РАЗДЕЛ VI

ФУНДАМЕНТАЛЬНАЯ МЕДИЦИНА

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CHOLESTEROL-CONJUGATED SMALL INTERFERING RNA TARGETING *IL6* ALLEVIATES ACUTE LUNG INJURY IN MICE *

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Abstract

Inflammatory diseases affect a substantial portion of the global population, and the current treatment is not universally effective. We demonstrate that locally administered cholesterol-conjugated siRNA targeting *Il6* alleviates the symptoms of acute lung injury, which makes it a promising strategy for the treatment of inflammatory diseases with potential applications beyond the lungs.

Inflammatory diseases affect 5–9 % of the global population, with their incidence increasing annually. The existing treatment is not effective for all patients and is often accompanied by side effects. Small interfering RNAs (siRNAs) sequence-specifically silence genes at the post-transcriptional level, representing a novel therapeutic modality. While their clinical application is currently limited to liver diseases, methods for targeting extrahepatic tissues with siRNA are advancing, with promising results achieved by conjugating siRNAs to lipophilic molecules. The lung is an attractive organ for siRNA delivery since it allows for local administration and has relatively low nuclease activity. Acute lung injury (ALI), a severe inflammatory condition, is characterized by damage to the vascular endothelium and alveolar epithelium, which leads to interstitial and pulmonary edema and, ultimately, alveolar collapse. Interleukin-6 (*Il6*) is one of the key regulators of inflammatory processes, and a reduction in *Il6* levels is associated with the alleviation of ALI symptoms. Thus, in this study, we designed a cholesterol conjugate of anti-*Il6* siRNA to reduce ALI manifestations.

First, we screened nine sequences of anti-*Il6* siRNAs selectively modified with 2'OMe in J774 murine macrophage cells. Four of the nine siRNAs transfected with Lipofectamine 2000 were inactive; other four reduced *Il6* mRNA to 50 %, and the best one reduced *Il6* mRNA to 35 %. The latter sequence was further modified with 2'OMe, 2'F, and phosphorothioate (PS) modifications for increased nuclease resistance and conjugated to cholesterol to allow for *in vivo* delivery.

The siRNA was administered intranasally to BALB/c mice. Four days later, LPS was administered intranasally to induce ALI. Six hours post-induction, mice were sacrificed, and *Il6* mRNA levels in bronchoalveolar lavage (BAL) fluid cells, as well as pro-inflammatory parameters in BAL fluid and lung tissue, were analyzed. The siRNA administered at doses of 2,1, 4,2, and 8,5 μg/g decreased *Il6* mRNA levels in BAL fluid cells by 30, 78 and 71 %, respectively, and the number of total leukocytes in BAL fluid by 1,9, 2,7, and 5-fold compared to the untreated control, respectively. The differential leukocyte count showed the reduction of inflammation-associated granulocyte recruitment, particularly at a dose of 8,5 μg/g. Histological study of the lung tissue demonstrated a 2,2- and 3,2-fold decrease in inflammatory infiltration intensity in the 4,2 and 8,5 μg/g groups. These groups also showed a 1,4- and 1,6-fold decrease in the volume density of alveolar septa compared to the untreated control, thus almost matching the levels observed in healthy mice.

In conclusion, we demonstrate the effective reduction of inflammation by cholesterol-conjugated anti-*Il6* siRNA. The present study was conducted using the ALI model, but our findings may aid in developing novel strategies to treat other inflammatory conditions, including but not limited to rheumatoid arthritis, psoriasis, inflammatory bowel disease, and sepsis.

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