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Cerium dioxide nanoparticles increase immunogenicity of the influenza vaccine

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ABSTRACT

We have demonstrated the influence of cerium dioxide nanoparticles on the immunogenicity of the influenza vaccine on an example of liquid split inactivated Vaxigrip vaccine. Antibody titers were analyzed using the hemagglutination inhibition (HI) assay. Seroprotection, seroconversion, the geometric mean titers (GMTs) and the factor increase (FI) in the GMTs were calculated. The effect of nano-ceria surface stabilizer on the enhancement of immunogenicity was shown. The vaccine modified by citrate-stabilized nano-ceria, in contrast to a non-modified Vaxigrip vaccine, did not provide an adequate level of seroprotection, and seroconversion after vaccination was 66.7% on days 49–63 for virus strain A(H1N1) and 100% on day 49 for virus strain B/Yamagata. For the low immunogenic influenza B virus, the rise in antibody titers (GMT/IF) was 24.38/3.28 after the first injection and 50.40/6.79 on day 49. For the vaccine modified by non-stabilized nano-ceria, for all virus strains under study, on day 63, upon immunization notable levels of seroprotection, seroconversion and GMT/IF were registered (higher than for the non-modified Vaxigrip vaccine). The successful attempt to modify the influenza vaccine demonstrates the possible ways of increasing the specific activity of vaccines using nano-ceria.

1. Introduction

Influenza and acute respiratory infections are viral infections for which a tendency for fast epidemic spread is inherent. The 2009 “swine flu” pandemic caused by the H1N1 flu virus has revealed the need not only for a detailed study of patterns and causes of previous pandemics, properties of viruses, and new strains emergence, but also of an improvement in the characteristics of existing antiviral vaccines [Guan et al., 2010; Taubenberger and Morens, 2010; Heikkinen et al., 2014; Kidd, 2014; Kissling et al., 2014]. Various types of vaccines are currently used in clinical practice, differing in the development of the immune response and in the level of side effects. Live-attenuated (LAIV) vaccines are used preferentially for influenza preimmunization, and trivalent inactivated vaccines (TIV) are considered to be effective for the improvement of already-existing immune response [Christopher et al., 2011]. However, their administration is accompanied by several side effects: fever, respiratory symptoms, sustained tenderness in the place of injection. The use of split and subunit vaccines results in these effects more rarely [Information sheet ‘Observed rate of vaccine reactions. Influenza vaccine’, 2012]. Split vaccines are most widely used for the effective prevention of influenza [Soema et al., 2015], such brands as Vaxigrip/Fluzone and Fluarix are key currently marketed flu vaccines [Belsey et al., 2006; Kresse and Rovini, 2009]. All vaccine formulations possess local and systemic reactogenicity, but it is not always combined with high immunogenicity [Squarcione et al., 2003; Baxter et al., 2010; Beyer et al., 2011; Petousis-Harris et al., 2011]. Therefore, the development of methods of increasing the immunogenicity of existing vaccines is a vital task. Conventional techniques for enhancing the immunogenicity of vaccines include

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the use of adjuvants and/or immunostimulatory compounds [Nordly et al., 2009; Foged, 2011; De et al., 2013; Garcia, De Sanctis, 2014]. Unfortunately, the use of such an approach quite often results in local reactions [Beyer et al., 2011]. For example, the reactogenicity of two pandemic influenza A (H1N1)pdm09 vaccines (AS03-adjuvanted and a non-adjuvanted) was compared among the 494 patients [Cerbino-Neto et al., 2012]. Adverse events following immunization (AEFI) were compared via prevalence ratio (PR) with confidence interval (CI). The group receiving the AS03-adjuvanted vaccine had a higher frequency of local reactions at 2 h (PR: 3.01, CI 95%: 2.12–4.29), 24 h (PR: 4.57, CI 95%: 3.29–6.37) and 7 days (PR: 6.05, CI 95%: 2.98–12.28) post-vaccination. The effect of the MF59 adjuvant on the efficacy of trivalent inactivated influenza vaccine (TIV) in 4707 healthy children was also studied [Vesikari et al., 2011]. Systemic events in the older age group were more frequent after administration of ATIV (63%) than after administration of TIV (44%) or the control vaccine (50%). Adjuvants have been recently implicated in the new syndrome named "ASIA – Autoimmune/inflammatory Syndrome Induced by Adjuvants". The most frequent clinical findings were pyrexia 68%, arthralgias 47%, cutaneous disorders 33%, muscle weakness 16% and myalgias 14% [Cerpa-Cruz et al., 2013].

Recently, the effectiveness of vaccines has been shown to be boosted by combining antigens with carrier nanoparticles [Gregory et al., 2013; Wibowo et al., 2014]. Among a plethora of currently available nanomaterials potentially suitable for combining with vaccines, cerium dioxide nanoparticles (nano-ceria) possess a unique set of physical and chemical properties that make them especially promising for biomedical applications [Ivanov et al., 2009; Karakoti et al., 2010; Shcherbakov et al., 2011; Zholobak et al., 2011]. It is generally accepted that nano-ceria possesses very low toxicity. In our studies of nano-ceria toxicity we have established that the dose of 45 mg/kg for 3–5 days is non-toxic for old female mice [Spivak et al., 2012], and a dose of 100 mg/kg for 10 days is non-toxic to old male rats [Spivak et al., 2013]. Moreover, the biomedical application of nano-ceria can reduce greatly the damaging effects of oxidative stress, thus protecting living beings from adverse environmental factors: UV irradiation, viral, bacterial, fungal lesions, and pathological conditions, including those associated with ageing [Babenko et al., 2012; Kim, Hyeon, 2014; Shcherbakov et al., 2014; Zholobak et al., 2014a, 2014b; Kalashnikova et al., 2015]. In our previous studies, we have shown that the use of nano-ceria could significantly enhance the biological activity of interferon (IFN) [Zholobak et al., 2010; Spivak et al., 2011]. Considering that both IFN and the components of the influenza vaccines which are responsible for the induction of a specific immune reaction (hemagglutinin (HA) and neuraminidase (NA)) are glycoproteins, we suggested that the characteristics of the existing influenza vaccines could also be improved by their modification with nano-ceria. Thus, the aim of the present paper is to elucidate the possible influence of nano-ceria on the immunogenicity of the Vaxigrip influenza vaccine *in vivo*.

2. Materials and methods

2.1. Vaccine

The influence of nano-ceria on the immunogenicity of flu vaccine was studied on an example of Vaxigrip liquid split inactivated vaccine during the 2012–13 epidemic seasons (individual packages were made by JSC "Farmex Group", Ukraine, using an "in bulk" form of the vaccine produced by Sanofi Pasteur SA, France). The vaccine contained the following inactivated flu split-virus strains:

- A/California/7/2009/(H1N1)pdm09 – derivative (NYMC X-179A);
- A/Victoria/361/2011/(H3N2) – derivative (IVR-165);
- B/Wisconsin/1/2010 – similar (NYMX BX-39, derivative B/Hubei-Wujiagang/158/2009).

Each of the individual packages (.5 ml) contained HA of all listed strains of influenza virus (15 μg of each HA). Sodium chloride, sodium hydrophosphate dihydrate, potassium dihydrophosphate, potassium chloride, water for injection (buffer components) and formaldehyde (30 mg) were used as adjuvants. Triton X-100, sucrose, neomycin and ovalbumin were present in residual amounts. The vaccine contained no thiomersal.

2.2. Viruses

To study the efficiency of the immune response of vaccinated mice the influenza virus strains were used, namely:

- A/Ukraine/348/2013 (H1N1)pdm – corresponds to A/California/07/2009 (H1N1)pdm;
- A/Ukraine/316/2013 (H3N2) – corresponds to A/Victoria/361/2011 (H3N2);
- B/Odessa/442/2013 – corresponds to A/Wisconsin/1/2010 – B/Yamagata-branch.

2.3. Ceria nanoparticles

Nano-ceria aqueous sol containing no stabilizer ("naked" ceria nanoparticles) was synthesized by the method proposed by Ivanov et al. [Ivanov et al., 2010b], which comprises microwave hydrothermal processing of the precursor CeO_2 sol formed by the anionite treatment of cerium (III) nitrate aqueous solutions. According to transmission electron microscopy (TEM) data, mean CeO_2 particle size in this sol is about 6 nm; the particles are well-crystallized and have an octahedral shape [Ivanov et al., 2010b].

Citrate-stabilized aqueous sol of CeO_2 was prepared by the method proposed by Ivanov et al. [Ivanov et al., 2010a]. Briefly, 2.0 g of citric acid was mixed with 25 mL of a .4 M aqueous cerium (III) chloride solution. The resulting solution was rapidly poured, under stirring, into 100 mL of a 3 M aqueous ammonia solution, and then exposed for 2 h at ambient conditions and further boiled for 4 h. Then, the solution was cooled to room temperature and purified from precursors and by-products by sedimentation and further redispersion. According to TEM data, the average CeO_2 particle size in this sol was about 2 nm.

2.4. Modified vaccine

The vaccine was modified by the addition of nano-ceria aqueous sols (.01 M) which differed by production method and the presence/absence of a stabilizer. To prepare ceria modified flu vaccine, non-stabilized or citrate-stabilized aqueous ceria sols were added to Vaxigrip vaccine. The weight ratio of hemagglutinin (HA) to nano-ceria was 22.5/8.6 μg/μg. The HA/nanoceria ratio was selected based on previously obtained data concerning the efficiency of ceria–interferon combination [Zholobak et al. 2010].

2.5. Analysis of efficiency and immunogenicity of vaccines in vivo

An immunogenicity study of non-modified and ceria-modified vaccines was performed on albino female mice (Mus musculus) weighing 20–22 g. The animals were marked and grouped seven days prior to vaccination, in order to minimize stress. Animals were