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Adsorption of histones on natural polysaccharides: The potential as agent for multiple organ failure in sepsis

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ABSTRACT

Histones are intracellular proteins that are structural elements of nuclear chromatin and regulate gene transcription. However, the extracellular histones released in response to bacterial challenges have been identified as mediators contributing to endothelial dysfunction, organ failure, and death during sepsis. In the present study, the adsorption of histones as well as plasma proteins (α_1-acid glycoprotein (AGP), albumin, and γ-globulin) on alginic acid, pectin, dextran, and chitosan was examined in order to evaluate the potential of natural polysaccharides as therapeutic agents for multiple organ failure in sepsis. Alginic acid and pectin strongly adsorbed histones, whereas the adsorption abilities of dextran and chitosan toward histones were very low or negligible. Among the natural polysaccharides examined, only alginic acid did not adsorb any of the plasma proteins. These results demonstrated that alginic acid strongly adsorbed histones, but not plasma proteins; therefore, it has potential as a candidate drug for the treatment of multiple organ failure in sepsis.

1. Introduction

Sepsis is a systemic deleterious host response to infection, and is one of major causes of death in intensive care units (ICUs) worldwide. This lethality is due to a systemic hyperinflammatory response and subsequent multiple organ failure [1,2]. A previous study reported that extracellular histones released in response to bacterial challenges functioned as mediators for endothelial dysfunction, organ failure, and death during sepsis [3]. Histones are intracellular proteins that are structural elements of nuclear chromatin and regulate gene transcription. They are released from dying cells, and contribute to antimicrobial defenses during infections.

Eukaryotic DNA is packaged into a chromatin structure consisting of repeating nucleosomes formed by the wrapping of DNA around an octamer of four core histones. Histones are subject to a large number of posttranslational modifications, and these modifications have been implicated in gene expression [4,5]. In innate immune responses, platelets activate neutrophils to make neutrophil extracellular traps (NETs). NETs are made of DNA fibers with histones. Microbes have been shown to bind to NETs and are killed by the antimicrobial actions of histones [6,7]. However, histones also possess cytotoxic properties against microorganisms and eukaryotic cells. Histones released from injured cells and NETs have been shown to trigger more inflammation and tissue injury, leading to multiple organ failure [8,9].

The aim of the present study was to identify new sepsis therapeutic agents. A schematic presentation of this study was shown in Fig. 1. Histones are released from cells in sepsis, and injure vascular endothelial cells, leading to multiple organ failure. We propose that neutralizing histones by their adsorption on natural polysaccharides may prevent multiple organ failure in sepsis. In order to achieve this, the adsorption of histones on natural polysaccharides such as alginic acid, pectin, dextran, and chitosan was investigated (Fig. 2). The adsorption of plasma proteins on these natural polysaccharides was also examined.

2. Materials and methods

2.1. Materials

Albumin from human serum, α_1-acid glycoprotein (AGP) from human plasma, and γ-globulin from human blood were purchased

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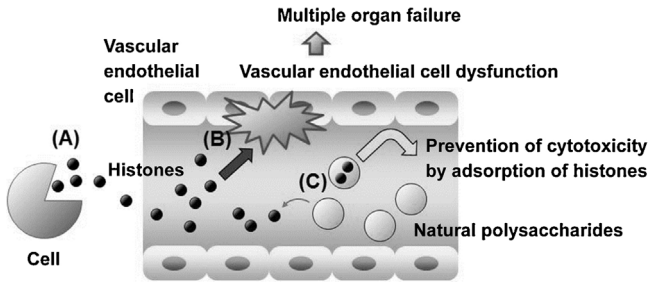

Fig. 1. Schematic presentation of this study. (A) In sepsis, histones are released from injured cells and neutrophil extracellular traps. (B) Histones injure vascular endothelial cells, leading to multiple organ failure. (C) Neutralizing histones by their adsorption on natural polysaccharides may prevent multiple organ failure in sepsis.

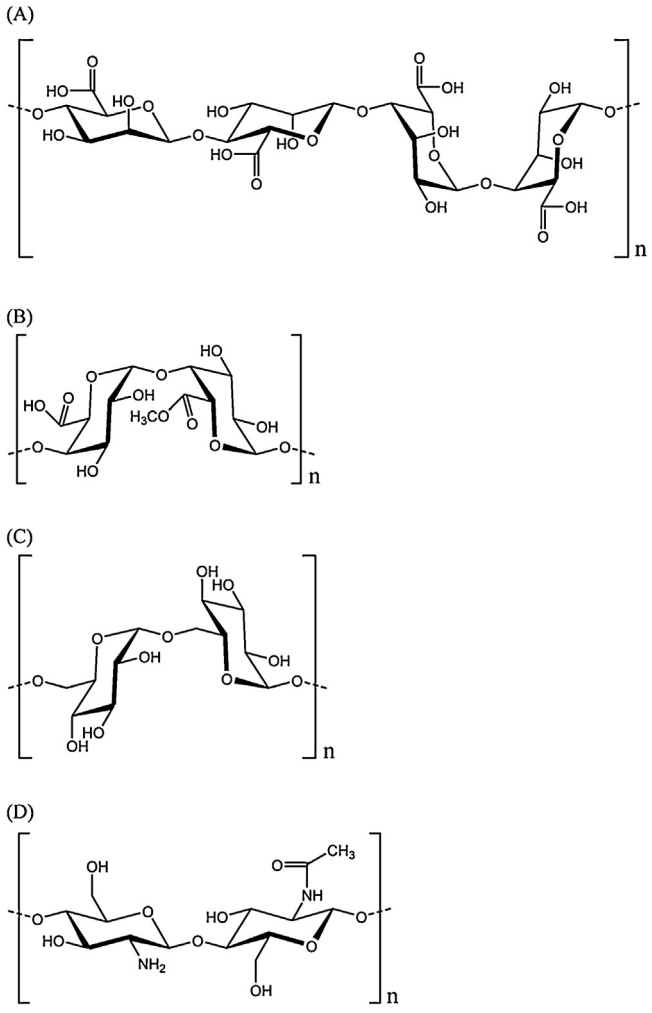

Fig. 2. Chemical structures of natural polysaccharides. (A) Alginic acid. (B) Pectin. (C) Dextran. (D) Chitosan.

from Sigma–Aldrich Co. LLC. (St. Louis, MO, USA); histones from the calf thymus were from Worthington Biochemical Corporation (Lakewood, NJ, USA); alginic acid 20 M was from Kibun Food Chemifa Co. (Tokyo, Japan); pectin from Citrus was from Nacalai Tesque Inc. (Kyoto, Japan); and dextran 40,000 and Chitosan 100 were from Wako Pure Chemical Industries (Osaka, Japan). All other reagents were of analytical grade.

2.2. Adsorption of histones on natural polysaccharides

The adsorption of histones on natural polysaccharides was determined as described previously with some modifications [10]. The incubation solution containing 0.1 mg/mL histones (20 mL) was placed in a plastic dish and maintained at 37 °C. Solutions containing 0–0.6 mg of each natural polysaccharide were added to the incubation solution, and shaken at 300 rpm for 60 min in a shaker incubator at 37 °C. A 0.4-mL aliquot of each incubation solution was removed periodically, and centrifuged at 10,000 rpm for 5 min at room temperature. The concentration of histones in the supernatant was measured using the BCA protein assay kit (Thermo Scientific, Rockford, IL, USA) at 562 nm with a spectrophotometer. The amounts of histones adsorbed on the natural polysaccharides were calculated by the difference between the initial and residual amounts of histones in the solution.

2.3. Adsorption of plasma proteins on natural polysaccharides

The adsorption of plasma proteins on natural polysaccharides was determined according to the procedure for histones described above. The incubation solution contained 0.1 mg/mL for AGP, 0.1 mg/mL for albumin, or 0.1 mg/mL for γ-globulin. The amount of each polysaccharide was 0.4 mg for alginic acid, 0.4 mg for pectin, 0.4 mg for dextran, and 0.4 mg for chitosan.

3. Results

3.1. Adsorption of histones on natural polysaccharides

The adsorption of histones on natural polysaccharides was initially examined (Fig. 3). Alginic acid dose-dependently adsorbed histones, the doses at 0.2, 0.4, and 0.6 mg were 0.25, 0.64, and 1.30 mg, respectively. The adsorption of histones on pectin also occurred in a dose-dependent manner, but was 28–75% that on alginic acid. In contrast, the adsorption of histones was not observed on dextran, while their adsorption on chitosan was very low.

3.2. Adsorption of plasma proteins on natural polysaccharides

In order to determine whether natural polysaccharides interacted with plasma proteins, the adsorption of AGP, albumin, or γ-globulin on natural polysaccharides was examined. The dose of each polysaccharide was set to 0.4 mg. The results obtained are shown in Fig. 4. Alginic acid did not adsorb any of the plasma proteins. Pectin only adsorbed 0.12 mg of γ-globulin. Dextran adsorbed

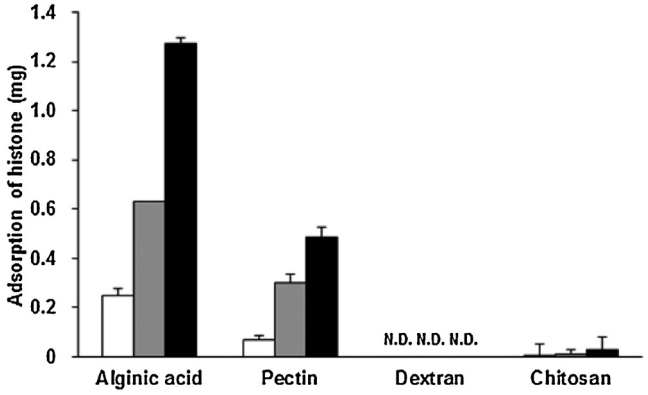

Fig. 3. Adsorption of histones on natural polysaccharides. The doses of natural polysaccharides used were as follows: □, 0.2 mg; ▒, 0.4 mg; ■, 0.6 mg. N.D.: not detected. Each column represents the mean ± S.D. ($n = 3$).