virus titre of $>10^8$ plaque forming units (pfu)/ml in a phosphate-buffered, glycerol-containing solution. For preparing the threefold and sixfold dilutions at the vaccination centre the same diluent was used.

The vaccine was administered with a bifurcated needle using the 15-puncture technique [14] in the deltoid region of the upper arm or in the inguinal region considering participants' preference. Subsequently, the vaccination site was covered with a semi-occlusive bandage (Tegaderm, 3M Health Care, St. Paul, MN, USA) [15]. Detailed instruction was given on how to care for the vaccination site to prevent spread of the virus either within the vaccinees or to a close contact by inadvertent inoculation. Bandage material was provided until resolution of the local reaction. For questions regarding adverse events, health status or contact transmission a professional hotline was available 24/7.

Study design and subjects

A prospective triple-blind, randomised, parallel-group clinical trial was conducted at the Vaccination Centre of the University of Zurich, Zurich, Switzerland. Healthy subjects, aged 18–65 years, were eligible in Switzerland if they reported an occupational exposure risk, such as laboratory workers working with non-highly attenuated vaccinia viruses, military personnel or humanitarian personnel designated to serve in the Iraq crisis according to expert advice. To ensure practicability and to set higher power at the diluted vaccines batches of 1:1, 1:3 and 1:6 dilutions were randomly assigned to the vaccination dates in a ratio of 1:4:2. Simple randomisation was used to allocate the vaccination dates to the vaccine dilutions with a computer generated randomisation list. The allocation sequence was concealed by a pharmacist as a "third party". Study subjects, study staff administering the vaccine as well as the laboratory personnel were unaware of the vaccine dose assigned.

Participants were invited to sign an informed consent and they were carefully screened for the presence of the following exclusion criteria: smallpox vaccination in the past three years, acute febrile illness (>38.0 °C axillary temperature), history of atopic dermatitis, eczema or an acute exfoliative or chronic skin condition, history of seizure disorders or malignancy, pregnancy or breast feeding and altered immunocompetence among both vaccinees or their close contacts. History of heart disease or at least three risk factors, known allergy to vaccine components and administration of any live vaccines in the past 30 days were assessed for study subjects only [16]. By using questionnaires baseline demographic characteristics and the medical history were assessed while general and vaccinia vaccination history were based on vaccination certificates or on the presence of a vaccination scar (vaccinia).

Vesicle formation, health status and adverse events were monitored immediately (15 min post-vaccination) and on days 7, 14 and 28 post-vaccination by follow-up visits or by questionnaires. In case of systemic adverse events follow-ups were done until *restitutio ad integrum*. Serum samples were collected at baseline (day 0) and 24 to 35 days post-vaccination (day 28) to determine antibody-based seroconversion rate. Due to time constraints no preimmunisation serum samples were collected from the subjects receiving the undiluted vaccine.

Approval was given by the Ethical Committee of the Canton of Zurich and the study was registered at Swissmedic, Berne, Switzerland.

Clinical assessment

Primary endpoint was the clinical response rate according to the WHO criteria [14]. A major reaction was defined as a vesicular or pustular lesion or an area of definite palpable induration or congestion surrounding a central lesion that might be a crust or an ulcer. Vaccine "take" corresponded to the presence of a major reaction examined 6–8 days after vaccination. An overall take rate ≥90% was considered to be sufficient. Local or systemic adverse events and serological testing were recorded as secondary endpoints.

Laboratory assessment

By the previously described plaque reduction assay titres of vaccinia-specific neutralising antibodies were measured in pre- and post-vaccination sera [17, 18]. In short, two-fold serial dilutions of pre- and postimmunisation serum samples with 50 pfu of vaccinia Lister were incubated for two days and plaque formation analysed following staining with crystal violet solution. Antibody titre was determined as the dilution step causing a 50% vaccinia plaque reduction compared to the number of plaques obtained with a negative serum.

Statistical methods

Statistical analyses were done with Stata version 8.2. Two-sided Fisher's exact test (for binary outcomes) or Wilcoxon rank sum tests (for continuous outcomes) were used for pair-wise comparisons of differences between groups. The level of statistical significance was set at $p < 0.05$ and all tests were two-sided. Overall effects were examined by multivariate logistic regression analyses.

Results

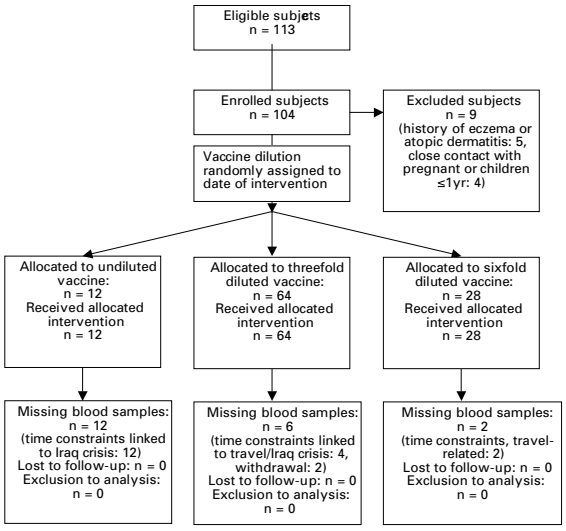
Study population

Between the end of 2001 and 2007, a total of 113 subjects presented themselves; 104 were recruited and 9 exhibited exclusion criteria (fig. 1). There was an average of 6.5 vaccinees on the 16 vaccination dates (range: 3–13). Of those, 56 (53.8%) were re-vaccinees (vaccinia non-naïve, last vaccination 6–55 years ago with a median of 33.7 years) and 48 (46.2%) primary vaccinees (vaccinia-naïve) with 102 (98.1%) being Western Europeans, one participant originating from China and another one from New Zealand.

Among the primary vaccination cohort the median age was 28 years (range 18–51 yrs) and there were 23 (47.9%) females. The demographic structure significantly differed with respect to age and gender in the re-vaccinated cohort with a median age of 45 years (range 28–65 yrs) and with 9 (16.1%) females (p = 0.001). Regarding the demographic characteristics no statistically significant differences between the dilution groups were observed (table 1).

Figure 1

Recruitment scheme.



Clinical reaction

All but three subjects showed a major reaction at the vaccination site (101, 97.1%) on day 7. There was no evidence for a vaccination failure for these re-vaccinated participants (one vaccinated once, 15 years ago, the two others were vaccinated twice each, 51 and 30 and 47 and 38 years ago, respectively). They showed pre-existing neutralising antibodies and a twofold titre increase post-vaccination. In addition, no other characteristics distinguished these three re-vaccinees from the cohort. Overall, there was no statistical difference in the take rate between the groups receiving different vaccine dilutions (table 2).

The average size of the vaccination lesion peaked in the second week following smallpox vaccination. Most of the primary vaccinees described vaccination site progressing from vesicle to pustule and scab formation. Probably due to the semi-occlusive dressing, drying was delayed for a couple of days until formation of a scab was observed. In addition, redness due to the adhesive of the bandage was occasionally reported. Previously vaccinated subjects showed on average not only a smaller pustule but also a more rapid resolution of the vaccination site than the primary vaccinees. All vaccination lesions had resolved by days 28 to 35.

Antibody response to vaccinia vaccination:

The majority of the vaccinia-naïve participants exhibited an at least fourfold titre increase (32/38, 84.2%) whereas this proportion was lower among re-vaccinees (29/46, 63.0%, p = 0.048). The average titre of neutralising antibodies post-vaccination was similar among primary and re-vaccinated individuals and significantly higher than before vaccination. No significant difference in the seroconversion rate between the threefold and sixfold diluted vaccine dose was observed (p = 0.447) (table 2). Six primary vaccinees and 17 pre-

Table 1

Demographic and medical characteristics of the study participants (n = 104).

Factor	Total	Dilution 1:1	1:3	1:6	p-value[1]
	n = 104 (%)	n = 12 (%)	n = 64 (%)	n = 28 (%)	
Gender: male	72 (69.2)	7 (58.3)	47 (73.4)	18 (64.3)	0.457
Age (years): median (range)	33.0 (18–65)	42.2 (30–64)	39.5 (24–65)	33.1 (18–64)	0.001
Origin					
European	102 (98.1)	12 (100.0)	63 (98.4)	27 (96.4)	0.518
Actual / chronic disease	8 (7.7)	3 (25.0)[2]	3 (4.7)[3]	2 (7.1)[3]	
Medication at enrolment	6 (5.8)	1	3 (4.7)	2 (7.1)	
Topical (skin)	2	1[4]	1[5]	0	
Oral	4	0	2[6]	2[7]	

[1] p-values for the comparison of 1:3 and 1:6 dilutions by use of Fisher's exact test
[2] arm fracture, hay fever, acne
[3] hypertension, prostate hyperplasia, hypothyroidism, hypertension, shingles
[4] acne lotion
[5] antiviral lotion
[6] antihypertensive medication, αι-inhibitor
[7] antihypertensive and anti-hypothyroidism-related medication

Table 2

Clinical reaction and antibody response after vaccination with undiluted or diluted vaccinia vaccine Lister strain (n = 104).

Vaccine	Major reaction (take; %)	Vesicle Mean diameter (mm)	At least 4fold increase of antibody titre (%)[1]	Titre pre-vaccination Mean±SD[2]	Titre post-vaccination Mean±SD[2]	p-value[3]
Total (n = 104)	101 (97.1)	9.4±4.2	61/84 (72.6)	0.98±0.98	4.39±2.14	0.000
Undiluted (n = 12)	12 (100.0)	9.7±1.5	n/a	n/a	n/a	
1:3 diluted (n = 64)	62 (96.9)	9.7±4.7	43/58 (74.1)	0.97±0.97	4.18±2.11	0.000
1:6 diluted (n = 28)	27 (96.4)	8.6±2.8	18/26 (69.2)	1.02±1.04	4.85±2.18	0.000

[1] The total per group is below n = 64 and n = 28, respectively due to missing blood samples as depicted in figure 1
[2] data presented as $\log_2$ mean titre. Samples with no detectable activity at 1:2 dilution are assigned to a value of 0.5 $\log_2$
[3] comparison of pre-/post-vaccination titres by Wilcoxon sign rank test
n/a: data not available

Table 3

Frequency of local and systemic adverse events following smallpox vaccination (n = 104).

Symptom	Dilution n (%)				
	Total (n = 104)	1:1 (n = 12)	1:3 (n = 64)	1:6 (n = 28)	p-value
Any symptom excluding itch	98 (94.2)	12 (100.0)	61 (95.3)	25 (89.3)	0.436
No. of symptoms reported					
0	2 (1.9)	0 (0.0)	1 (1.6)	1 (3.6)	
1	33 (31.7)	7 (58.3)	14 (21.9)	12 (42.9)	
>1	69 (66.4)	5 (41.7)	49 (76.5)	15 (53.5)	
Any local symptom	95 (91.3)	12 (100.0)	59 (92.2)	24 (85.7)	0.547
Itching	50 (48.1)	2 (16.7)	34 (53.1)	14 (50.0)	0.065
Lymphadenopathy	95 (91.3)	12 (100.0)	59 (92.2)	24 (85.7)	0.547
Any systemic symptom	42 (40.4)	1 (8.3)	29 (45.3)	12 (42.8)	0.051
Gastrointestinal	3 (2.9)	1 (8.3)	1 (1.6)	1 (3.6)	
Fever	21 (20.2)	1 (8.3)	14 (21.9)	6 (21.4)	0.627
Flu-like symptoms	29 (27.9)	1 (8.3)	20 (31.2)	8 (28.6)	

viously vaccinated subjects failed to show a fourfold increase in antibody titres but for 20 (87%) of them a major reaction was observed. In addition, baseline neutralising antibodies were more frequently detected among previously vaccinated individuals than among primary vaccinees (16/46, 34.8% *vs* 5/38, 13.1%; p = 0.022). The increases in neutralising antibody titres were correlated neither to the size of the cutaneous reaction nor to the vaccine dilution administered nor to the rate of adverse events reported.

Adverse events following vaccinia vaccination:

A total of 98 (94.2%) participants reported any adverse events not considering itching (table 3). Most of them were confined to anticipated, more local reactions including lymphadenopathy. Itching was more prominent among subjects receiving diluted vaccine doses. Notably, systemic adverse events occurred in 40.4% (42/104) mostly referring to flu-like symptoms such as headache, fever and muscle pain. Interestingly, there was no significant difference among vaccinia-naïve and non-naïve vaccinees neither in the distribution of reported side effects nor with respect to the number of adverse events recorded. Although, a tendency was observed towards less frequent local adverse events among vaccinia-experienced subjects compared to primary vaccinees. These differences were mainly restricted to local complaints of pain and tenderness at the vaccination site. No significant difference of adverse events was observed when considering three- or sixfold vaccine dilution.

Five participants consulted a physician, all these primary vaccinees suffered from systemic symptoms such as headache, chills or fever. Four of them were diagnosed with a robust take according to the definition for suspected cases [19] and one with a suspected superinfection at the injection site following smallpox vaccination [19]. Intake of medication was reported by eight vaccinees with five individuals having taken analgesics and three having declared antibiotic treatment for potential superinfection. One episode of vaccination-related transient arthritis resolved completely following a short-term high-dose corticosteroid therapy.

Multivariate logistic models were constructed for all groups of adverse events because gender, age and history of smallpox vaccination were strongly interrelated due to the previous national vaccination recommendations. None of these variables were significantly related to the presence of any local or systemic adverse events as was already shown in the bivariate analysis.

Discussion

Most importantly, our results suggest that the current stocks of vaccinia virus Lister strain can be diluted at least sixfold and still induce a major cutaneous reaction in more than 95% of the vaccinees. This finding is generally in accordance with recent studies from Israel, Taiwan, and Korea [11–13] which report take rates of less than 100% for a tenfold dilution of the Lister strain vaccine corresponding to an estimated virus titre of at least $10^{6.6}$ pfu/ml. Similar success rates were also described for the New York City Board of Health strain as well as in studies performed decades ago [6–10, 20–21]. As a limitation of this study and due to the small dilution group sizes a difference could not be established in a superiority trial. From a practical point of view, the high take rates among all vaccinees are essential.

There was no clear correlation between antibody responses and cutaneous reaction at the vaccination site. Whereas the clinical take rate as a marker of protection following smallpox vaccination has been validated throughout its history, immunological correlates of protection are not exactly defined but are considered to involve a combination of humoral and cell-mediated responses [5, 9]. Of note, also individuals vaccinated more than 25 years ago with low pre-existing antibody titres revealed a clinical take rate exceeding 90% which demonstrates potency of the diluted vaccine in this group. It is controversial whether this detectable but declined humoral immunity would protect against smallpox or would reduce disease severity [22]. Therefore, the characteristics of the population unresponsive to the vaccine need further exploration. In this context, emphasis has to be placed on instructing the vaccination technique carefully. Actually, there exist no similar, routinely applied immunisation procedures and therefore, training and practical experience are essential.

Adverse events

The reported rate of local and systemic adverse events is high when compared to previous studies [6-9, 11, 12, 23–25] but not unusual [10, 13]. However, the local reactions at the vaccination site were anticipated and their duration was limited. Due to the small sample size, the high proportion of previously vaccinated subjects and the under-representation of younger people comparisons between different vaccine dilution groups are limited. Especially, we fail to demonstrate that less severe or a lower rate of systemic adverse events are attributed to the diluted vaccine doses. However, it seems plausible and was shown several times, that vaccine dilution as well as vaccination history reduce not only the rate of adverse events but also the severity of side effects [6, 7, 10, 20]. Here, no significant difference in the rate of adverse events was detected among three- or sixfold diluted vaccine doses.

None of the study participants experienced any of the rare, severe adverse events previously associated with smallpox vaccines, such as eczema vaccinatum, postvaccinial encephalitis or progressive vaccinia. However, these complications generally occurred at a rate of one to 25 cases per million [26] and therefore, would not be likely to be found in a study of this size. In addition, careful pre-vaccination screening has been shown to efficiently reduce the rate of these known severe adverse events [10, 27]. By excluding individuals at risk of cardiac-related events, atopic dermatitis and immunosuppressive disorders smallpox vaccine can be administered with minimised risk. However, the rate of adverse events reported for this smallpox vaccine would not be acceptable for the actual routinely used vaccines. In the view of these concerns about vaccine safety there is demand for the development of newer, safe but still efficacious smallpox vaccines especially to achieve bioterrorist preparedness.

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