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Development and pre-clinical evaluation in the swine model of a mucosal vaccine tablet for human influenza viruses: A proof-of-concept study

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ABSTRACT

Liquid vaccine formulations present some disadvantages such as stability problems, cold chain requirement or administration by trained personnel. Vaccine formulated as tablets would present a wide range of progress such as an increase stability that would facilitate the administration, the distribution and the storage of vaccine formulations. This work investigates the possibility to develop a mucosal tablet vaccine for human influenza viruses. The tablets were tested *in vitro* for biological efficacy and stability and *in vivo* in swine as a model for influenza A virus immunity. First, the ability to produce by compaction a stable vaccine with a preserved antigen was demonstrated. In a second part, vaccine tablets were used to immunize pigs. After positioning the tablets on the buccal mucosa, the animals were challenged by inoculation of the A/H1N1 pandemic virus. The responses were compared to those observed in animals vaccinated intramuscularly with the commercial liquid vaccine. It was observed signs of priming of the pig's immune system with vaccine tablets, even if the immune response stayed lower than vaccination by intramuscular route. Thus, we present attractive results that indicate a promising potential for mucosal vaccine tablets.

1. Introduction

In most cases, vaccines on the market are liquid formulations for parenteral administration. These formulations present numerous disadvantages such as stability problems, cold chain requirement or administration by trained personnel. To overcome these disadvantages, two ways can be explored, the route of administration (such as the mucosal routes (Gebril et al., 2012; Kraan et al., 2014; Slütter et al., 2008; Tonnis et al., 2012) and the vaccine formulations (with for example the development of dry vaccine formulations (Amorij et al., 2008; Liebowitz et al., 2015; Tomar et al., 2016). Mucosal administration of vaccines is an attractive alternative to the parenteral route (Brandtzaeg, 2010; Çuburu et al., 2007; Kraan et al., 2014). The main benefits concern the patient compliance due to the avoidance of painful injections and the facility of a self-administration. Moreover, a majority of pathogens infect their hosts through the mucosa. Then, mucosal vaccination is interesting since it can induce immune responses at the way of entry of most infectious pathogens (Holmgren and Czerkinsky, 2005; Kraan et al., 2014; Kweon, 2011; Shim et al., 2013; Song et al., 2008). Among mucosal routes for vaccine delivery, buccal and sublingual routes have received less attention compared to the most popular oral, nasal and pulmonary routes (Gebril et al., 2012; Slütter et al., 2008; Tomar et al., 2016; Tonnis et al., 2012). With the oral route, buccal and sublingual routes present also the advantage to make possible the use of tablets. Vaccine formulated as tablets would present a wide range of progress such as an increased stability that would facilitate the administration (non-invasive nature of delivery), the distribution and the storage of vaccine formulations (allowing a wide scale use, in particular in vaccination campaigns in developing countries).

Vaccines are usually presented as liquid dispersions that are not suitable for tableting process as it is. As a consequence, the first step will consist in the production of solid (dry) products (Tomar et al., 2016). Freeze-drying (or lyophilization) is a method of choice for drying fragile products like proteins, viruses or bacteria (Baheti et al., 2010; Wang, 2000). The dry and porous products can then be processed by compaction after being mixed with appropriate excipients (Murugappan et al., 2014). These excipients should be chosen to make possible tableting but also to determine tablet functionalities (e. g.

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active compound release, resident time of the formulation). Considering a mucosal administration, flu was chosen as a model to design a muco-adhesive tablet for a buccal delivery of flu vaccine. In fact, influenza virus infects its host through the respiratory mucosa. IM inactivated vaccines are poor stimulators of secretory IgA at respiratory mucosal sites (Barackman et al., 1999) (inactivated vaccines induce serum IgG). By exploring mucosal immunization, vaccines are expected to induce significant secretory IgA. In the development of a vaccine tablet, several challenges must be met. The first one is those encountered with other fragile products, *i.e.* the maintenance of viability during the product life cycle up to the time of delivery (Borde et al., 2016, 2012; Murugappan et al., 2014; Muller et al. 2014, 2013). For flu vaccine, the antigens must be preserved during processes with an immunogenicity maintained. Murugappan et al. (2014) obtained some promising results on mice with sublingual vaccine tablets containing H5N1 inactivated virus. Nevertheless, stability was not tested although production of a tablet with a sufficient self-life without the requirement of constraining cold chain conditions is a key point to consider. Since mucosal delivery is targeted, the tablet vaccine must be kept at the site of delivery for a sufficient time using suitable tablet formulations. This point is not considered in Murugappan et al. work since dry vaccine powder was reconstituted in PBS and pipetted under the tong of mice. The last challenge is to elicit an immune response making possible a protection against influenza virus infection. Once again, the work of Murugappan et al. shown the potential of vaccine tablets for buccal routes. Nevertheless, this study lacked by the choice of a non-appropriate animal models. As pointed out by Tomar et al. (2016), better preclinical models must be used. Due to the similarities between humans and swine (genetics, anatomy and physiology), pigs is more suitable for influenza virus vaccination and infection than mice (Rajao and Vincent, 2015).

In this work, commercial flu vaccines were freeze-dried. The obtained dry product was further formulated with muco-adhesive excipients before tableting under controlled conditions. *In vitro* experiments were performed on tablets to investigate the impact of the processes on the antigens and the preservation of their biological activity. In parallel, stability studies were performed. Finally, an *in vivo* study was carried out using a porcine model since it has been successfully used for influenza A virus infection and flu vaccine efficacy (Rajao and Vincent, 2015). The flu vaccine tablets were tested for the buccal immunization of SPF pigs in comparison to the commercial liquid vaccine administrated intramuscularly. The humoral immune response and the protection conferred against an influenza A virus infection were evaluated and analyzed.

2. Materials and methods

2.1. Influenza virus vaccines

The flu vaccine used in this work was a commercial quadrivalent vaccine for use in the 2014–2015 Northern hemisphere influenza season, Fluarix® (AFLUA813AA, GlaxoSmithKline France, Rueil-Malmaison, France). A dose consists of a 0.5 mL injectable suspension containing 15 µg of each hemagglutinin from A/California/7/2009 (H1N1)pdm09-like virus, A/Texas/50/2012 (H3N2)-like virus, B/Massachusetts/2/2012-like virus and B/Brisbane/60/2008-like virus (Cox and Subbarao, 1999; Who, 2014).

2.2. Freeze-drying of vaccine doses

Fluarix® vaccines were freeze-dried in the presence of a bulking agent, mannitol (Parteck M200, Merck Chimie SAS, Fontenay-sous-bois, France) and a lyoprotectant, trehalose (Treha® 16,400, Cargill France SAS, Paris-La Défense, France). Fifteen vaccine doses were introduced and weighted in a 50 mL tube (Falcon® centrifuge tube, Corning®, New York, USA) (weighed mass of 8.1 g per tube). Masses of mannitol and trehalose corresponding to 1% w/w of the vaccine dose mass was then introduced in each 50 mL tube. Samples were frozen 30 min at liquid nitrogen temperature and afterwards subjected to freeze-drying (Cryodos 80, Telstar, Terrassa, Spain). Tubes were dried outside the ice condenser chamber at ambient temperature during 24 h. The pressure was controlled at $5 \cdot 10^{-2}$ mbar. At the end of freeze-drying, all tubes were manually closed and stored at 4 °C one night until usage. In parallel, freeze-dried placebos were obtained by replacing the vaccine doses by 7.5 mL of phosphate buffered saline (PBS) solution (Sigma-Aldrich, Saint-Quentin Fallavier, France).

Table 1
Composition of mucoadhesive tablets used in the study.

	Tablets with vaccine		Placebo tablets
	Formula 1 (% w/w)	Formula 2 (% w/w)	Formula 3 (% w/w)
Freeze-dried vaccine	58.5	58.5	0
Freeze-dried placebo	0	0	58.5
Milk protein concentrate (Fronterra)	20	0	0
Mannitol (Emprove® Parteck® M200, Merck Chemicals)	0	20	20
HPMC (90 SH-4000SR, Shin Etsu Chemical Co., Ltd.)	20	20	20
Fumed silica (Aerosil® 200 Pharma, Evonik Industries)	0.5	0.5	0.5
Magnesium stearate (Cooper Industrie)	1	1	1

2.3. Tableting of freeze-dried vaccines

Freeze-dried samples were blended with magnesium stearate (Cooper, Melun, France), fumed silica (Aerosil® 200 Pharma, Evonik Industries, Essen, Germany) and mucoadhesive excipients. The resulting tableting blend contained 58.5% w/w of freeze-dried vaccine or freeze-dried placebo, 40% w/w of mucoadhesive excipients, 1% w/w of lubricant and 0.5% w/w of glidant. Two tablet formulations were tested differing by mucoadhesive excipients (Table 1). The first formula (Formula 1) is based on the Lauriad® technology funded on the association of a polymer and a milk protein concentrate to achieve mucoadhesion (Attali et al., 2012; Downing et al., 2014). HPMC was used in the second formula (Formula 2) since it has been widely investigated as mucoadhesive excipient (Shaikh et al., 2011; Smart, 2005). When it was associated with mannitol in tablets, it was observed an increased rate of water transport into the tablets (Tajarobi et al., 2009) (Formula 2).

To preserve the immunogenicity of antigens, tableting process must be fully controlled and stresses applied on antigens have to be known. Mucoadhesive tablets were produced using a StylOne Evolution tableting press monitored by Analis software (MedelPharm, Beynost, France). This press is instrumented for force (accuracy of 10 N) and displacement (accuracy of 0.01 mm) measurements. The mechanical system makes possible to drive the press in force or in displacement. Force driven mode is helpful to obtain tablets when a small quantity of powder is available (for example for early formulation stage). It was the compression mode used in this study. The press was tooled with beveled-edged Euro B punches (diameter of 6 mm). The filling height was adjusted to obtain tablets of about 50 mg. This mass corresponds to tablets containing two doses of commercial vaccine (*i.e.* 30 µg of each hemagglutinin type). The compaction pressure was set to 200 MPa in order to produce tablets with the required properties for the rest of the study (tablets with a mean diameter of 6.05 ± 0.01 mm and a mean thickness of 1.35 ± 0.02 mm). It corresponded to tablets with a friability lower than 1% (European Pharmacopeia (9th edition), 2017) and with a sufficient tablet strength (mean tensile strength of about 1.3 MPa