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The effect of mucoadhesive excipient on the nasal retention time of and the antibody responses induced by an intranasal influenza vaccine

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ABSTRACT

Introduction: Recently, we reported that intranasal vaccination of humans with whole inactivated influenza vaccine in the absence of mucosal adjuvant induced neutralizing antibody responses in the serum and nasal mucus. The mucoadhesive excipient carboxy-vinyl polymer (CVP) increases the viscosity and therefore mucoadhesiveness of intranasal medicaments and is an authorized excipient in Japan. In the present study, we analyzed the effect of adding CVP on intranasal whole inactivated influenza vaccine antigen dynamics and antibody responses.

Methods: Mice and nonhuman primates (NHPs) were intranasally administered the [^{18}F]-radiolabeled vaccine and subjected to positron emission tomography analysis for 6 h. Dendritic cells were stimulated *in vitro* with the vaccine mixed with or without a mucosal adjuvant (Ampligen) and/or CVP, after which the tumor necrosis factor (TNF)-α and interferon (IFN)-β levels in the supernatants were measured. Cynomolgus monkeys were immunized intranasally with the vaccine mixed with Ampligen and/or CVP and their vaccine-specific serum IgG and IgA titers were measured on days 0 and 33.

Results: The vaccine was retained significantly longer in the nasal cavity of both mice and NHPs when it was delivered with CVP rather than PBS. Accumulation of the radiolabeled vaccine in the central nervous system was not detected in either model regardless of whether CVP was used. CVP only very weakly increased the TNF-α production of vaccine-stimulated dendritic cells. IFN-β production was not observed regardless of the presence or absence of CVP. CVP increased the vaccine-specific IgA antibody responses of the intranasally vaccinated cynomolgus macaques.

Conclusion: CVP increased intranasal retention of whole inactivated influenza vaccine, did not promote antigen redirection to the central nervous system, and improved mucosal antibody responses. The mechanism probably relates to its mucoadhesive properties rather than its ability to directly stimulate the immune system. Intranasal vaccines with CVP may be a promising candidate vaccine formulation for humans.

1. Introduction

Influenza virus infection causes a highly contagious acute respiratory disease in humans. Vaccination is considered the most ideal approach to control epidemics and pandemics. At present,

Abbreviations: BM-DC, bone marrow-derived dendritic cell; CVP, carboxy-vinyl polymer; CNS, central nervous system; LPS, lipopolysaccharide; MRI, magnetic resonance imaging; NHP, nonhuman primate; PET, positron emission tomography; SUV, standardized uptake value.

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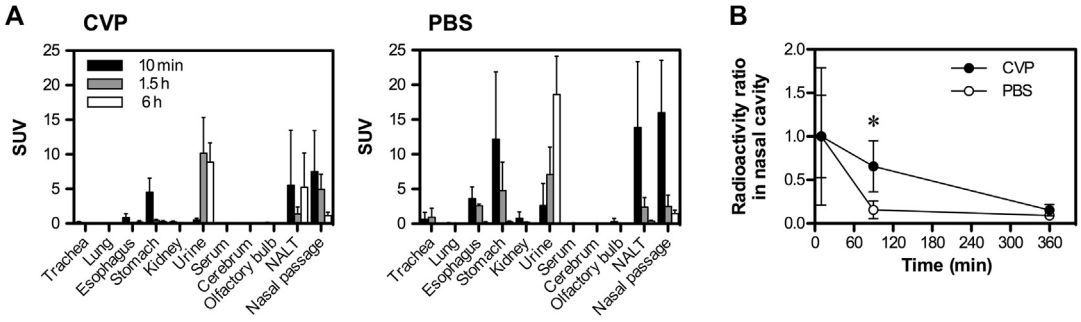

Fig. 1. Tissue distribution of radiolabeled vaccine mixed with carboxy-vinyl polymer or PBS after intranasal administration in mice.
After intranasal administration of a whole inactivated A(H1N1)pdm09 virus vaccine labeled with [^{18}F] and mixed with carboxy-vinyl polymer (CVP) or PBS ($n = 9$ mice per group), three mice from each group were sacrificed 10 min, 1.5 h, and 6 h later. (a) The isotope activity in each mouse tissue was measured directly using a gamma counter and displayed as standardized uptake value (SUV). NALT, nasopharynx-associated lymphoid tissue. (b) Radioactive counts in the nasal cavity at the indicated time points were expressed as a ratio to the total count 10 min after vaccination with the two preparations. The CVP and PBS groups were compared in terms of remaining radioactivity at each time point by two-tailed unpaired *t*-tests. The data were analyzed by one-way ANOVA with Tukey's multiple comparisons test. The error bar indicates standard deviation. The asterisk indicates $p < 0.05$.

to protect people from epidemics of seasonal influenza virus, inactivated influenza virus vaccines that are mainly composed of chemically disrupted products are administered subcutaneously or intramuscularly [1]. This vaccine primarily induces systemic hemagglutinin-specific serum IgG antibodies that effectively reduce mortality and morbidity after homologous virus infection. However, the antibodies are less effective against heterologous virus infections. Notably, natural infection with influenza virus elicits not only systemic IgG antibodies but also local secretory IgA antibodies on the mucosal surfaces of the respiratory tract that are targeted by influenza viruses. The infection-induced secretory IgA antibodies both prevent virus entry on re-infection with the homologous virus and provide cross-protection against antigenically heterologous viruses that are the result of "antigenic drift" [2–4]. This suggests that a better vaccination approach might be one that mimics the natural infection. Indeed, live attenuated influenza virus vaccines are superior to conventional influenza vaccines. However, they can only be administered to healthy children and adults between 2 and 49 years old because of safety concerns [5]. Thus, at present, the most protective and safest influenza vaccine may be intranasally administered inactivated influenza virus vaccine [6–9].

Intranasal administration of a split-virion vaccine alone induces almost no antibody responses. Although the addition of an immune stimulator (namely, an adjuvant) can improve the immunogenicity of the vaccine [10–14], a mucosal adjuvant has not yet been authorized for practical use anywhere in the world. However, our previous study showed that when healthy adult volunteers are intranasally immunized with a whole inactivated influenza vaccine without any mucosal adjuvant, they develop neutralizing antibody responses in the serum and nasal mucus [15]. Another way to improve immune responses to vaccines is to employ vaccine delivery systems, which aim to efficiently target antigens to immune cells [10,16–21]. In relation to this, it was recently reported that, when a vaccine antigen is mixed with a cationic hydrogel before intranasal delivery, it enhances the antigen-specific antibody responses [16–18,20]. This cationic gel acts by increasing the viscosity of the vaccine, thus causing the vaccine to stick to the mucosal surface in the upper respiratory tract and prolonging the retention of the vaccine in the nasal cavity. Another material that could increase antigen viscosity is carboxy-vinyl polymer (CVP), which is a nasal mucoadhesive excipient that is used to increase the viscosity of medicaments; this polymer is authorized for use as a nasal spray excipient in Japan. Although it has been shown to increase antibody responses induced by an intranasal inactivated influenza vaccine in mice [22], the effect of CVP on the dynamics of the vaccine in the nasal cavity remains poorly understood. The information of vaccine dynamics in the nasal cavity is an important issue considering that several reports show that intranasal vaccines combined with a heat-labile enterotoxin as a mucosal adjuvant may cause Bell's palsy as an adverse event in some participants [23,24]. Thus, studies that assess whether intranasally administered vaccine accumulates in central nervous system (CNS) tissues such as the olfactory bulb and cerebrum are warranted.

Table 1
Standardized radioactive counts in the cerebrum and olfactory bulb of mice that were intranasally administered radiolabeled whole inactivated influenza virus vaccine mixed with carboxy-vinyl polymer or PBS.

	Time	Mean values ± standard deviations		p value [a]
		CVP	PBS	
Cerebrum	10 min	0.0057 ± 0.0031	0.0147 ± 0.0165	0.3581
	1.5 h	0.0023 ± 0.0006	0.0047 ± 0.0021	0.9694
	6 h	0.0007 ± 0.0006	0.0000 ± 0.0010	0.9992
Olfactory bulb	10 min	0.0480 ± 0.0654	0.2903 ± 0.4609	0.3753
	1.5 h	0.0067 ± 0.0021	0.0373 ± 0.0040	0.9964
	6 h	0.0107 ± 0.0116	0.0137 ± 0.0283	>0.9999

CVP, carboxy-vinyl polymer.
[a] *p*-values were obtained using two-way ANOVA with Sidak's multiple comparisons test.
There were three mice in each group at each time point.

The present study assessed the qualitative and quantitative antigen dynamics and retention time of a whole inactivated influenza virus vaccine that was administered intranasally in mice and nonhuman primates (NHPs) in the presence and absence of CVP. In addition, the effect of CVP on vaccine-stimulated dendritic cells *in vitro* and serum antibody responses in intranasally vaccinated cynomolgus monkey was evaluated.

2. Materials and methods

Detailed materials and methods are supplied as Supplementary information.

2.1. Animals

Female BALB/c mice, male rhesus monkeys (*Macaca mulatta*), and male cynomolgus monkeys (*Macaca fascicularis*) were used in this study. Animal experiments using mouse and nonhuman primates were conducted in compliance with Japanese legislation (Act on Welfare and Management of Animals, 1973, revised in 2012) and guidelines under the jurisdiction of the Ministry of Health, Labour

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