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The impact of cationic solid lipid nanoparticles on human neutrophil activation and formation of neutrophil extracellular traps (NETs)

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ABSTRACT

Cationic solid lipid nanoparticles (cSLNs) are extensively employed as the nanocarriers for drug/gene targeting to tumors and the brain. Investigation into the possible immune response of cSLNs is still lacking. The aim of this study was to evaluate the impact of cSLNs upon the activation of human polymorphonuclear neutrophil cells (PMNs). The cytotoxicity, pro-inflammatory mediators, Ca^{2+} mobilization, mitogen-activated protein kinases (MAPKs), and neutrophil extracellular traps (NETs) as the indicators of PMN stimulation were examined in this work. The cSLNs presented a diameter of 195 nm with a zeta potential of 44 mV. The cSLNs could interact with the cell membrane to produce a direct membrane lysis and the subsequent cytotoxicity according to lactate dehydrogenase (LDH) elevation. The interaction of cSLNs with the membrane also triggered a Ca^{2+} influx, followed by the induction of oxidative stress and degranulation. The cationic nanoparticles elevated the levels of superoxide anion and elastase by 24- and 9-fold, respectively. The PMN activation by cSLNs promoted the phosphorylation of p38 and Jun-N-terminal kinases (JNK) but not extracellular signal-regulated kinases (ERK). The imaging of scanning electron microscopy (SEM) and immunofluorescence demonstrated the production of NETs by cSLNs. This phenomenon was not significant for the neutral SLNs (nSLNs), although histones in NETs also increased after treatment of nSLNs. Our results suggest an important role of cSLNs in governing the activation of human neutrophils.

1. Introduction

Nanomedicine provides potential benefits in the fields of drug delivery, medical diagnosis, cancer therapy, and tissue engineering [1]. Within these fields, solid lipid nanoparticles (SLNs) have gained much attention during the last decade. SLNs are colloidal nanosystems made up of crystalline lipids that are solid at room and body temperature [2]. Owing to the unique features of the particulate structure, SLNs show the advantages of controlled drug release, increased drug bioavailability, improved stability, and industrial-scale production [3]. The nanoparticles with positive charges are necessary in the cases of increasing tumor targeting, penetrating the blood–brain barrier (BBB), and promoting gene transfection. Previous studies have demonstrated the capability of cationic SLNs (cSLNs) as the vectors of DNA and RNA [4,5]. The cSLNs also augment the gastrointestinal absorption and brain delivery of the small-molecular drugs [6–8].

Despite the extensive interests in using cationic colloids for biomedical application, an increasing safety concern is raised due to the possible toxicity of the cationic additives. The nanoparticles can be considered as xenobiotics when they are being administered into the body. Polymorphonuclear neutrophil cells (PMNs) are the first line of defense against the xenobiotics [9]. The cationic nanocolloids can activate PMNs through the respiratory burst and degranulation pathways [10]. The activation of PMNs can increase the immunity and prevent pathogen invasion; however, the overwhelming activation may lead to inflammatory disorders.

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Cationic nanoparticles are similar to a double-edged sword, functioning not only as profitable nanocarriers but also as toxic materials. Due to the insufficiency of information concerning the effect of cSLNs on PMNs, the aim of this study was to systematically investigate the interaction between cSLNs and PMNs and the subsequent activation of PMNs by cSLNs.

In order to develop cSLNs, cetyltrimethylammonium bromide (CTAB) was employed as the cationic surfactant in this work because of its wide application in nanomedicine [11,12]. To achieve the purpose of the present study, lactate dehydrogenase (LDH) release, cell viability, superoxide anion production, and elastase release of PMNs after stimulation by cSLNs were examined. Mitogen-activated protein kinases (MAPKs) are important for the regulation of the inflammatory process, including reactive oxygen species (ROS) fabrication and cytokine release [13]. MAPKs, which consist of p38, extracellular signal-regulated kinases (ERK), and c-Jun N-terminal kinases (JNK) were also determined after treatment of cSLNs on PMNs. Recently, a novel pathogen-killing mechanism of neutrophil extracellular traps (NETs) was elucidated. This mechanism was involved in the elimination of foreign microorganisms and materials by the highly activated PMNs [14]. NETs are composed of a core DNA element attached with histones and proteases. We elucidated whether cSLNs activated the formation of NETs. The experimental results of this report are helpful to understanding the role of cSLNs in affecting the immune system.

2. Materials and methods

2.1. Preparation of SLNs

Cetyl palmitate (12%) and soybean phosphatidylcholine (1%) were blended as the lipid matrix, while the aqueous phase was created by mixing water and Pluronic F68 (3.5%). CTAB (2%) was added to the lipid matrix when fabricating cSLNs. The lipid and aqueous phases were separately heated at 85 °C for 15 min. The aqueous phase was combined with the lipid phase and homogenized by a high-shear homogenizer for 20 min at 12,000 rpm. The blend was further mixed with a probe-type sonicator for 15 min.

2.2. Average diameter and zeta potential

The mean diameter (z-average) and zeta potential of the SLNs were measured using a laser-scattering method (Nano ZS90, Malvern, Worcestershire, UK). The colloidal dispersion was diluted 100-fold with water before testing. The determination was repeated three times per sample for three batches.

2.3. Human PMNs

Human neutrophils from healthy volunteers (20–30 years old) were purified using a protocol approved by the Institutional Review Board at Chang Gung Memorial Hospital. All subjects were required to provide written informed consent. Neutrophils were isolated with sedimentation prior to centrifugation in a Ficoll Hypaque gradient and hypotonic lysis of erythrocytes [15].

2.4. Membrane damage determined by LDH

LDH value after colloidal treatment was detected using a commercially available method (CytoTox 96®, Promega, Madison, WI, USA). PMNs (6×10^5 cells/ml) were equilibrated at 37 °C for 2 min and subsequently treated with SLNs for 15 min. The membrane damage was displayed by LDH release in a cell-free medium as a percentage of the total LDH released. The total LDH release was measured by treating neutrophils with 0.1% Triton X-100.

2.5. Cell viability determined by WST-1

The survival rate of PMNs was measured using a WST-1 cell proliferation kit (Roche, Indianapolis, IN, USA). The neutrophils (1×10^6 cells/ml) were treated with SLNs for 15 min at 37 °C. The treated medium was centrifuged at 200×*g* for 8 min. The supernatant was discarded. WST-1 reagent was added, and after a 1-h incubation at 37 °C, the absorbance was determined at 450 nm.

2.6. Intracellular superoxide anion production

Hydroethidine (HE) is a probe for detecting intracellular superoxide anion. The PMNs (2.5×10^6 cells/ml) were loaded with HE (10 μM) at 37 °C for 5 min. SLNs were added into the cell medium for 15 min at 37 °C. Subsequently, Hank's balanced salt solution (HBSS) at 4 °C was used to stop the reaction, and the fluorescence was monitored by flow cytometry.

2.7. Degranulation determined by elastase

The elastase substrate used was MeO-Suc-Ala-Ala-Pro-Val-p-nitroanilide. After supplementation with the substrate (100 μM), the PMNs (6×10^5 cells/ml) were equilibrated at 37 °C for 2 min and then treated with SLNs for 15 min. The absorbance at 405 nm was determined for 15 min to measure the elastase release.

2.8. Intracellular Ca^{2+} ($[Ca^{2+}]_i$)

PMNs (3×10^6 cells/ml) were loaded with Fura-2/AM reagent (2 μM) at 37 °C for 40 min. After being washed, the PMNs were resuspended in HBSS. SLNs were added into the cell medium for 15 min at 37 °C. The fluorescence spectrometry at λ_{ex} of 340/380 and λ_{em} of 505 nm was examined with continuous stirring. The detailed processes were described by Hwang et al. [16]. 1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA-AM) at a concentration of 20 μM was joined into medium for suppressing Ca^{2+} homeostasis if necessary.

2.9. MAPKs determined by immunoblotting

After incubation with SLNs for 5 min, the suspensions of PMNs were centrifuged at 14,000×*g* for 20 min at 4 °C. The whole-cell lysates were produced based on the previous method [16]. For the separation of proteins, sodium dodecyl sulfate–polyacrylamide gel electrophoresis with 12% polyacrylamide gels was used. The samples were blotted onto a nitrocellulose membrane. Immunoblotting was carried out utilizing the indicated antibodies and horseradish peroxidase-conjugated secondary anti-rabbit antibodies. The immunoreactive bands were observed using an enhanced chemiluminescence system. Phorbol myristate acetate (PMA) at a concentration of 10 nM was employed as the positive control. Each experiment was repeated three times.

2.10. Scanning electron microscopy (SEM)

PMNs (1.8×10^7 cells/ml) were incubated with SLNs for 5 min. The suspension was centrifuged at 200×*g* at 4 °C for 8 min. Glutaraldehyde (0.5%) was added to the PMNs after supernatant removal. The PMNs were fixed and dehydrated before SEM visualization. Gold palladium alloy was used as a sputter coating.