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Immunogenicity and immunomodulatory properties of HPMA-based polymers ☆

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ABSTRACT

HPMA copolymers are one of the most promising drug carriers as their biophysical and biochemical properties, including their immunocompatibility, are very favorable. So far, there is no evidence that HPMA copolymers can interact with the immune system in a way that would lead either to suppression of some of its crucial functions or to inappropriate activation with possible serious side-effects and thus we can conclude that HPMA copolymers are convincingly proved to be "immunologically" safe. Moreover, it was shown both in mice and humans that HPMA copolymer-bound doxorubicin (DOX–HPMA) conjugates possess besides powerful anti-tumor effect also various immunomodulatory properties and exert significantly decreased side-toxicities, minimized bone marrow toxicity and cardiotoxicity being the most important ones. The possibility to induce potent and long-lasting tumor-specific immunity during the treatment with these compounds which is capable to provide protection against minimal residual disease is one of the most important and therapeutically valuable features of these conjugates.

Contents

1. Introduction

The potential for treating cancer patients by immunologic approaches has held great promise for oncologists and immunologists for many years. One of the hallmark features of an effective immunotherapy is its ability to stimulate lasting tumor-specific immunity. Immunopharmacological agents or immunomodulators can conventionally be divided to immunosuppressive and immunostimulating drugs which correspond to the therapeutic need. The medicinal purpose of immunostimulators may be to support rejection of cancer cells. The field of pharmacological immunostimulation, especially with nanotherapeutics, is still at a very early stage of development and systems with dual, i.e. cytostatic and immunomodulating activity are rare and at the very beginning of their fundamental understanding and wide exploitation. Polymeric therapeutics based on HPMA is reported to have such a dual activity. They were proven not to be immunogenic/antigenic in the sense that they do not stimulate cellular or humoral immune response against itself. Originally it was thought that they mediate their tumoricidal activity only by exerting a direct effect on tumor cells. The recent evidence indicates, however, that they are not immunologically silent and that

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the treatment triggers in host specific systemic anti-cancer immune response contributing greatly to the therapy outcome.

2. Immunogenicity of poly(HPMA) and HPMA copolymers

An antigen is any substance that may be specifically bound to an antibody molecule or T cell receptor. Antibodies can recognize as antigens almost every kind of biological molecule, including saccharides, lipids, nucleic acids and, as we will see later, synthetic polymers. This is in contrast to T cells which recognize solely peptides. Although all antigens are recognized by specific lymphocytes or by antibodies, only some of them are capable of stimulating immune response against them. Such antigens are called immunogens.

Antibodies bind to only a portion of the macromolecule which is called the immunodominant epitope or determinant. Macromolecules typically contain multiple determinants, some of which may be repeated. The presence of multiple determinants in an antigen is referred to as polyvalency or multivalency.

The initiation and development of an adaptive immune response require that antigens are captured and displayed to specific lymphocytes. The cells that serve this role are called antigen-presenting cells (APCs). The other cells of the adaptive immune system are T lymphocytes (mediators of cellular immunity) and B lymphocytes (mediators of humoral immunity). B lymphocytes, which can develop in antibody producers (plasma cells) can be effectively activated either by monovalent antigens but only with the help of T lymphocytes or by polyvalent antigens that induce clustering of the B cell receptor.

The factors important for polymer–drug conjugate design are now rather well established. The polymeric carrier must be biocompatible and immunocompatible (immunoacceptable). Thus, the homopolymer of *N*-(2-hydroxypropyl)methacrylamide (HPMA) and its copolymers, which differ in the structure and number of introduced side chains, were studied for their possible use in human medicine as carriers. Factors such as composition and size of the polymeric carrier, dose, and the way (subcutaneous, intravenous, intraperitoneal or oral) and frequency of its administration were carefully analyzed as well as the inherited ability (Ir genes) of the host to respond to a tested material [1–5].

The homopolymer poly(HPMA) was developed as blood expander under the commercial name "DUXON" [6]. Soon after that, however, copolymers of HPMA were considered to be promising drug carriers. It was revealed that the homopolymer poly(HPMA) with an estimated average molecular weight around 30 kDa is not recognized as an antigen and also does not act as an immunogen. No defense reaction against it was recorded [1–4]. ^{14}C-pHPMA showed lack of mitogenicity, hepatotoxicity and immunogenicity [7] and when a non-radioactive derivative was injected *in vivo*, afferent lymph nodes were not activated [8]. Intraperitoneal administration of alum precipitate in doses of 1–100 μg did not induce the formation of detectable level of antibodies in any of the five inbred strains of mice tested with four different haplotypes (C3H = H-2^k; A/J = H-2^a; BALB/c = H-2^d; C57BL/ScSn = H-2^b; C57L/J = H-2^b) and in two strains of the same haplotype (C57L/J and C57BL/10ScSn) but differing in the remaining genetic background [1–4]. In this respect poly(HPMA) resembles the synthetic polymers of one or two alpha amino acids, which also were proven to be non-immunogenic in mice. It was revealed that an important attribute for the immunogenicity of an injected material is a certain degree of its heterogeneity as more complex polymers were more immunogenic [9].

The attachment of side oligopeptidic sequences to the HPMA backbone bestows a certain degree of immunogenicity on such a copolymer molecule. The immunogenicity and mitogenic activity of copolymers differing in oligopeptide side chains (–Gly-Gly–OH; –Acap-Phe–OH; –Acap-Leu–hexamethylenediamine (HMDA), –Gly-Phe-Tyr–OH and –Gly-Phe-Leu-Gly) or in their content (1%, 3.5% and 8.4% mole of –Gly-Gly–OH) was tested in the same five inbred strains of mice as mentioned above. Immunization with doses ranging from 1–100 μg induced regularly only a very weak antibody response despite the fact that not only solution and alum precipitate but also complete Freund's adjuvant was used as a vehicle. On average, the titer of the antibodies was lower by four orders of magnitude than that of antibodies against the reference bovine gamma globulin (BGG). The number and composition of side oligopeptidic chains was not so important for the intensity of the reaction, as a very similar response to different polymeric conjugates was obtained. Also, no substantial effect of H-2 haplotype (Ir genes) on the intensity of the reaction was seen [1–5].

If the end of the oligopeptidic side chain was modified with a drug-resembling substance that behaved as a hapten (dinitrophenyl, DNP; arsanilic acid, ARS; fluorescein isothiocyanate, FITC) a significant immune response was detected by plaque-forming (antibody-forming) cells (PFC), ELISA and haemagglutination. Haptens are small chemicals which may bind antibodies and are therefore antigens but cannot activate B cells on their own, i.e. they are not immunogens. Most of these antibodies were aimed against the modifying haptenic group and only a low amount against the side oligopeptidic sequences of the carrier. This means that the main immunodominant epitope (determinant) of the copolymer molecule is a derivatized oligopeptide side chain which also determines the specificity of formed antibodies [1–5].

The dependence of the immune reaction on the molecular weight of the copolymer was studied in experiments where a copolymer with –Acap-Leu–HMDA-ARS side chains was used as an antigen and the immune reaction was expressed by the number of antibody-forming cells (PFC). Compared with the fraction of molecular weight 5 kDa, fraction with molecular weight between 150–200 kDa brought about a 2- to 5-fold increase in the number of antibody-forming cells in the spleen [2,10]. The most likely explanation is that the low molecular weight fractions are rapidly removed from the blood circulation which decreases the chance of immunocompetent cells to come into contact with tested foreign material.

Before introducing polymeric prodrugs based on HPMA into phase I and II clinical trials both non-targeted (PK1; FCE 28068) and galactosamine-targeted (PK2; FCE 28069) derivatives were thoroughly tested for their anti/immunogenicity. Antibody response of two different inbred strains of mice (high IgG responders A/J = H-2^a, Th_2-prone and low IgG responders C57BL/10 = H-2^b, Th_1-prone [11] was compared by ELISA test on days 3 and 6 after the treatment. The increase in the antibody production did not exceed one or two dilutions of sera even after multiple immunizations, which confirmed that the immunogenicity of the injected material is very low. Intravenous, subcutaneous and oral immunization produced similar antibody titers [12].

Determination of the potential to activate/inhibit complement can be used as one criterion in testing the biocompatibility of synthetic materials. It was reported that only a very high concentration of 20 g/l of the homopolymer and/or copolymers differing in oligopeptidic side chains (–Gly-Gly; –Acap-Phe; –Gly-Phe-Tyr; –Gly-Leu-Phe and –Acap-Leu) terminating in carboxylic acidic groups, amine groups, aromatic units or puromycin activates complement pathways while lower concentrations (2 g/l or 0.2 g/l) do not activate/inhibit either the classical or the alternative complement activation pathway [13]. The obtained results proved that the inhibition of both pathways of complement system occurs only at concentrations highly exceeding the dose needed for therapeutic purposes.

The possible harmful effect of massive and chronic treatment with synthetic material on its recipient is still the subject of an intensive discussion as large doses of foreign synthetic and sometimes only partially degradable material may induce short or long-lasting blockage of the reticuloendothelial system. We have injected a water-soluble copolymer based on HPMA copolymer once a week (a total dose 2 g/kg body weight was administered during two months) and an insoluble polymer based on 2-hydroxyethyl methacrylate (HEMA) (2.5×10^9 particles/mouse). During the time course of prolonged large dose