International Journal of Pharmaceutics 483 (2015) 110–114

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical nanotechnology

Anti-cancer vaccination by transdermal delivery of antigen peptide-loaded nanogels via iontophoresis

Mao Toyoda [a], Susumu Hama [a], Yutaka Ikeda [b], Yukio Nagasaki [b], Kentaro Kogure [a,*]

[a] Department of Biophysical Chemistry, Kyoto Pharmaceutical University, Kyoto 607-8414, Japan
[b] Graduate School of Pure and Applied Sciences, University of Tsukuba, Ibaraki 305-8573, Japan

ARTICLE INFO

Article history:
Received 8 December 2014
Received in revised form 25 January 2015
Accepted 10 February 2015
Available online 11 February 2015

Keywords:
Iontophoresis
Anti-cancer vaccine
Transdermal delivery
Nanogel

ABSTRACT

Transdermal vaccination with cancer antigens is expected to become a useful anti-cancer therapy. However, it is difficult to accumulate enough antigen in the epidermis for effective exposure to Langerhans cells because of diffusion into the skin and muscle. Carriers, such as liposomes and nanoparticles, may be useful for the prevention of antigen diffusion. Iontophoresis, via application of a small electric current, is a noninvasive and efficient technology for transdermal drug delivery. Previously, we succeeded in the iontophoretic transdermal delivery of liposomes encapsulating insulin, and accumulation of polymer-based nanoparticle nanogels in the stratum corneum of the skin. Therefore, in the present study, we examined the use of iontophoresis with cancer antigen gp-100 peptide KVPRNQDWL-loaded nanogels for anti-cancer vaccination. Iontophoresis resulted in the accumulation of gp-100 peptide and nanogels in the epidermis, and subsequent increase in the number of Langerhans cells in the epidermis. Moreover, tumor growth was significantly suppressed by iontophoresis of the antigen peptide-loaded nanogels. Thus, iontophoresis of the antigen peptide-loaded nanogels may serve as an effective transdermal delivery system for anti-cancer vaccination.

1. Introduction

Anti-cancer vaccination is expected to become an effective anti-cancer therapy (Aranda et al., 2013). Induction of an immune response against a specific tumor requires the administration of antigen proteins or peptides derived from cancer cells directly into the skin or muscle. Intradermal injection is especially an effective administration method, because the antigen presenting Langerhans cells (LCs) exist in the epidermis (Tay et al., 2013). However, injection of antigen solution with a needle is invasive. Moreover, as intradermal injection is also difficult, special techniques are required for assured administration of the antigen solution into the epidermis. Iontophoresis has attracted attention in this regard as an effective and non-invasive transdermal delivery technology that can overcome the barriers associated with intradermal injection (Schoellhammer et al., 2014; Wong, 2014; Gratieri et al., 2013). In fact, non-invasive transdermal delivery of insulin by iontophoresis has been reported (Dixit et al., 2007). Iontophoresis is known as an effective technology for the physical stimulation of transdermal permeation of charged substances by the application of a small electric current (Schoellhammer et al., 2014; Wong, 2014; Gratieri et al., 2013). However, substances, such as antigen proteins and peptides, delivered by iontophoresis can also diffuse into the skin and muscle. As accumulation of the antigen in the skin is required for effective exposure to Langerhans cells, devices that can concentrate the antigen within restricted regions of the skin may be useful for effective induction of immune responses mediated by antigen capture of Langerhans cells. Carriers such as liposomes and nanoparticles, which are used in the intravenous administration of drugs, can also be valuable in the transdermal delivery of antigens into the skin. Recently, we succeeded in the iontophoretic transdermal delivery of hydrophilic macromolecules, such as siRNA and CpG oligoDNA, and liposomes (Kigasawa et al., 2011, 2010; Kajimoto et al., 2011). Based on a mechanistic analysis, transdermal penetration of such macromolecules and nanoparticles induced by applying a small electric current was attributed to cleavage of the intercellular junction via Ca^{2+}-mediated activation of cellular signaling (Hama et al., 2014). Thus, it is expected that iontophoresis can deliver antigen-loaded carriers into the epidermis, where Langerhans cells exist.

It was previously reported that Langerhans cells extend their dendrites to capture antigens located within the stratum corneum (Kubo et al., 2009). Thus, effective exposure of antigens by

* Corresponding author at. Department of Biophysical Chemistry, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan. Tel.: +81 75 595 4663; fax: +81 75 595 4762.
E-mail address: kogure@mb.kyoto-phu.ac.jp (K. Kogure).

http://dx.doi.org/10.1016/j.ijpharm.2015.02.024
0378-5173/

Langerhans cells may be accomplished by accumulation of antigen-loaded carriers within the surface region of the epidermis via iontophoresis. With regard to carriers, accumulation of nanogels exhibiting small (<100 nm) and rigid core structures is more preferable in the stratum corneum than liposomes that exhibit flexible structures (Hama et al., 2014), because rigid nanogels can hook into the intercellular space.

Based on these considerations, we hypothesize that cancer antigen-loaded nanogels accumulated in the surface region of the epidermis by iontophoresis should exhibit the ability to be captured by Langerhans cells, resulting in the subsequent induction of an anti-cancer effect via activation of an immune response. To validate our hypothesis, we herein examine the iontophoretic delivery of nanogels modified with antigen peptides into the epidermis, and the subsequent effect on tumor growth of mouse melanoma.

2. Materials and methods

2.1. Materials

Poly(ethylene glycol) (PEG)-modified nanogels were synthesized by the redox-emulsion copolymerization of 2-(*N*,*N*-diethylamino) ethyl methacrylate (DEAMA), using methoxy-PEG macromonomers as both a surfactant and a comonomer and ethylene glycol dimethacrylate (EGDMA) as the cross-linker in the presence of potassium persulfate (KPS), as previously reported (Tamura et al., 2009). Antigen peptide hgp-100_{25-33} containing a tetra glutamic acid moiety (KVPRNQDWL-EEEE) and KVPRNQDWL-EEEE labeled with the fluorescent dye fluorescein amidite (FAM-labeled KVPRNQDWL-EEEE) were synthesized by Scrum Inc. (Tokyo, Japan). Anti-mouse MHC class II (I-A/I-E) biotin antibody was purchased from eBioscience Inc. (San Diego, CA). Streptavidin–FITC from *Streptomyces avidinii* was purchased from Sigma Aldrich Co., LLC. (St. Louis, MO). The devices used for iontophoresis (TCTTM) were manufactured by TTI ellebeau Inc. (Tokyo, Japan). 2-Morpholinoethanesulfonic acid, monohydrate (MES) was purchased from Dojindo Laboratories (Kumamoto, Japan). B16-F1 cells, a mouse melanoma cell line, were obtained from DS Pharma Biomedical Co., Ltd. (Osaka, Japan). Male Hos:HR-1 hairless mice were purchased from SHIMIZU Laboratory Supplies Co., Ltd. (Kyoto, Japan). All mice were maintained and used in accordance with the animal protocol approved by the institutional Animal Care and Use Committee, Kyoto Pharmaceutical University (Kyoto, Japan).

2.2. Iontophoresis

Iontophoresis of nanogels was conducted according to our previously published reports (Hama et al., 2014). For preparation of the antigen peptide-loaded nanogels, 46.5 μl of the antigen peptide solution (10 mg/ml) was added to 307 μl of the nanogel suspension (45 mg/ml), and gently mixed with a micropipettor. After mixing, 46.5 μl of 10 mM 2-morpholinoethanesulfonic acid (MES) buffer (pH 6.5) was added to the suspension. The ratio of peptide to nanogel was optimized so as to avoid a significant decrease in the surface charge of nanogels, based on measurements of zeta-potentials of various peptide/nanogel mixtures. Briefly, mice were anesthetized by intraperitoneal injection of Somnopentyl. For administration of nanogels, nonwoven fabric (0.785 cm^2) containing a suspension of nanogels or peptide-loaded nanogels (14 mg) was placed on the dorsal skin and a second nonwoven fabric moistened with saline was placed 1 cm away. The nonwoven fabrics containing the nanogels and saline were connected to the anode and cathode, respectively, of a power supply (TTI ellebeau, Inc., model TCCR-3005, Tokyo, Japan), with Ag–AgCl electrodes, and the connections were covered with tape. A current density of 0.4 mA/cm^2 was applied for 1 h. The skin was excised 3 h after application of the electric current, and used for cross section preparation.

2.3. Confocal laser scanning microscopy

To evaluate nanogel and peptide delivery, mouse skin tissue following iontophoresis was embedded into optimal cutting temperature (O.C.T) compound, and tissue sections (16 μm thickness) were prepared with a LEICA CM1100 cryostat (Leica Microsystems, Germany). Images were obtained by confocal laser scanning microscopy (CLSM) with a LSM 510 META microscope (Carl Zeiss Co., Ltd., Germany) equipped with an objective lens (EC Plan-Neofluar 10 × 0.3 M27).

2.4. Immunohistochemistry

Following treatment, tissue sections were fixed in phosphate-buffered saline (PBS) containing 4% paraformaldehyde for 15 min at room temperature, washed with PBS, permeabilized in PBS containing 1% Triton X-100 for 10 min at room temperature, and blocked in PBS containing 0.5% fetal bovine serum (FBS). Tissue sections were then incubated with anti-mouse MHC class II (I-A/I-E) biotin antibodies overnight at room temperature in a moist chamber. Streptavidin–FITC was used for labeling anti-mouse MHC class II (I-A/I-E) biotin antibodies (1 h incubation at 37 °C). After washing, tissue sections were mounted in PBS containing 80% glycerol and VECTASHIELD (Vector Laboratories, Inc., Burlingame, CA). Images were observed by CLSM, as described above.

2.5. Tumor growth inhibition

The B16-F1 cell-bearing mice were prepared according to our previously published report (Hama et al., 2012). The B16-F1 cell suspension (1×10^6 cells) was mixed with ECM gel obtained from Sigma Aldrich Co., LLC. (St. Louis, MO) at a ratio of 5:1 (v/v), and the cells were inoculated under the skin of mice that were six to eight weeks old (day 0). From three to nine days after tumor inoculation, iontophoresis of antigen-loaded nanogels was performed three times every three days. Tumor volume was determined according to the formula T_{vol} = length × width^2 × 0.5.

2.6. Statistical analysis

Statistical significance was determined using one-way ANOVA followed by Turkey–Kramer HSD test. P values <0.05 were considered to be significant.

3. Results and discussion

3.1. Iontophoresis of nanogels

Accumulation of the nanogels in the surface region of the epidermis was confirmed by iontophoresis of nanogels labeled with rhodamine. The physicochemical properties of the nanogels are summarized in Table 1. The average size and zeta-potential of

Table 1
Physicochemical properties of nanogels and antigen peptide-loaded nanogels.

	Size (nm)	Zeta-potential (mV)
Nanogel	67.3 ± 11.4	17.7 ± 7.6
Nanogel/peptide	67.8 ± 15.1	15.2 ± 7.5

The data are average (±SD) of three measurements.