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Research paper

Specific uptake mechanisms of well-tolerated thermoresponsive polyglycerol-based nanogels in antigen-presenting cells of the skin

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ABSTRACT

Engineered nanogels are of high value for a targeted and controlled transport of compounds due to the ability to change their chemical properties by external stimuli. As it has been indicated that nanogels possess a high ability to penetrate the stratum corneum, it cannot be excluded that nanogels interact with dermal dendritic cells, especially in diseased skin. In this study the potential crosstalk of the thermoresponsive nanogels (tNGs) with the dendritic cells of the skin was investigated with the aim to determine the immunotoxicological properties of the nanogels. The investigated tNGs were made of dendritic polyglycerol (dPG) and poly(glycidyl methyl ether-co-ethyl glycidyl ether) (p(GME-co-EGE)), as polymer conferring thermoresponsive properties. Although the tNGs were taken up, they displayed neither cytotoxic and genotoxic effects nor any induction of reactive oxygen species in the tested cells. Interestingly, specific uptake mechanisms of the tNGs by the dendritic cells were depending on the nanogels cloud point temperature (Tcp), which determines the phase transition of the nanoparticle. The study points to caveolae-mediated endocytosis as being the major tNGs uptake mechanism at 37 °C, which is above the Tcp of the tNGs. Remarkably, an additional uptake mechanism, beside caveolae-mediated endocytosis, was observed at 29 °C, which is the Tcp of the tNGs. At this temperature, which is characterized by two different states of the tNGs, macropinocytosis was involved as well. In summary, our study highlights the impact of thermoresponsivity on the cellular uptake mechanisms which has to be taken into account if the tNGs are used as a drug delivery system.

1. Introduction

In recent years, nanomaterials have been increasingly introduced in pharmaceutical and medical use. These nanomaterials are of interest as carriers of specific drugs and compounds due to their unique characteristics [1]. Especially nanogels, which are able to change their chemical properties by external stimuli, may enable a targeted and controlled drug transport. Nanogels can significantly increase drug penetration depth compared to conventional formulations, in order to overcome strong biological barriers like the human skin [2–4]. Therefore, nanogels are a powerful drug delivery candidate for the treatment of severe skin diseases. As nanogels are able to penetrate into viable layers of human skin, it cannot be excluded that penetrating nanogels are taken up by antigen-presenting cells of the skin [5,6]. This emphasizes the need of a comprehensive toxicological characterization and identification of uptake and interaction mechanisms of nanogels in dendritic cells. Dendritic cells are a highly potent antigen-presenting cell population. In skin epidermis, Langerhans cells (LCs) are the most prominent dendritic cell subset and represent the first immunological barrier for nanoparticles, which have surmounted the skin barrier. It is known that nanoparticles can induce an immune response in LCs and thereby induce the expression of costimulatory molecules and secretion of proinflammatory cytokines [7]. In contrast to vaccine formulations, the induction of an immunological response caused by nanoparticle-based treatment of skin diseases is undesired and can result in adverse reactions [8–10]. Therefore, if used for the treatment of skin diseases, it is

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crucial, that nanogels are highly biocompatible and do not cause an adverse response in LCs of the skin.

The term gel refers to polymer chains which are interconnected in a crosslinked network and are able to swell in a good solvent [11,12]. The existence of such network in particulate formulation in a nanometer scale is referred as nanogels. Thermoresponsive nanogels (tNG) in particular are nanogels fabricated of thermoresponsive polymers possessing, for instance, a lower critical solution temperature [13]. Poly(glycidyl methyl ether-co-ethyl glycidyl ether) (p(GME-co-EGE)), a thermoresponsive linear polyglycerol derivate (tPG) was the thermoresponsive polymer utilized for the synthesis of the tNGs tested in this study. The thermoresponsive polymer tPG, provide unique characteristics to the tNGs which facilitate their swelling in a hydrophilic state, below the cloud point temperature (Tcp) of the polymer, and their shrinkage into a hydrophobic state above the Tcp. This feature makes the tPG-based nanogels very interesting for specific drug transport and release. Besides, the tPG-based nanogels possess high loading capacity, biocompatibility, and physical stability in biological environment [14]. Moreover, tPG-based nanogels can be loaded with water-soluble drugs as well as biomacromolecules, which can be achieved by covalent conjugation, incorporation or by adsorption.

To the best of our knowledge the crosstalk between tPG-based nanogels and LCs has not been examined, so far. Yet, the topical use in dermatotherapy will become possible only, if biocompatibility of these nanogels with the antigen-presenting cells is safeguarded.

2. Materials and methods

2.1. Synthesis of nanogels

Thermoresponsive nanogels were synthesized and characterized according to previously reported methods [14]. Briefly, for the synthesis of tPG-based nanogels (tNG_dPG_tPG$_{(GME:EGE)}$), dPG functionalized with (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethyl carbonate (dPG-BCN$_{8\%}$) (10 mg) and di-azide functionalized tPG (tPG-$(N_3)_2$) (20 mg) were mixed in 1 mL of dimethylformamide (DMF), cooled in an ice bath and injected with a syringe into 20 mL of water at 45 °C. tNG_dPG_tPG$_{(3:1)}$ nanogels were synthesized in the exact same manner while the polymer solution in DMF was injected into water at 65 °C. The mixture was stirred for three hours and the unreacted alkynes were quenched with azidopropanol or alternatively with indodicarbocyanine azide (IDCC-N_3). The products were purified by dialysis (Molecular weight cut-off (MWCO) 50 kDa membrane) in water for at least two days. Benzoylated regenerated cellulose membrane purchased from Sigma-Aldrich (Schnelldorf, Germany), 2 kDa MWCO, and regenerated cellulose membrane purchased from Spectra/Por®, 50 kDa MWCO were used to perform dialysis. Typically dialysis was carried out for 48 h with 1 L of solvent that was exchanged after first 6 h of the process. Size exclusion chromatography (SEC) was performed with Sephadex G 25 Fine from GE Healthcare (Freiburg, Germany). The material was activated by swelling in the respective eluent prior to performing chromatography. Thin layer chromatography (TLC) was performed on Merck aluminum sheets with silica (corn size 60) and fluorescence marker (F254).

3. Characterization of nanogels

All tNGs were characterized by ^{1}H nuclear magnetic resonance (^{1}H NMR), dynamic light scattering (DLS), and UV–Vis spectroscopy. Size, size distribution, and ζ potential of tNGs were measured at various temperatures by dynamic light scattering using a Nano-ZS 90 (Malvern, UK) equipped with a He–Ne laser (λ = 633 nm) under scattering of 173°. All the samples were maintained for stabilization at the designed temperature for 5 min before testing. Particle sizes and size distribution are given as the average of 3 measurements from the intensity distribution curves.

Cloud point temperatures were measured on a Cary 100 Bio UV–Vis spectrophotometer equipped with a temperature-controlled, six-position sample holder. Nanogel solutions in phosphate buffer pH 7.4 were heated at 0.2 °C/min while monitoring both the transmission at 500 nm (1 cm path length) and the solution temperature (from 20 to 60 °C), as determined by the internal temperature probe. The Tcp of each nanogel is defined as the temperature at the inflection point of the normalized transmission curves.

3.1. Materials

Iscove's modified Dulbecco's medium (IMDM) with GlutaMAX® was obtained from Thermo Fisher (Darmstadt, Germany). Fetal calf serum (FCS) superior, penicillin and streptomycin were from Biochrom (Berlin, Germany). Recombinant mouse granulocyte-macrophage colony-stimulating factor (rm-GM-CSF) was purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Phorbol myristate acetate, dynasore hydrate, LY294002 hydrochloride, Genistein, Rottlerin and silver nanoparticles sized 40 nm in aqueous buffer with sodium citrate as stabilizer were obtained from Sigma-Aldrich (Schnelldorf, Germany). Sodium dodecyl sulfate (SDS) and bovine serum albumin (BSA) were obtained from Carl Roth (Karlsruhe, Germany).

3.2. Cell culture

The long term immature LC cell line XS52 was kindly provided from G. Müller (Mainz, Germany). The NS47 cell line was kindly provided from A. Takashima (University of Toledo, OH, USA). The XS52 cells were cultivated as described elsewhere [15]. Briefly, XS52 cells were cultured in IMDM with GlutaMAX® supplemented with 10% FCS superior, 100 IU/mL penicillin, 100 µg/mL streptomycin, 50 U/mL rm-GM-CSF and 10% NS47 fibroblast supernatant in a humidified incubator with 5% CO_2 at 37 °C. The NS47 fibroblast supernatant was obtained by the culture of the fibroblastic stromal NS47 cells in IMDM with GlutaMAX® supplemented with 10% FCS superior, 100 IU/mL penicillin and 100 µg/mL streptomycin in a humidified 5% CO_2 incubator at 37 °C until reaching a confluence grade of 90%. Then the supernatants were collected and used as a supplement for the XS52 culture medium.

3.3. MTT assay

The cytotoxic effects of the tNGs were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay, which is based on the ability of viable cells to reduce MTT to formazan by cellular oxidoreductase enzymes. Thus, the amount of produced formazan reflects the number of viable cells [16]. In short, 10×10^3 cells/well were seeded in 96-well tissue culture plates and incubated for 24 h. The medium was then replaced with fresh basal medium (2.5% FCS superior, 100 IU/mL penicillin and 100 µg/mL streptomycin) containing the tNGs with the respective concentrations (50, 500 µg/mL). After 24 h, MTT was added to each well at a final concentration of 0.5 mg/mL for 4 h. The medium was removed and the formazan crystals were dissolved by incubation in 50 µL DMSO for 10 min. UV absorbance of formazan was measured at 540 nm using a FLUOstar Omega (BMG Labtech, Ortenberg, Germany). Cell viability was expressed as the absorbance of cells exposed to the tNGs compared to the absorbance of cells grown under identical conditions but not exposed