Bioinspired DNase-I-coated melanin-like nanospheres for modulation of infection-associated NETosis dysregulation

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Abstract

We report that DNase-I-coated melanin-like nanospheres (DNase-I pMNSs) mitigate sepsis-associated NETosis dysregulation, thereby preventing further progression of the disease. Recombinant DNase-I and poly(ethylene glycol) (PEG) were used as coatings to promote the lengthy circulation and dissolution of NET structure. Our data indicate that the application of bioinspired DNase-I pMNSs reduce neutrophil counts and NETosis-related factors in the plasma of SARS-CoV-2 sepsis patients, alleviates systemic inflammation, and attenuates mortality in a septic mouse model. Altogether, our findings suggest that these nanoparticles have potential applications in the treatment of SARS-CoV-2-related illnesses and other beta-CoV-related diseases.

Background

A recent study has proposed that neutrophilia might be possibly linked to poor outcomes in patients with severe COVID-19; it is believed to play a critical role in COVID-19 pathogenesis. NETosis, a special type of neutrophil-specific programmed cell death, is a process in which reticular structures consisting of chromatin and granular proteins are formed. It has been suggested that excessive amounts of NETosis, which lead to a hyperinflammatory response are associated with organ damage and multiple organ failure. Therefore, it is postulated that aberrant activation of neutrophils followed by release of neutrophil extracellular traps (NETs) and extracellular DNAs (eDNAs) in the peripheral blood may contribute to organ damage and mortality in sepsis-related diseases; it is predicted that similar outcomes may arise in COVID-19 patients.

Inspired by our observations and based on a recently published report, we suggest that the dissolution of a basic constituent of NET structure—DNA —using DNase-I may be appropriate for preventing NET-related pathogenesis in SARS-CoV-2 patients. Previously, it had been demonstrated that the delivery of a recombinant DNase-I by inhalation led to the dissolution of NETs in the airways of cystic fibrosis (CF) patients, that resulted in clear mucus and ameliorated symptoms. In addition, the use of an actin-resistant DNase in CF patients in Phase I and II clinical trials showed promising results. When tested using animal models, delivery of DNase-I through the airways resulted in increased survival, leading us to believe that DNase-I may help dissolve NETs and prevent further progression to ARDS and sepsis in severe COVID-19 patients. However, exogenously administered recombinant DNase-I showed only a modest effect, presumably due to the short half-life of DNase-I in the blood plasma. Previous studies have reported that conjugation of DNase-I onto the surface of nanoparticles enhances the stability and preserves the activity of DNase-I in blood plasma.

Methods

Figure.1 Bare melanin-like nanospheres (bMNSs) were synthesized by dopamine hydrochloride, as presented in our previous report (Figure 1). Dopamine hydrochloride (100 mg) was dissolved in deionized water (DW, 50 mL). Then, NaOH solution (50 0 μL (1 N) was added to the dopamine hydrochloride solution. The reaction was stirred for 24 h, and the color gradually changed to black as the reaction proceeded. For purification, the prepared bMNSs were collected via centrifugation at 17,000 rpm (27,237 ×x g-force) for 20 min and washed with deionized water (DW) three times. To prepare DNase-I pMNSs, surface engineering of bMNSs with DNase-I was performed according to our previous reports.[17-18] The resulting bMNSs (10 mg) were re-suspended in Tris buffer (5 mL, 10 mM, pH 8.5) containing DNase-I (2,

5, 10, 20, or 50 mg) and poly(ethylene glycol) (50 10 mg, PEG, 4 four arm-amine termini, HCL salt), and stirred at 4 ℃ for 3 h. The prepared DNase-I pMNSs were purified in the same manner as above and washed with DW multiple times. We also prepared PEG-coated MNSs (pMNSs) as a control, using the same method as for DNase-I pMNSs , but without DNase-I addition.

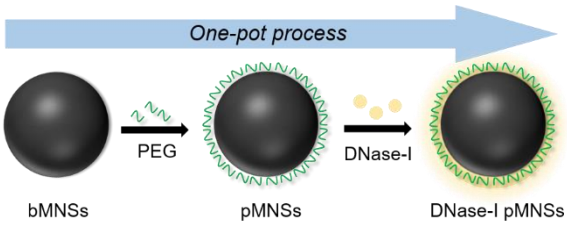


Figure 1. Preparation of DNase-1 pMNSs.

Results and Discussion

We therefore validated the effects of DNase-I pMNSs on reduction of the neutrophils in NETosis. To demonstrate the effects of DNase-I on DNA degradation, we treated the plasma of severe COVID-19 patients with either free DNase-I or DNase-I pMNSs. The results showed that both forms of DNase-I significantly reduced the eDNA levels (Figure 2), and that exposure of DNase-I to the plasma of severe COVID-19 patients increased the activity of DNase-I (Figure 2b). We also observed markedly reduced NET levels, MPO activity, and NE levels in neutrophils of severe COVID-19 patients upon treatment with DNase-I pMNSs (Figure 2). We also evaluated the effects of DNase-I pMNSs on NF-kB activation and cytokine secretion from neutrophils. The results showed that the activity of NF-κB and secretion of cytokines IL-1β, IL-6, IFN-γ, and TNF-α were slightly reduced upon treatment with free DNase-I, and were further drastically reduced upon treatment with DNase-I pMNSs (Figure 2).

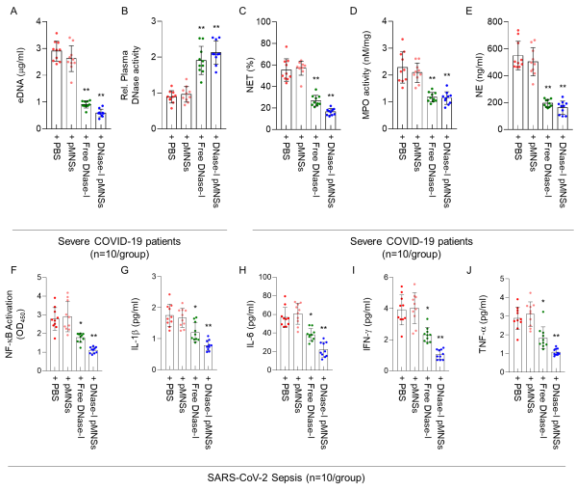
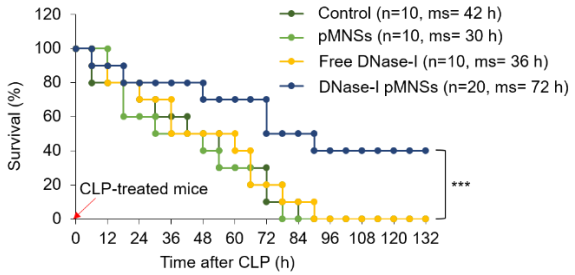


Figure 2. DNase-I pMNSs suppress NETosis factors and cytokine storm via down-regulation of NF-κB activation.

We further validated the effects of DNase-I pMNSs in vivo. Based on a recent report that neutrophil activity is a valid target for preventing or reducing sepsis and enhancing survival, we tested the exogenous administration of the DNase-I pMNSs in an in vivo setting: cecal ligation and perforation (CLP)-treated septic mouse model (Figure 3). All of the CLP-operated mice died within 90 h of CLP induction when PBS, PEG-Nano, or free DNase-I were administered. This demonstrated the high mortality rate of the CLP-operated sepsis model. Previously, the pharmacodynamics of recombinant human DNase I in the serum was analyzed, indicating a short half-life, and a lack of in vivo effect of free DNase-I. Interestingly, DNase-I pMNSs demonstrated a 40% survival rate for the CLP-operated septic mice for over 132 h, leading to the full recovery of these mice. We then confirmed the effect of DNase-I pMNSs on the lungs, and we found a significant reduction in the morphological changes caused by CLP, including pulmonary edema, hemorrhage, alveolar collapse, and inflammatory cell infiltration (Figure 3).



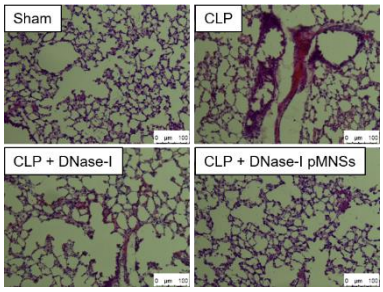


Figure.3. Anti-NETosis effects of DNase-I pMNSs in the sepsis mouse model.

Acknowledgements

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