Cell-Penetrating Peptide-Linked Polymers as Carriers for Mucosal Vaccine Delivery

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ABSTRACT SUMMARY

We evaluated the potential of poly(N-vinylacetamide-co-acrylic acid) modified with D-octaarginine as a carrier for mucosal vaccine delivery. When a physical mixture of ovalbumin (OVA) and the polymer was nasally administered repeatedly to mice, the production of serum OVA-specific immunoglobulin G (IgG) and intranasal secreted immunoglobulin A (IgA) was induced. A similar immunization profile was observed when OVA was replaced with influenza virus HA vaccines. The polymer-induced immune response was inferior to that induced by an equivalent dose of the cholera toxin B subunit (CTB), which was used as a positive control; however, dose escalation of the polymer completely overcame this disadvantage.

INTRODUCTION

Intranasal vaccines have been studied as one of the most effective and adaptive immunization strategies for protecting hosts against infectious pathogens that invade epithelial cells at the mucosa such as the influenza virus. This strategy is expected to provide significant production of antigen-specific IgG and secreted IgA in the serum and at the mucosa, respectively. IgG, whose production is also enhanced by systemic immunization with subcutaneous or intramuscular vaccine administration, prevents severe infection-related symptoms. Mucosal immunity is mainly mediated by secreted IgA, whose production is not stimulated by conventional parenteral immunization.

Secreted IgA represents the first immunological barrier to pathogens that infect the epithelial surface. However, because most antigens are poor immunogens when solely applied to the mucosa, adjuvants/antigen carriers, which are co-administered with vaccines, are essential for significant induction of immunity. Toxin-based adjuvants, such as CTB, were first used as adjuvants for mucosal vaccines. The entero-toxins enhanced the production of antigen-specific IgG in the serum and secreted IgA at the mucosa; however, the clinical use of them is not practical because of their toxicity. Safe and effective adjuvants/antigen carriers are needed for establishment of mucosal vaccines.

We have been independently investigating cell-penetrating peptide-linked polymers as a new class of penetration enhancers. Cell-penetrating peptides are oligopeptides that are mainly composed of cationic amino acids such as arginine, and most typical ones are the HIV-1 Tat protein (48–60) and oligoarginine. It is considered that cell-penetrating peptides are predominantly internalized into cells via macropinocytosis. Fig. 1 shows the chemical structure of our polymer: poly(N-vinylacetamide-co-acrylic acid) (PNVA-co-AA) modified with oligoarginine. This biocompatible polymer was designed with a unique strategy that the polymers enable poorly membrane-permeable bioactive molecules physically mixed with them to effectively penetrate cell membranes without their concomitant cellular uptake.

Our previous studies¹² revealed that oligoarginine-linked PNVA-co-AA significantly enhanced in vivo permeation of insulin through the nasal membrane in mice. The order of the penetration-enhancing function of oligoarginine anchored to PNVA-co-AA was 8 arginine residues ≥ 12 residues and D-form ≥ L-form. No penetration enhancement was observed when the polymer was substituted with its individual components such as intact D-octaarginine; this demonstrated that only D-octaarginine anchored chemically to the polymer platform enhanced membrane permeation of poorly membrane-permeable bioactive molecules. In vitro cell studies indicated that D-octaarginine-linked PNVA-co-AA remained on the cell membrane and the bioactive molecules were continuously internalized into cells mainly via macropinocytosis repeated for the individual peptidyl branches in the polymer backbone, as expected.

The unique characteristics of oligoarginine-linked polymers potentially facilitate the intracellular delivery of bioactive molecules. Here, we report the potential of our polymer as a carrier for mucosal vaccine delivery.

![Fig. 1. Chemical structure of oligoarginine-linked PNVA-co-AA.](image)

EXPERIMENTAL METHODS

D-Octaarginine was linked to PNVA-co-AA (Mw: ca. 1600 kDa, N-vinylacetamide/acrylic acid: 70/30) by coupling the amino groups of D-octaarginine with the carboxyl groups of the polymer backbone. Instrumental analysis confirmed that the D-octaarginine unit was introduced onto the polymer backbone with a linkage level of 11% (ca. 37% of carboxyl groups of PNVA-co-AA was occupied with D-octaarginine branches).

OVA and influenza virus HA vaccines were used as a model antigen. PBS was used as a vehicle. Mice were
nasally inoculated repeatedly every 7th day with PBS containing antigens (a negative control), PBS containing antigens and CTB (a positive control), or PBS containing antigens and D-octaarginine-linked PNVA-co-AA (n = 4 in each). Seven days after the final inoculation, mice were sacrificed and sera and nasal wash fluids were collected.

Expression levels of antigen-specific IgG and secreted IgA in sera and nasal wash fluids, respectively, were monitored. Titers of antigen-specific antibodies were measured using the ELISA method and endpoint titers of the antibodies were determined from the x-axis intercept of the dilution curve. Each value of endpoint titers, which increases with an elevation of the antibody level, is presented as the mean and standard deviation.

RESULTS AND DISCUSSION

Doses of OVA, D-octaarginine-linked PNVA-co-AA, and CTB were first set to 10 μg/mouse. OVA-specific IgG was detected in sera of a quarter of mice to which OVA was solely administered twice. When mice were inoculated twice with PBS containing OVA and CTB, the enterotoxin-induced IgG production was clearly observed in all mice. Three mice were immunized when OVA was co-administered twice with D-octaarginine-linked PNVA-co-AA; however, the polymer-induced IgG production was not observed in the remaining mouse. OVA-specific secreted IgA was not detected in nasal wash fluids when 2 inoculations of OVA alone were performed; an increase in the number of inoculations hardly ever affected the immune response. On the other hand, a clear elevation of the IgA level was observed in half the number of mice inoculated 4 times with PBS containing OVA and D-octaarginine-linked PNVA-co-AA.

A reduction of the OVA dose (1 μg/mouse) resulted in the complete disappearance of the immune response in mice inoculated with the antigen alone even when the number of inoculations was set to 4. The serum IgG level clearly elevated when OVA was co-administered 4 times with either D-octaarginine-linked PNVA-co-AA or CTB. The polymer-induced intranasal IgA production was also observed; however, the antibody level was considerably lower than that observed in mice inoculated 4 times with PBS containing OVA and CTB. As shown in Fig. 2, the IgA level clearly elevated with an increase in the polymer dose. A similar dose dependency was observed, irrespective of the antibody type. Antibody levels still differed significantly from those observed in the positive control; however, the difference was also comparable when the polymer dose was 4 times that of CTB. We considered that OVA transferred to systemic circulation enhanced IgG production and that OVA internalized into nasal epithelial cells stimulated IgA production.

In order to examine the effect of the antigen type on the D-octaarginine-linked PNVA-co-AA-induced immune response, influenza virus HA vaccines, which are clinically used, were substituted for OVA. The antibody production was barely induced when mice were inoculated 4 times with the vaccine alone at a dose of 0.05 μg/mouse. As shown in Fig. 3, D-octaarginine-linked PNVA-co-AA clearly induced both mucosal and systemic immune responses against the vaccines, as did CTB. No statistical significance was observed for the levels of both serum IgG and intranasal secreted IgA at a polymer dose of 100 μg/mouse. When the number of inoculations was doubled, further elevation of the IgA level was observed; however, the IgG level reached the plateau.

Fig. 2. Levels of OVA-specific IgG in sera (a) and OVA-specific secreted IgA in nasal wash fluids (b) of mice. Mice were nasally inoculated 4 times with PBS containing OVA (left hashed bar), PBS containing OVA and D-octaarginine-linked PNVA-co-AA (black bar), or PBS containing OVA and CTB (right hashed bar). Doses of OVA, D-octaarginine-linked PNVA-co-AA, and CTB were set to 1 μg, 5–40 μg, and 10 μg, respectively/mouse.

Fig. 3. Levels of influenza virus HA vaccine-specific IgG in sera (a) and the vaccine-specific secreted IgA in nasal wash fluids (b) of mice. Mice were nasally inoculated 4 times with PBS containing the vaccine (left hashed bar), PBS containing the vaccine and D-octaarginine-linked PNVA-co-AA (black bar), or PBS containing the vaccine and CTB (right hashed bar). Doses of the vaccine, D-octaarginine-linked PNVA-co-AA, and CTB were set to 0.05 μg, 40–100 μg, and 10 μg, respectively/mouse.

CONCLUSION

D-octaarginine-linked PNVA-co-AA is a potential candidate for antigen carriers that induce humoral immunity on the mucosal surface and in systemic circulation when nasally co-administered with antigens.

REFERENCES