The Therapeutic Potential of Peptide 6A Derived from Frog Skin Secretions in the Treatment of Sepsis

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Introduction

Sepsis is a common life-threatening condition that affects the health of millions of patients around the world and is defined as systemic inflammatory response syndrome (SIRS) of the host to infection. When an infectious disease is treated with conventional antibiotics, the antibiotics cause the bacteria to rupture and dissolve, releasing large amounts of endotoxins such as lipopolysaccharide (LPS) and activating a systemic inflammatory cascade. At the same time, the abuse of antibiotics has directly led to the serious problem of antibiotic resistance in related bacteria. Antimicrobial peptides (AMPs) are short cationic amphiphilic peptides with antimicrobial and immunomodulatory activities, which are a core part of the natural immune system and can effectively prevent and control infection by combining antibacterial and anti-inflammatory effects. In our previous study, a natural antimicrobial peptide with anti-staphylococcus aureus activity was obtained from the skin secretions of South American frogs and named Medusin-PT. After amino acid substitution, a double-acting peptide 6A with antibacterial and anti-inflammatory activity was obtained. First, it was tested for antimicrobial activity against a group of gram-positive, gram-negative, and drug-resistant bacteria. Subsequently, the cytotoxicity of the modified peptide was evaluated by cytocompatibility studies and its anti-inflammatory activity in vitro was evaluated by detecting the inhibitory effect of the modified peptide on the release of pro-inflammatory factors from the primary peritoneal macrophages of mice induced by lipopolysaccharide. The alleviating effect of 6A on inflammation in vivo was evaluated by cecal ligation and puncture induced sepsis model.

Secondary Structure of 6A

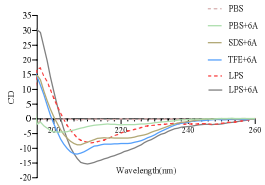

Figure 1. CD spectrum of 6A in solvent and LPS environment.

Table 1. Secondary structure parameters of 6A in different solvent environments

6A	PBS	SDS	TFE	LPS
Helix	6.7%	75.5%	70.6%	64.8%

The half-life of 6A in rats is approximately 7 hours

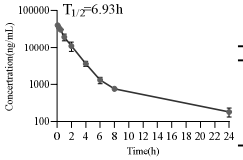

Table 3. Pharmacokinetic parameters of a single intravenous injection of polypeptide 6A

Parameter	Units	Mean
$T_{1/2}$	hr	6.93
T_{max}	hr	0.1389
C_{max}	ng/mL	38000
C_0	ng/mL	42165
Vz	mL/kg	1333
Cl	mL/hr/kg	132.3

Figure 2. The half-life of 6A in rats.

Cytotoxicity of 6A

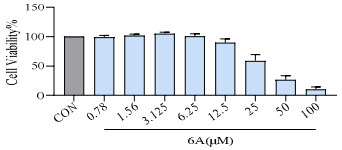

Figure 3. Cytotoxicity of peptides 6A on MPMs 24h.

Antiinflammatory activity of 6A

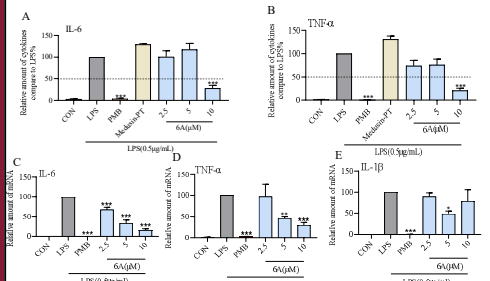

Figure 4. Polypeptide 6A was incubated with LPS for 2h and acted on MPMs cells. (A, B): The inflammatory cytokines IL-6 and TNF-α were detected after the cells were stimulated by 6A and LPS for 12h. (C, D, E): The mRNA of inflammatory factors was detected after 6A and LPS stimulation for 6h.

Mechanism of antiinflammatory activity of 6A

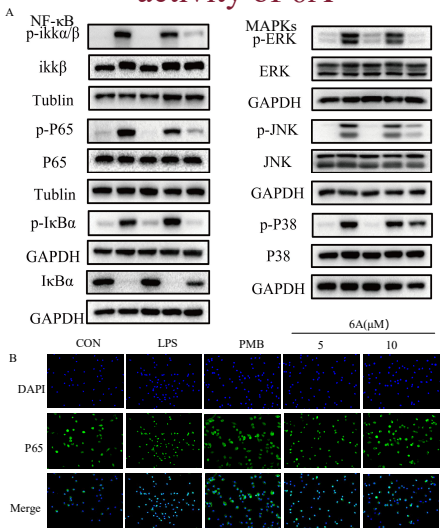

Figure 5. 6A inhibits LPS-induced NF-κB and MAPKs signaling pathways. (A): Protein levels in the MAPKs and NF-κB pathways were detected by Western blotting. (B): Immunofluorescence of P65 into the nucleus.

6A inhibited inflammation in mice with sepsis

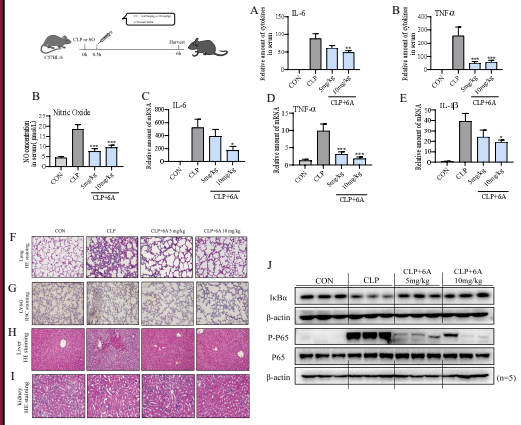

Figure 6. C57BL/6 mice underwent CLP operation or sham operation, and were injected with 6A (5 mg/kg, 10 mg/kg) or normal saline through the tail vein 30 min after operation, and killed 6h later. (A-C): Serum levels of IL-6, TNF-α and NO. (C-E): Levels of inflammatory cytokines mRNA in lung tissue. (F): HE staining of lung tissue. (G): Immunohistochemistry of lung tissue. (H, I): HE staining of liver and kidney tissue. (J): The western blot of NF-κB protein in lung tissue.

Conclusions

1. 6A has a high helicity in membrane and LPS environments, and a long half-life in vivo.
2. 6A reduces inflammation by inhibiting LPS-induced activation of NF-κB and MAPKs signaling pathways.
3. 6A can not only alleviate the systemic inflammatory response induced by aerocecal ligation and puncture (CLP), but also has a certain protective effect on other organs, including lung tissue, liver tissue and kidney tissue damage.

Information

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