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Effect of Molecular Weight and Degree of Acetylation on Adjuvantive Properties of Chitosan Derivatives

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Abstract—The hemostatic and immunostimulating activity and cytotoxicity were determined for a number of chitosans differing in molecular weight (from 3 to 510 kDa) and degree of acetylation (from 1 to 25 mol%) that were used as adjuvants in inactivated poliomyelitic, influenza, and live influenza vaccines. It has been shown that the hemostatic activity of chitosan increased sharply with an increase in its molecular weight. In oligochitosan with a molecular weight of <16 kDa, it was smaller by a factor of 15–100 than in chitosan with a molecular weight of 20–510 kDa. The level of increase in the immunogenicity of vaccines containing oligochitosan as adjuvants was not lower than that for the vaccine including high-molecular chitosan. However, the immunostimulatory activity of oligochitosan depended on the degree of acetylation, reaching a maximum value at 6 mol%. It was shown that all oligochitosans and chitosans with a molecular mass below ~50 kDa showed almost no cytotoxicity at a concentration of ≤2.5 mg/mL, which enable their use as adjuvants for inactivated and live vaccines at the optimal ratio of molecular weight to the degree of acetylation.

Keywords: chitosan, oligochitosan, vaccine, adjuvant, cytotoxicity, immunostimulating activity
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INTRODUCTION

Vaccination is one of the most effective means of preventing infections. However, the effectiveness of a number of vaccines is still not high enough, especially for the elderly. Thus, the problem of creating highly effective live and inactivated vaccines with increased immunogenicity remains relevant. Live vaccines, in contrast to inactivated ones, induce not only serum IgG antibodies but also secretory IgA antibodies; they are good stimulants of cellular immunity, have a long duration of protective action, and protect against drift variants. However, such vaccines have reduced prophylactic activity for some population groups, in particular, for the elderly, which is explained by their strong immunosuppressive effect. One possible means to increase the effectiveness of vaccines is the use of adjuvants (AD) [1].

Over the past decade, vaccinologists have started using the product of chitin deacetylation: chitosan, a polycationic, low-toxicity, biodegradable, and biocompatible polysaccharide consisting of glucosamine and N-acetylglucosamine units [2]. Chitosan can vary significantly in such characteristics as molecular weight (MW), polydispersity, the degree of deacetylation (DD), and the distribution of residual acetyl groups over the polymer chain, which determines its physicochemical and biological properties [3, 4]. Chitosan is not toxic for homoiothermal animals and plants; it has fungicidal and bactericidal properties. In the European Union, the use of chitosan hydrochloride in the medical, food, and cosmetic industries is permitted.

For the first time in global health practice, studies have been undertaken to increase the immunogenicity of live influenza, cold-adapted (CA) vaccine with high-molecular weight chitosan with an MW of more than 100 kDa as an AD for mucosal and parenteral administration [5–8]. It should be noted that, in practical use, chitosan is divided by MW into high-molecular weight (>500 kDa), medium weight (>100–500 kDa), low-molecular weight (>16–100 kDa), and oligochitosan, which is a product of deep depolymerization of chitosan with MW of 2–16 kDa [9, 10].

Chitosan with optimal properties is required for use in vaccine compositions in order to provide better solubility in physiological environments and low hemagglutination capacity, as well as high titers for the immunogenicity of vaccine preparations.