Renal Replacement Therapy

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In vitro evaluation of antibacterial nanomaterial-induced anaphylactoid reaction for indwelling catheters

Koji Umeda[1†], Masaji Tachikawa[1,2†], Yoshinao Azuma[3] and Tsutomu Furuzono[1*]

Abstract

Background: To prevent tunnel infection of indwelling catheters, impregnation with antiseptics or antibiotics is effective. However, 13 patients using chlorhexidine–silver sulfadiazine-impregnated catheters experienced serious anaphylactic shock in Japan. Thus, it is necessary to select a suitable evaluation method for allergic reactions and develop a novel antibacterial coating material that does not cause anaphylactic reaction.

Methods: Two types of highly dispersible and antibacterial nanoparticles—fluorine (F)-doped hydroxyapatite (HAp) and zinc (Zn)-doped HAp—were tested using of the system and compared with compound 48/80 (c48/80) as a histamine releaser and chlorhexidine gluconate (CHG) as an anaphylactic inducer.

Results: The histamine concentrations secreted from HMC-1 cells remained mostly the same even with the addition of F-HAp and Zn-HAp. On the contrary, the levels of the chemical mediators from the cells by the addition of F-HAp and Zn-HAp were significantly lower than those of only c48/80 and CHG without the addition of HAp.

Conclusions: The assay was a well-evaluated method for quantifying histamine concentrations released from HMC-1 cells. Our study induced HMC-1 cells accompanied with and without the nanomaterials; the potential of F-HAp and Zn-HAp to induce allergic reactions was found to be quite low. Therefore, the antibacterial nanomaterials are expected to hardly induce anaphylactoid reactions.

Keywords: Hydroxyapatite, Anaphylactoid reaction, Antibacterial, Nanomaterial

Introduction

Infection through percutaneous devices—vascular access catheter for hemodialysis, peritoneal dialysis catheter, and central venous catheter for nutrition or medication—is the most common type of device infection that induces sepsis or peritonitis. For example, it has been reported that vascular access-associated blood stream infection rates of tunneled catheters for hemodialysis are approximately eightfold higher than those of arteriovenous fistulas (4.2 vs. 0.5 per 100 patient months) [1]. To prevent tunnel infection of long-term indwelling catheters, impregnation with antiseptics or antibiotics—chlorhexidine, silver, vancomycin, rifampicin, or a mixture of these agents—is effective [2]. However, 13 patients using chlorhexidine–silver sulfadiazine-impregnated catheters experienced serious anaphylactic shock, including one potentially associated death in Japan [3]. It has been believed that chlorhexidine induces shock, because of the interaction of the chemical antiseptic with immunoglobulin E (IgE) antibodies, leading to anaphylaxis [4]. Thus, from the point of view of infection control for indwelling catheters, it is necessary to select

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a suitable evaluation method for allergic reactions and develop a novel antibacterial coating material that does not cause anaphylactic reaction.

Generally, animal models have been applied to analyze immunological responses to nanomaterials, such as devices, drugs, imaging agents, vaccines, and immunotherapies [5]. However, the diversity of animal models in terms of genetic, metabolic, and phenotypic properties often complicates animal selection and interpretation of the experimental results, as well as consideration of animal welfare. Therefore, it is necessary to develop or adapt an in vitro assay method to detect immunological responses for nanomaterials in a short time. About 15 years ago, Hamano and colleagues successfully developed an in vitro screening method for drug-induced anaphylactoid reaction by quantifying the histamine dose released from human mast cell-1 (HMC-1) after exposure to chemical agents and medicines, such as compound 48/80 (c48/80), 5-fluorouracil, amidotrizoate, and ofloxacin [6]. This unique method for evaluating immunological responses had not been applied to biomaterials and medical devices.

Recently, two types of highly dispersible, crystalline, and antibacterial fluorine (F)- and zinc (Zn)-doped hydroxyapatite [HAp, $Ca_{10}(PO_4)_6(OH)_2$] (F-HAp and Zn-HAp) nanoparticles were developed by our group using an anti-sintering method, to be used as coating nanomaterials for medical devices [7, 8]. Intact HAp is a biocompatible ceramic material because it is a component of bone and tooth in the living body [9]. In this study, an in vitro screening method for anaphylactoid reactions [6] was applied to these nanoparticles.

Materials and methods

Paraformaldehyde, ethanol, and 0.05% toluidine blue solution (pH 4.1) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). HAp used as the control (SHAp, average diameter of approximately 40 nm) was obtained from SofSera Corporation (Tokyo, Japan). Human mast cell-1 (HMC-1) line was kindly donated by Dr. Susumu Nakae (The University of Tokyo).

HMC-1 cells were cultured in RPMI-1640 (Sigma-Aldrich Japan, Tokyo, Japan) containing 10% fetal bovine serum (JRH Bioscience, KS, USA), 1% penicillin–streptomycin solution (×100) (Sigma-Aldrich Japan), MEM non-essential amino acids solution (×100) (FUJIFILM Wako Pure Chemical Corporation) at 37 °C and 5% CO_2. To observe histamine granules, HMC-1 cells cultured in phenol red-free RPMI-1640 were stained with toluidine blue and observed under a fluorescence microscope (Nikon ECRIPSE Ti, Nikon, Tokyo, Japan).

An in vitro detection system for the anaphylactoid reaction [6] was adopted in this study. Briefly, HMC-1 cells (with density of 1.0×10^4 in 150 μL) were seeded in a 96-well plate. After 6 h of incubation, the plate was centrifuged at 150×g for 5 min and histamine concentration in the supernatant was measured using a histamine test system (Kikkoman Biochemifa, Tokyo, Japan) [10] and histamine ELISA Kit (Bertin Pharma, MI, USA) [11]. c48/80 (MP Biomedicals, Tokyo, Japan) and 20% chlorhexidine gluconate solution (CHG, FUJIFILM Wako Pure Chemicals) were used as the positive control agents. Histamine-inhibition tests for HAp nanoparticles were also conducted using a histamine test system.

Data of histamine concentrations secreted from HMC-1 cells stimulated by the test materials were presented as mean ± standard deviation (SD: $n = 3$). Statistical comparisons were performed using Student's t test between the two groups and Tukey's test for multiple comparisons. The levels of statistical significance were defined as $p < 0.05$ and 0.01.

Results

Before carrying out the anaphylactoid reaction assay for novel antibacterial nanomaterials (F-HAp and Zn-HAp), we examined whether HAp worked as an inhibitor against the evaluation system. After treatment of the cultured HMC-1 cells with toluidine blue, intracellular particles were observed by fluorescence microscopy (Fig. 1). It has been suggested that HMC-1 cells used for the anaphylactoid reaction assay possess histamine granules. In the adsorption behavior of histamine molecules against HAp, the calibration curve with the addition of HAp closely overlapped with that of histamine standard, as shown in Additional file 1: Fig. S1. Subsequently, the influence of the existence/absence of HAp on histamine release from HMC-1 cells was examined. Figure 2 shows histamine concentrations secreted from HMC-1 cells by the addition of c48/80 (79.5 and 159 μM) as a typical histamine releaser, with or without HAp nanoparticles. There were no statistically significant differences in histamine concentrations with HAp addition and without, in both c48/80 concentrations. From these results, it is clear that HAp did not influence the histamine detection system.

Using the detection system of histamine with HAp, the impacts of F-HAp and Zn-HAp on the anaphylactoid reaction of HMC-1 cells were examined and compared to c48/80 and CHG as positive controls. F- and Zn-ion substitution contents in HAp structures of F-HAp and Zn-HAp nanoparticles were 84% [F/(F + OH) × 100] and 9% [Zn/(Zn + Ca) × 100], respectively [7, 8]. Antibacterial activity of the two types of nanoparticles, F-HAp and Zn-HAp, possessed that antibacterial activities against pathogenic micrograms have been reported [7, 8], as shown in Additional file 1: Fig. S2.

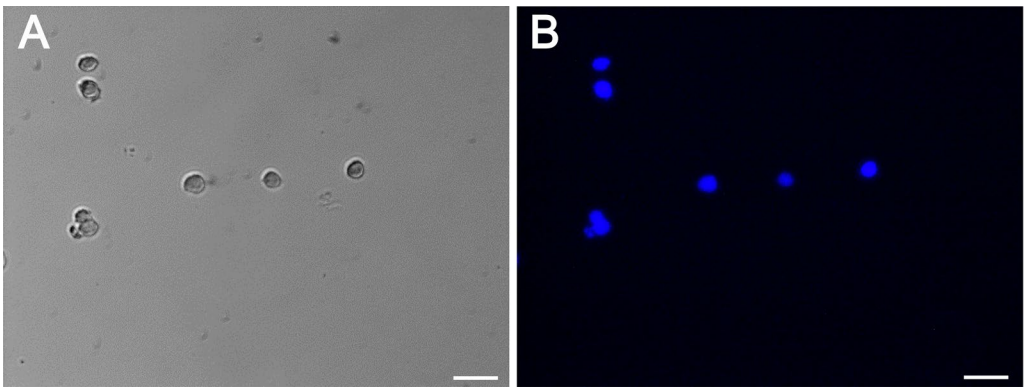

Fig. 1 Phase contrast **A** and toluidine blue stained-histamine granules **B** images of HMC-1 cells. Scale bar, 50 µm

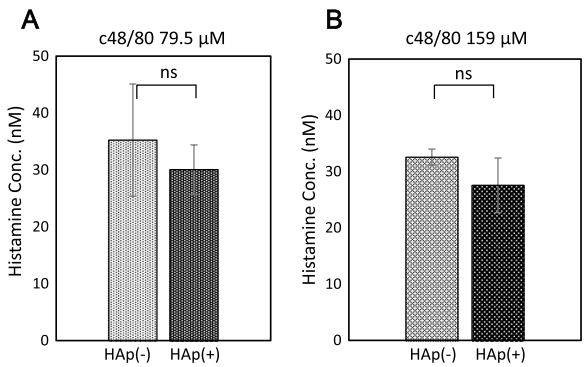

Fig. 2 Concentrations of histamine secreted from HMC-1 cells in c48/80 medium **A** 79.5 µM, **B** 159 µM with/without HAp nanoparticles (SD: $n = 3$, Student's t test; ns: not significant). HAp(−) and HAp(+) denote without/with HAp, respectively

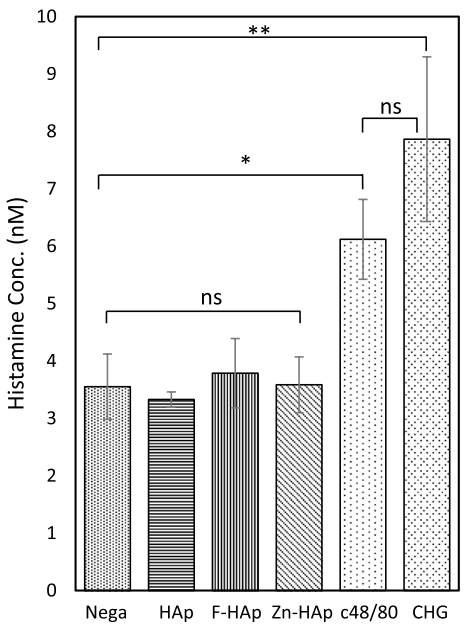

Fig. 3 Concentrations of histamine secreted from HMC-1 cells by addition of sample nanoparticles (HAp, F-HAp, and Zn-HAp) compared to c48/80 and CHG (SD: $n = 3$, Turkey's test; ns: not significant, $^*p < 0.05$, $^*p < 0.01$). Nega denotes no addition samples or the agents

Initially, two or three different of concentrations of either the sample or controls (HAp, F-HAp, Zn-HAp, c48/80, and CHG) were added to the detection system using HMC-1 cells to determine the ideal concentrations to be added (Additional file 1: Fig. S3). From the results, the highest values of histamine concentration released from the cells were selected for each sample and agent, as shown in Fig. 3. (Whole data are shown in Additional file 1: Fig. S3.) The histamine concentrations secreted from HMC-1 cells did not very much by addition of F-HAp and Zn-HAp. On the contrary, the values of histamine concentrations from the cells with the addition of F-HAp and Zn-HAp were significantly lower than these of c48/80 and CHG.

Discussion

Type I allergy, an immediate type, contains IgE-mediated and non-IgE-mediated reactions caused by the secretion of chemical mediators, such as histamine, platelet-activating factor, thromboxane, prostaglandin, and leukotriene, from mast cells, and results in anaphylactic and anaphylactoid reactions, respectively. Furthermore, severe allergies caused by anaphylactoid reactions often induce anaphylactic shock, ultimately resulting in death. Concerns about anaphylactic reactions induced by implantable medical devices with antibacterial coatings have disturbed the development of novel antibacterial materials as coating agents in Japan. This is because antibiotic-impregnated catheters induce serious anaphylactic shock [3]. Thus, the development of in vitro

toxicological screening tests for novel nanomaterials and chemical compounds showing antibacterial activity for use in medical implantable devices is necessary. In this study, an in vitro assay to detect histamine secretion from HMC-1 cells [6] was applied to novel antibacterial nanomaterials (F-HAp and Zn-HAp).

HMC-1 cells without the surface expression of high-affinity IgE receptors were established from a patient with mast cell leukemia. These cells have been widely used in studies of human mast cell functions due to the possession of many key properties of tissue mast cells, such as expression of histamine, tryptase, heparin, and similar cell surface antigens [12]. Under normal physiological conditions, an amino group of histamine (pKa = 9.75) is usually protonated, while the imidazole group is uncharged (pKa = 6.04) [13, 14]. On the other hand, the zeta potential of HAp is negatively charged in aqueous solution at a pH of approximately 6 [9]. Thus, there is sufficient possibility of interaction between histamine molecules and HAp nanoparticles. However, it was found that the adsorption of histamine on HAp nanoparticles is hardly observed in Additional file 1: Fig. S1. This might be because positively charged RPMI-1640 components (amino acids, vitamins, and other ions) used for HAp dilution were adsorbed on HAp surface before the histamine detection test.

It is well known that c48/80 functions as a Ca ion-dependent histamine releaser for HMC-1 cells, even lacking surface expression of high-affinity IgE receptors [15]. Regarding CHG as an antibacterial agent, it was clear that the agent could not only induce IgE-mediated allergy in the living body [4] but also stimulate to HMC-1 cells through an IgE-independent pathway. In contrast, F-HAp and Zn-HAp suppressed histamine release, similar to HAp and HMC-1 cells alone, as shown in Fig. 3. This might be because F-HAp and Zn-HAp were mainly constructed with HAp possessing biocompatibility in the living body [9], and F- and Zn-ions were slowly released from the crystalline HAp structures, causing little damage to HMC-1 cells.

Therefore, this histamine detection system worked as an in vitro primary screening test for antibacterial nanomaterials; the frequency of occurrence of the allergic reaction for the novel antibacterial nanomaterials, F-HAp and Zn-HAp, might be extremely low. In other words, it may be suggested that the nanoparticles are hypoallergenic materials that are suitable for antibacterial coatings on medical devices, such as long-term indwelling catheters.

Although this in vitro screening assay for allergic reactions is expected to be useful in developments of antimicrobial coatings on medical implantable devices, such as indwelling catheters, it is not necessarily warrant safety in vivo. Thus, regarding this novel assay, a further comparative study on the testing within a risk management process based on the biological evaluations of medical devices described in ISO 10993-1 [16] is needed.

Conclusions

An in vitro screening system of drug-induced anaphylactoid reaction was applied to antibacterial nanoparticles, F-HAp and Zn-HAp, as medical device coatings. The assay was a well-evaluated method for quantifying histamine concentrations released from HMC-1 cells. Our study induced HMC-1 cells accompanied with and without the nanomaterials; the potential of F-HAp and Zn-HAp to induce allergic reactions was found to be quite low.

Abbreviations

IgE: Immunoglobulin E; HAp: Hydroxyapatite; F-HAp: Fluorine-doped HAp; Zn-HAp: Zinc-doped HAp; HMC-1: Human mast cell-1; c48/80: Compound 48/80; CHG: Chlorhexidine gluconate.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41100-022-00424-5.

Additional file 1: Fig. S1 Calibration curves of the histamine standard (dotted line) and after HAp addition (solid line). The error bars denote SD ($n = 3$). r^2: the histamine standard = 0.9958, the calibration with HAp = 0.9944. HAp (0.9 μg/μL) was added into the histamine detection system. **Fig. S2** Relative survival rates of HAp and Zn-HAp (Zn content = 9% [Zn/(Zn+Ca) × 100] against *Staphylococcus aureus (S.a)* (SD: $n = 3$, Student's *t* test, $^{**}p < 0.01$). 100 μL of the bacterial cultured medium (OD_{600} adjusted to 0.1) was mixed with 1.0 mg/100 μL of the sample powders and incubated at 37 °C for 1 h. Subsequently, the diluted samples were spotted onto the LB medium plate and incubated at 37 °C for 15 h. Finally, the colony numbers were counted. **Fig. S3** Concentrations of histamine released from HMC-1 cells by addition of two or three steps of concentrations of sample solutions into the evaluation system ($n = 3$).

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Author contributions

KU, MT, and TF were involved in the implementation, data analysis, and article writing related to the study. YA evaluated the antibacterial test results. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available upon corresponding request to the corresponding author.

Declarations

Ethics approval and consent to participate
The in vitro study did not involve human or animal subjects; therefore, no approval was needed.

Consent for publication
Not applicable.

Competing interests
T. Furuzono is a scientific adviser of SofSera Corporation. M. Tachikawa is an employee of SofSera Corporation. The other authors declare no conflict of interest.

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