Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Morning vaccination enhances antibody response over afternoon vaccination: A cluster-randomised trial

Joanna E. Long[a], Mark T. Drayson[b], Angela E. Taylor[d], Kai M. Toellner[b], Janet M. Lord[c,1], Anna C. Phillips[a,*,1]

[a] School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham B15 2TT, UK
[b] Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham B15 2TT, UK
[c] Institute of Inflammation and Ageing, University of Birmingham, Birmingham B15 2TT, UK
[d] Institute of Metabolism and Systems Research, University of Birmingham, Birmingham B15 2TT, UK

ARTICLE INFO

Article history:
Received 1 February 2016
Received in revised form 4 April 2016
Accepted 12 April 2016
Available online xxx

Keywords:
Ageing
Antibodies
Cluster-randomised
Influenza vaccine
Time of day
Vaccination

ABSTRACT

Objectives: Older adults are less able to produce a protective antibody response to vaccinations. One factor that contributes to this is immune ageing. Here we examined whether diurnal variations in immune responses might extend to the antibody response to vaccination.
Design: We utilised a cluster-randomised trial design.
Setting: 24 General Practices (GPs) across the West Midlands, UK who were assigned to morning (9–11 am; 15 surgeries) or afternoon (3–5 pm; 9 surgeries) vaccination times for the annual UK influenza vaccination programme.
Participants: 276 adults (aged 65+ years and without a current infection or immune disorder or taking immunosuppressant medication).
Interventions: Participants were vaccinated in the morning or afternoon between 2011 and 2013.
Main outcome measures: The primary outcome was the change in antibody titres to the three vaccine influenza strains from pre-vaccination to one month post-vaccination. Secondary outcomes of serum cytokines and steroid hormone concentrations were analysed at baseline to identify relationships with antibody responses.
Results: The increase in antibody levels due to vaccination differed between morning and afternoon administration; mean difference (95% CI) for H1N1 A-strain, 293.3 (30.97–555.66) $p = .03$, B-strain, 15.89 (3.42–28.36) $p = .01$, but not H3N2 A-strain, 47.0 (−52.43 to 146.46) $p = .35$; those vaccinated in the morning had a greater antibody response. Cytokines and steroid hormones were not related to antibody responses. No adverse events were reported.
Conclusions: This simple manipulation in the timing of vaccine administration to favour morning vaccination may be beneficial for the influenza antibody response in older adults, with potential implications for vaccination strategies generally.
Trial registration: This trial is registered with the ISRCTN (ISRCTN70898162).

1. Introduction

The influenza vaccination is part of the seasonal vaccination programme carried out by General Practice (GP) surgeries across the UK and in many other countries, with patients aged 65+ years being the majority of recipients. Despite this, the influenza virus is responsible for 250,000–500,000 thousand deaths annually [1] and older adults are the highest proportion of the hospitalisations and influenza-related mortalities [2]. Although the contributing factors are varied, the age-related decline in immunity reduces the ability of older adults to produce adequate antibody responses following vaccination [3,4], compromising the protection given against the influenza virus.

A number of interventions have sought to improve the antibody response to vaccination. For example, the addition of adjuvants to the vaccine preparation, but these can have adverse side effects [5]. More recently, behavioural interventions prior to vaccination have been used, such as aerobic exercise [6–8], with some success. However, such interventions may be impractical in a public health setting.

* Corresponding author. Tel.: +44 121 414 4398; fax: +44 121 414 4121.
E-mail address: a.c.phillips@bham.ac.uk (A.C. Phillips).
[1] These authors contributed equally to the work described here.

http://dx.doi.org/10.1016/j.vaccine.2016.04.032

Recent developments in chronobiology have revealed that the response of the immune system to challenge varies significantly with the time of day [9,10] and 56 of the top 100 best-selling drugs in the United States target the product of a circadian gene [11] suggesting that the timing of vaccinations may also influence antibody responses. Indeed, circadian variations in responses to antigen have been observed in mice [12,13]. The scant previous research in humans has produced mixed results. An attenuated Venezualan equine encephalomyelitis vaccine administered at 8 am resulted in peak antibody titres 4 days earlier than the peak in those vaccinated at 8 pm [14]. However, a hepatitis B vaccine administered in the afternoon between 1 and 3 pm yielded a higher antibody response, compared to vaccination between 7.30 and 9 am [15]. More recently, a convenience sample of 164 men and women showed that men exhibited a higher antibody response when vaccinated in the morning [16]. However, this study was not randomised and used a relatively small mixed sample of young and elderly populations and hepatitis A and influenza vaccinations, respectively. It is possible that diurnal variations in immune cell responses and/or levels of hormones with immune modifying properties, such as cortisol or inflammatory cytokines, provide an advantageous period for vaccination responses to occur. Therefore, adjusting the timing of vaccination may be a simple, cost neutral and effective public health intervention to improve vaccination responses, particularly in older adults. However, it is possible that the best time of day for vaccination may be different for different vaccines, as they stimulate different types of immune response for protection, e.g. thymus-dependent versus thymus-independent responses.

1.1. Rationale for cluster design and hypothesis

The present cluster-randomised trial aimed to determine whether randomising GP surgeries to administering the influenza vaccination to older adults in the morning (9–11 am) or afternoon (3–5 pm) impacted upon the magnitude of antibody responses at four weeks post vaccination. Timings were chosen to represent the two extremes of routine morning versus afternoon clinics, in keeping with GP surgery opening hours for practicality of future application. A cluster design was chosen to fit the practicalities of organising GP surgery vaccination clinics. It was hypothesised that morning vaccination would be more beneficial for antibody responses than afternoon vaccination, at both individual and cluster level.

2. Methods

2.1. Participants and eligibility criteria

298 participants were recruited from 24 Primary Care General Practices within the West Midlands, UK, with 276 being eligible for full data analysis. Eligibility criteria to participate in the study were: ≥65 years old, taking no medication which could influence immune function e.g. immune-suppressants, no current acute infections and no current cancer, diabetes, chronic inflammatory disease or immune disorder. There were no eligibility criteria for clusters except being an NHS GP surgery within the West Midlands UK area willing to take part in the trial and be randomised to vaccinating participants in one of the two time slots.

2.2. Trial design

This was a non-blinded cluster-randomised trial. This study was approved by the South Birmingham Local Research Ethics committee and funded by an MRC Lifelong Health and Wellbeing Collaborative Research Grant. This trial is registered with the ISRCTN as a controlled trial (ISRCTN70898162). The protocol is available from the corresponding author or in the trial registry.[2]

2.3. Intervention

Participants were invited to take part in the study by a letter sent from their GP surgery on behalf of the research sponsor (University of Birmingham (UB)) and they returned the signed written informed consent form to the research team at UB. GP surgeries (clusters) where participants had returned consent forms were then notified of which arm of the trial (morning or afternoon) they had been randomised to. Participants were then invited to attend on two separate occasions, one month apart. The initial session involved providing a blood sample and receiving the trivalent influenza vaccination as standard practice (administered intra-muscularly) between either 9 and 11 am or 3 and 5 pm. In accordance with standard GP practise, the standard influenza vaccine used routinely during each influenza season was administered using the standard single dose (0.5 ml), route of administration (intramuscularly into deltoid) and common commercially available inactivated preparations in pre-filled syringes in 2011/12: Pfizer Ltd Enzira® split virion or Sanofi Pasteur split virion; in 2012/13: Pfizer Ltd Enzira® split virion, Sanofi Pasteur split virion, BGP Products Ltd Imuvac® surface antigen or GlaxoSmithKline FLUARIX® split virion; and in 2013/14: Pfizer Limited Enzira® or generic split virion, Sanofi Pasteur split virion, BGP Products Ltd Imuvac® surface antigen, GlaxoSmithKline FLUARIX® split virion, or Janssen-Cilaq Viroflu® surface antigen; the exact influenza components these contained are detailed below. A questionnaire pack was given to participants to complete at home and return by mail. One month later, participants returned to their GP practice to give a morning fasted blood sample and have weight, height and waist to hip ratio measurements taken. Number of previous influenza vaccinations the participant had received was gained from GP electronic records.

2.4. Questionnaires

Participants completed a battery of questionnaires at baseline to assess socio-demographics and health behaviours. Health behaviours (smoking, alcohol consumption and sleep duration) were assessed using a questionnaire adapted from the Whitehall II study [17]; smoking and drinking alcohol were dichotomised into yes/no variables.

2.5. Blood sampling and analysis

Blood was collected in to anti-coagulant free tubes (BD Vacutainer, UK) and clotted at room temperature before centrifugation at 2000 × *g* for 5 min. The separated serum was frozen at −20 °C for later analysis.

2.5.1. Haemagglutination inhibition assay

Anti-influenza antibody titres were measured using an in-house haemagglutination inhibition test as described in the

[2] Following completion of the trial in 2013, the sponsor (University of Birmingham) through a query to the MHRA, reclassified this trial as a Clinical Trial of an Investigational Medicinal Product (CTIMP). The study was retrospectively submitted to an ethics committee appropriate for a CTIMP, Haydock NRES Committee North West, and received a favourable opinion through special ethics review. Following consideration by the sponsor and MHRA, it was deemed not justifiable to prevent the use of the trial data given that patients were to receive this medication regardless of whether this was done as part of the trial or as per routine practice. There were no adverse events reported, and the data and analyses were deemed reliable and appropriate by the Primary Care Clinical Trials Unit statistician at the University of Birmingham.

Please cite this article in press as: Long JE, et al. Morning vaccination enhances antibody response over afternoon vaccination: A cluster-randomised trial. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.04.032