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ORIGINAL ARTICLE

Effects of slow-releasing colistin microspheres on endotoxin-induced sepsis

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Abstract Lipopolysaccharide (LPS) is a major contributing factor to endotoxic shock. Colistin specifically binds to LPS. However, it has the disadvantages that adverse reactions are common and it has a short half-life. To overcome these disadvantages, we prepared slow-releasing colistin microspheres and examined the efficacy of these colistin microspheres in a mouse model of endotoxin-induced sepsis. We prepared the colistin microspheres using poly-lactic-*co*-glycolic acid. For acute toxicity investigations, mice were overdosed with colistin sulfate or colistin microspheres. The group administered with colistin microspheres was associated with less acute toxicity and fewer nephrotoxic changes on histopathological examination compared to the group administered with colistin sulfate alone. For pharmacokinetic analysis, mice were subcutaneously administered with colistin microspheres or colistin sulfate alone. The plasma concentration of colistin was higher in the colistin microspheres group than in the colistin sulfate group at 12 and 24 h after administration. Moreover, mice were intraperitoneally injected with LPS and then immediately subcutaneously administered with blank microspheres, colistin microspheres or colistin sulfate alone. The levels of endotoxin in the sera and cytokine in the spleens were then measured. A significant reduction in the serum endotoxin level in the colistin microspheres group was observed at 24 h. The reduced endotoxin levels in the sera were correlated with the lower cytokine levels in the spleens of mice treated with colistin microspheres. Our results suggest that the use of colistin microspheres may help to maintain a higher colistin concentration in blood, reduce the levels of endotoxin and cytokines in endotoxin-induced sepsis, and lead to decreased toxicity.

Keywords Colistin · PLGA microsphere · Endotoxin · Toxicity

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Introduction

Sepsis, defined as the systemic host response to microorganisms, is a condition related to systemic inflammatory response syndrome that results in end-organ dysfunction in organs away from the primary site of infection [1, 2]. Severe sepsis and septic shock are common problems encountered in the intensive care unit (ICU), with an estimated annual incidence of 750,000 cases and a mortality rate of 25–80 % in the United States [3–6].

While almost any microorganism can be associated with sepsis and septic shock, Gram-negative bacteria are the usual etiologic pathogens. Lipopolysaccharide (LPS), a bacterial endotoxin, is a component of the outer membranes of Gram-negative bacteria [7–9]. Gram-negative sepsis is mediated by macrophages and monocytes and is caused by the excessive production of several cytokines in response to LPS, rather

than to LPS toxicity itself [7]. LPS triggers the release of cytokines such as tumor necrosis factor (TNF), interleukin 1 (IL-1), and IL-6, and activates complement and coagulation factors [2, 7, 10]. Further, high levels of LPS activity are associated with poor clinical outcomes [11, 12].

Polymyxins are a group of cyclic cationic polypeptide antibiotics derived from *Bacillus polymyxa*. They are characterized by a heptapeptide ring, a high content of diaminobutyric acid, and a side chain ending in fatty acid residues [9, 13]. Over 40 years ago, researchers found that the lethal effects of endotoxins in animal models can be neutralized by polymyxin B [14–19]. However, the toxicity of intravenous polymyxin limited the extension of these findings to human patients.

Colistin is a class of polymyxin also known as polymyxin E. It was first used for therapy in Japan and Europe during the 1950s [20]. Colistin is different from polymyxin B because it has D-leucine instead of D-phenylalanine in the polypeptide ring [21]. However, like polymyxin B, it binds to bacterial LPS outer membranes and bacterial endotoxins and thereby deactivates them [16].

Despite its proven efficacy, the use of colistin in parenteral therapy is limited because of concerns related to adverse reactions, such as nephrotoxicity and neurotoxicity. Moreover, colistin and colistin methanesulfonate (CMS), which is the colistin prodrug, have relatively short terminal half-lives (55.7 ± 19.3 and 23.6 ± 3.9 min, respectively) [22] and are required to be administered intravenously and frequently. Therefore, less toxic polymyxins with longer terminal half-lives are highly desirable.

In our previous study (unpublished data), we prepared colistin microspheres using poly-lactic-*co*-glycolic acid (PLGA), which are in widespread clinical use in products such as Leuplin® SR, and we confirmed that these microspheres continued to release colistin after seven days under in vitro conditions. Moreover, in a preliminary experiment, we evaluated the antibiotic efficacy of these colistin microspheres in a septic mouse model of *Pseudomonas aeruginosa* (data not shown), and the results were positive. A disadvantage of this method, however, is that because of sustained release, the plasma concentration is maintained below the minimum inhibitory concentration (MIC) over an extended period, and this may lead to colistin resistance. Therefore, the aim of this study was to examine the efficacy and toxicity of the colistin microspheres in a mouse model of endotoxin-induced sepsis.

Materials and methods

Animals

C3H/HeN mice (male, four weeks old) and Balb/c mice (female, six weeks old) were purchased from Charles River in Japan and then quarantined for 1–2 weeks. They were housed in separate cages at a constant temperature (26 °C) with a 12 h light/dark cycle and given standard laboratory food and water ad libitum. All animal experiments were performed with the approval of the animal center of Toho University (approval number: #11-53-54).

Chemicals

LPS purified from *Escherichia coli* 055:B5 was used in the experiments (Sigma–Aldrich Japan, Tokyo, Japan). Colistin sulfate was purchased from Sigma–Aldrich Japan and its potency was as determined by the European Pharmacopoeia. We measured its weight in milligrams and the activity unit of colistin sulfate was considered to be 10000 units/mg [23].

PLGA (PLGA7520) and the sodium salt of carboxymethylcellulose were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Normal saline solution was obtained from Otsuka Seiyaku, Ltd. (Tokushima, Japan). The colistin sulfate, colistin microspheres, or blank microspheres were suspended in 10 mg/mL carboxymethyl cellulose in saline before administration to the mice.

Microsphere development

Colistin microspheres were prepared and purified based on the method described previously [24]. Briefly, to prepare a water-in-oil emulsion, PLGA was dissolved in dichloromethane (DCM), colistin sulfate was added to this mixture, and the mixture was agitated. The emulsion was taken up into a syringe with a needle (Terumo, Tokyo, Japan) and dropped into polyvinyl alcohol (PVA) solution with agitation. Then, to evaporate the oil phase, the entire suspension was agitated and passed through a sieve with a pore size of 74 μm. The sifted colistin microspheres were washed using purified water and freeze-dried.

Analysis of the plasma concentrations of colistin and colistin microspheres

Forty Balb/c mice were divided into two large groups. One group received colistin sulfate and the other received colistin microspheres, both via subcutaneous administration at 1.0 mg per mouse. Blood samples were collected by cardiac puncture from 5–6 animals in each group using heparin-coated syringes following CO_2 asphyxia at 3, 6, 12, and 24 h after colistin injection. The samples were centrifuged at 2400×*g* for 15 min to separate the plasma, and the plasma samples were stored at −80 °C until analysis. The plasma colistin concentration was determined by high-performance liquid chromatography (HPLC) (Nemoto Science Co., Ltd., Ibaraki, Japan) [25]. If the

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