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Exogenous deoxyribonuclease has a protective effect in a mouse model of sepsis

Lucia Lauková[a], Barbora Konečná[a], Janka Bábíčková[a], Alexandra Wagnerová[b], Veronika Mešíková[a], Barbora Vlková[a], Peter Celec[a,c,d,*]

[a] Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia
[b] Institute of Physics, Biophysics, Informatics and Telemedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia
[c] Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia
[d] Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

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ABSTRACT

Sepsis is associated with the activation of white blood cells (WBCs) that leads to the production of extracellular traps. This process increases extracellular DNA (ecDNA) that can be recognized by the innate immune system and leads to inflammation. Previous studies have shown that by cleaving ecDNA deoxyribonuclease (DNase) prevents the antibacterial effects of extracellular traps, but also has beneficial effects in sepsis. The aim of our study was to analyze the effects of DNase on WBCs *in vitro* and on ecDNA in a mouse model of sepsis. Our results confirmed that DNase decreases ecDNA by 70% and prevents the antibacterial effects of WBCs *in vitro*. Sepsis was induced in mice by intraperitoneal injection of *E. coli*. DNase was subsequently administered intravenously. In comparison to untreated septic mice DNase treatment improved the survival of septic mice by 60%, reduced their weight loss as well as inflammatory markers. Increased plasma DNase activity led to ecDNA concentrations in plasma comparable with the control group. In conclusion, the study showed that intravenous DNase improves survival of septic mice by cleavage of ecDNA, especially of nuclear origin. Further mechanistic studies are needed to prove the potential of DNase in the treatment or prevention of septic complications.

1. Introduction

Sepsis is a life-threatening disease characterized by activation of inflammatory and coagulation pathways in response to infection [1,2]. Severe sepsis is accompanied by the multiple organ dysfunction syndrome or failure. Septic infection can be caused by various types of microorganisms, including fungi and viruses, but bacteria are the most common culprits. The inflammatory reaction is associated with the release of cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukins, from various immune cells. The concentration of these markers can be used for the monitoring and prognosis of sepsis [3].

Sepsis has two stages. The first, early phase is characterized by activation of the immune response and expression of genes encoding inflammatory chemokines and cytokines. In this stage it is possible to improve the prognosis with a right treatment. The subsequent later phase is characterized as immunoparalysis and interventions have currently very limited effects [4]. Therefore, antibiotic treatment of sepsis needs to be performed as soon as possible and new alternative approaches are needed to make the later phase treatable efficiently.

White blood cells (WBC) are central mediators of the innate immune defense system during sepsis. One of the mechanisms of their antibacterial activity is the release of extracellular traps (ETs) – ETosis [5]. ETosis is a form of cell death associated with the release of chromatin fibrils by neutrophils and other WBCs (eosinophils, macrophages, mast cells) into the extracellular space producing ETs. This has been identified as an important evolutionarily conserved mechanism of vertebrate innate immune defense [6]. ETs are formed in response to a variety of pro-inflammatory stimuli such as microorganisms in order to trap and kill them. However, components of ETs can be detected by specific receptors as danger-associated molecular patterns or alarmins. One of these compounds released into the extracellular space is DNA.

Extracellular DNA (ecDNA) is able to activate the NLRP3 (nucleotide-binding oligomerization-like receptor family)

* Corresponding author at: Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Sasinkova 4, 811 08 Bratislava, Slovakia.
E-mail address: petercelec@gmail.com (P. Celec).

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inflammasome and to induce synthesis of pro-inflammatory cytokines as part of the immune response [7]. Endogenous ecDNA can be of nuclear or mitochondrial origin. The mitochondrial DNA (mtDNA), similarly to bacterial DNA and in contrast to nuclear DNA (nDNA) is not protected by histone proteins and contains unmethylated CpG islands that are recognized by toll-like receptor 9 stimulating the innate immunity [8,9]. The quantity of ecDNA and more specifically mtDNA is increased in sepsis and several studies proved its predictive value regarding the clinical outcome [10–12]. A recent study showed that higher plasma ecDNA in septic patients inhibits fibrin degradation suggesting that ecDNA is not just a biomarker, but it is actively involved in the pathogenesis of septic complications [13]. EcDNA could, thus, be a potential therapeutic target.

One way of reducing ecDNA could be the use of deoxyribonuclease (DNase). DNase cleaves the phosphodiester bonds of DNA strands. If ecDNA is bound to histones many cleavage sites are hidden. Such protected ecDNA is either not cleaved at all or less efficiently than ecDNA without histones. During inflammation, the elevated ecDNA is mainly derived from neutrophils that produce neutrophil ETs [14]. This ecDNA is bound to histones and other DNA-binding proteins [5,15]. However, studies have shown that DNase is able to cleave ETs and to inhibit their antibacterial effects. In addition, DNase protects tissues from inadequate ETs release in sepsis [16]. Recently, in a lipopolysaccharide (LPS)-induced animal model of sepsis it was shown that the disruption of ETs by DNase protects animals against septic complications in the gut [17]. Another study proved that delayed DNase treatment decreased ecDNA and reduced mortality of mice with polymicrobial sepsis induced by cecal ligation and puncture [18].

The aim of our study was to analyze the effects of DNase on WBCs *in vitro* and on ecDNA in a mouse model of *E. coli*–induced sepsis. Concentrations of nDNA and mtDNA were analyzed in plasma to decipher the effects of sepsis induction and DNase treatment on the particular type of ecDNA.

2. Material and methods

All methods were carried out in accordance with relevant guidelines and regulations. All procedures and animal experiments have been conducted in accordance with Slovak legislation and were approved by the ethics committee of the Institute of Molecular Biomedicine, Comenius University in Bratislava, Slovakia. Informed consent was obtained from all people before the collection of blood samples.

2.1. Animals

Adult, 4 months old C57BL/6 mice (13 males and 12 females) as well as adult, 6 months old CD1 mice (62 males and 24 females) were used (Anlab, Prague, Czech Republic). The mice were housed in conventional cages under standard conditions (21–24 °C environmental temperature and 55–65% humidity) with a 12/12 h light-dark cycle and had free access to food and water.

2.2. Bacterial strain and growth conditions

An uropathogenic strain *E. coli* 536 (clinical uropathogenic isolate, O6:K15:H31) was used for the induction of sepsis in animals. Bacteria were grown aerobically at 37 °C in liquid LB medium overnight before application. The concentration of bacteria was measured using a spectrophotometer at 600 nm and was verified on solid LB agar.

2.3. In vitro *experiment*

WBCs were isolated from human blood with Lymphoprep™ (Stemcell Technologies, Grenoble, France), seeded on 96-well plates and left to sit down for 1 h. Then WBCs were stimulated with *E. coli* (MOI 0.1) for 3 h (Fig. 1a). DNase I (Sigma-Aldrich, St. Louis, Missouri, USA) (0.5 mg/ml) was added after 30 min. After 3 h of incubation, cell supernatants were mixed vigorously, diluted and inoculated on solid LB agar. Colonies were counted after overnight incubation. Supernatants were used for DNA isolation.

2.4. In vivo *experiments*

In the first experiment, C57BL/6 mice were used (13 males and 12 females). Mice of this strain are smaller in comparison to other strains and it was not possible to obtain sufficient volume of blood for all analyses (results marked with *). To obtain sufficient plasma volume for DNA isolation and measurement of inflammatory markers the experiment was repeated with CD1 mice that were larger and two months older. However, the administration of the same dose (10^7 CFU) did not have any observable effect on CD1 mice in a preliminary experiment (9 males and 9 females). The dose was, thus, increased to 10^8 CFU (15 males and 15 females) and later optimized to 5×10^7 CFU to not be lethal within the first 24 h in order to have enough mice per group for further analysis. In the last experiment, only male animals (n = 38) were used, as no sex differences were observed in the previous experiments.

Mice were randomized into three groups: control, *E. coli* and *E. coli* + DNase. The mice from *E. coli* and *E. coli* + DNase groups were intraperitoneally injected with 10^7 CFU *E. coli* in 100 μl phosphate-

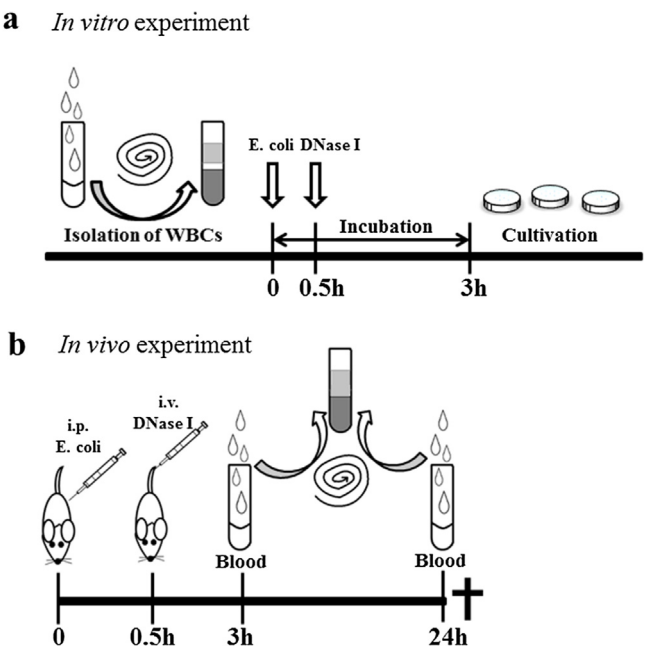

Fig. 1. Schematic representation of the experimental setup of *in vitro* (a) and *in vivo* (b) experiments. *In vitro* experiment (a): Human white blood cells (WBCs) were isolated and stimulated with uropathogenic *E. coli* for 3 h. Deoxyribonuclease I (DNase I) was added 0.5 h after the beginning of WBCs stimulation. Cell supernatants were inoculated on solid LB medium or stained with propidium iodide and Hoechst. *In vivo* experiment (b): Mice were divided into three groups: Control, *E. coli* and *E. coli* + DNase. The mice from *E. coli* and *E. coli* + DNase groups were intraperitoneally (i.p.) injected with *E. coli* to induce sepsis. Control animals were i.p. injected with PBS. Thirty minutes later, the mice from *E. coli* + DNase group were intravenously (i.v.) injected with DNase I, while the same volume of PBS was applied to mice from the other two groups. Blood was collected after 3 and 24 h and mice were sacrificed.