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Rabies pre-exposure prophylaxis elicits long-lasting immunity in humans

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ABSTRACT

Despite the availability of safe and effective human vaccines, rabies remains a global threat, with an estimated 60,000 human deaths annually attributed to rabies. Pre-exposure prophylaxis against rabies infection is recommended for travelers to countries where rabies is endemic, and also for those with a higher risk of exposure. In this study, the rabies-specific neutralising antibody responses in a cohort of rabies-vaccinated recipients over a period of twenty years have been assessed. In particular, the antibody response to primary vaccinations and boosters, and the waning of antibody post primary vaccination and post booster were investigated. The significance of gender, age at vaccination, vaccine manufacturer and vaccination intervals were also evaluated. These data confirm that rabies vaccination can elicit a neutralising antibody response that can remain at detectable levels for a number of years, without additional booster vaccinations. The antibody response following both primary vaccination and booster was significantly influenced by the gender of the subject ($p = 0.002$ and 0.03 respectively), with supportive data that suggests an effect by the make of vaccine administered following primary vaccination, with significantly higher VNA titres observed for one vaccine manufactured prior to 2006 ($p < 0.001$) in a small subset of recipients ($n = 5$). Additionally, the decay rate was demonstrated through the overall decline in antibody titre for all individuals, which was a 37% and 27% reduction per 2-fold change in time following primary and booster vaccination respectively. Individuals within older age groups demonstrated a significantly faster decline in antibody titre following the primary vaccination course ($p = 0.012$). Rate of decline in antibody titre was also significantly influenced by the vaccine make following primary course ($p < 0.001$). The assessment of neutralising antibody titre decline has also provided an insight into the most appropriate timing for booster administration, and enabled the prediction of long term titres from post-vaccination antibody titres.

1. Introduction

Rabies remains a threat to global public health, despite the availability of safe and effective human vaccines [22,14]. The number of human fatalities as a result of rabies infection is estimated to be almost 60,000 per year worldwide, and this is likely to be an underestimate [21,31,10]. The majority of human rabies cases occur in resource-limited countries where rabies is endemic, therefore pre-exposure prophylaxis against rabies is recommended for travelers to these countries [11,35]. In the absence of pre- or post-exposure prophylaxis, rabies is the only known virus with a 100% case fatality rate once clinical symptoms are observed [14].

There are two inactivated rabies vaccines currently licensed for human use in the United Kingdom (UK). The first is Rabies vaccine BP (Sanofi Pasteur), a human diploid cell culture vaccine (HDCV), which is based on the Wistar rabies virus strain. The second vaccine is Rabipur® (Novartis/Chiron), a purified chick embryo vaccine (PCECV) based on the flury LEP-25 strain and produced in primary chick fibroblast cell cultures [5]. However, there are many rabies vaccines available worldwide, and there are strict guidelines for vaccines approved by the WHO. Additional WHO-approved vaccines include Verorab™, which is also produced by Sanofi Pasteur, and is

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a purified Vero cell rabies virus vaccine (PVRV) based on the Wistar rabies strain but produced in Vero cells. HDCV is generally the most expensive due to higher production costs [4], and has therefore been superseded by PCECV and PVRV [13]. The current pre-exposure schedule for rabies vaccination in the UK is described in the 'Immunisation against infectious disease handbook' [25], and is three doses administered intramuscularly on days 0, 7 and 28, followed by a booster one year later for those at regular and continued risk. Further reinforcing doses should be given at three to five years thereafter, or as needed, assessed by regular serological testing (Public Health England [PHE], 2015). Additionally, for laboratory workers, regular serological testing for immunity is recommended following vaccination, and booster doses administered where necessary.

Following vaccination, the principal correlate of protective immunity against rabies infection is the development of virus neutralising IgG antibodies (VNA) against the viral glycoprotein [32,16]. The WHO recommendations state that a VNA titre of greater than or equal to 0.5 IU/ml will likely confer protective immunity against rabies virus infection following an exposure [36]. However, the cell-mediated immune response to rabies vaccination has not been thoroughly studied and gaps in our knowledge, especially with respect to longevity of the immune response following rabies vaccination remain unanswered [19]. Whilst the stimulation of VNA is universally considered to be a pre-requisite for protective immunity against rabies infection, the stimulation of memory T-cells are likely to play a role in any anamnestic response following exposure to rabies virus and to long-lived immunity following vaccination [17]. Vaccine-induced immunity is thought to last between 3 and 5 years [34], but rabies virus-specific neutralising antibodies have been shown to be detectable for up to ten years [28]. Rabies virus and the other lyssaviruses can be differentiated into phylogroups based upon their antigenic and genetic characteristics. Although WHO-recommended rabies vaccines are known to provide some protection against phylogroup I lyssaviruses, including the European bat lyssaviruses (EBLV) [6], there is limited or no cross-protection against phylogroup II lyssaviruses, or more divergent lyssaviruses such as Ikoma lyssavirus (IKOV) [12,15,18,3]. This variable efficacy suggests a reduction in protection as viruses become more antigenically distinct from the vaccine strains [1,18].

In this study, human rabies vaccine longevity was investigated in a cohort of laboratory workers over a period of twenty years, to assess the response to primary vaccinations and boosters, the waning of antibody post-primary vaccination and post-booster, and to investigate whether immunity may be elicited beyond 5 years post-vaccination without booster. Factors that were considered included gender, age at vaccination, vaccine manufacturer and vaccination intervals. Of particular interest was an assessment of VNA titre decline, to provide an insight into the most appropriate timing for booster administration, and to determine whether post-vaccination VNA titres may be utilised to predict longer term titres.

2. Methods

2.1. Ethical statement

The work described in this study has been conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Informed consent was obtained from each individual whose age, gender, vaccination and blood test data was used for this study. All results have been anonymised to ensure that privacy rights have been maintained.

2.2. Sample population

These data were derived from a sample population of 280 laboratory workers and support staff who were previously vaccinated against rabies for occupational purposes within the workplace. In order to monitor the rabies-specific VNA titre, blood samples were taken at intervals, and tested using the validated and accredited fluorescent antibody virus neutralisation test (FAVN) as previously described [8]. VNA titres were determined as International Units per millilitre of serum (IU/ml). The earliest serum sample dated from 1995, and the latest from 2015, therefore the study period encompassed twenty years of data. Over the course of the study period, any subject who was shown to have an antibody titre below the occupationally-defined minimum received an additional booster vaccination; 5 IU/ml for 'high-risk' workers (laboratory workers handling divergent lyssaviruses including IKOV) or 1 IU/ml for laboratory workers undertaking routine work with rabies virus (RABV). It should be noted that primary containment (biological safety cabinets), and not vaccination, remains the principal mode of preventing exposure for laboratory workers handling infectious rabies virus. These minimum titres were determined through cross-neutralisation studies with human vaccinated sera, where a VNA titre against CVS of $\geqslant$4.5 IU/ml was necessary in order to cross-neutralise EBLV-1, EBLV-2 and Australian bat lyssavirus (ABLV) [6]. Therefore some subjects had received more than one additional booster since their primary course of vaccination.

Of the 280 subjects, 272 had dates of vaccination recorded and post-vaccination blood test results available. All blood samples were taken at least seven days after a vaccination. Two individuals were excluded due to the likelihood of missing vaccination information, therefore there were a total of 270 individuals who had provided at least one post-vaccination blood sample. Four outlying blood results were identified and excluded as potential transcription errors, therefore a total of 1624 blood testing results were available for assessment.

2.3. Data analysis

To assess post-primary and post-first and second booster vaccination responses, only samples taken within 2 months (61 days) of a vaccine dose were included. In addition to calculating the overall geometric mean response, results were stratified by gender (male/female), age at vaccination (<30, 30–39, 40–49, 50+) and vaccine make (Rabies Vaccine BP, Rabipur® [pre- and post-1996, when the manufacturer changed], Verorab™, and an unidentified Sanofi Pasteur vaccine), although some individuals did not have vaccine make recorded. Adjusted fold effects were also calculated using a multiple regression model on logged titres with gender, age and vaccine make included.

Decline in titre by time since primary and booster vaccination was then assessed using $\log_2$-titre regressed against $\log_2$-time. This model allows for initial rapid declines then slower declines and the slope can be represented (when anti-logged) as the percentage decline per doubling in time since vaccination. The interactions between the time effect and gender, age and vaccine make were assessed to investigate whether these factors influenced decline in antibody titre. In this analysis, repeated measures were evaluated using random intercept models, and were restricted to those who received either Rabies Vaccine BP or Rabipur® from 1996 due to small numbers receiving the other vaccines. In addition to modelling the antibody decline, the titre immediately post-primary and post-booster was used to predict the titre at later time-points (one year, two years and three to seven years), as was the titre one year post-primary and post-booster on later titres. This was undertaken within those individuals with samples taken at these time points to assess how predictive recent post vaccination results were of longer term titres. This was assessed by plotting the paired results and calculating the line of best fit and R^2 value, with a high R^2 indicative that results are predictive.

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