SHORT COMMUNICATION

Anti-inflammatory effect of streptocholorin in cellular and mouse models through TRIF

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ABSTRACT

Streptochlorin, a tiny chemical produced from a marine actinomycete, possesses anti-angiogenic, anti-tumor, and anti-allergic properties. The anti-inflammatory effects and underlying mechanisms, however, have yet to be discovered. The effect of streptochlorin on Lipopolysaccharide (LPS)-induced inflammatory reactions in vitro and in vivo was examined in this work. Streptochlorin inhibited the Toll/Interleukin-1 receptor (TIR)-domain-containing adaptor, which reduced the generation of proinflammatory mediators such as nitric oxide, cyclooxygenase-2, pro-interleukin (IL)-1, and IL-6 in LPS-stimulated RAW264.7 cells. TRIF-dependent signalling mechanism that induces interferon In the LPS-induced Acute Lung Injury (ALI) animal model, streptochlorin inhibited the infiltration of immune cells such as neutrophils into the lung and the generation of proinflammatory cytokines such as IL-6 and TNF- in Broncho-Alveolar Lavage Fluid (BALF).

Streptochlorin exhibits powerful anti-inflammatory effects by modulating TRIF-dependent signalling pathways, suggesting that it could be a useful therapeutic method for a variety of inflammatory illnesses.

Key Words: *Streptochlorin; Lps; Anti-inflammation; Trif; Ali*

INTRODUCTION

Chronic inflammation is linked to numerous disorders, including inflammatory bowel disease and rheumatoid arthritis [1]. Inflammation is a host defence mechanism against microorganisms. Macrophages play a key role in inflammation and are used as an in vitro model to assess the efficacy of anti-inflammatory medicines and investigate their mechanisms of action [2]. Toll-like Receptor 4 (TLR4) recognises LipoPolysaccharide (LPS) and activates two distinct signal pathways, MyD88- and TIR-domain-containing adapter-inducing. The Interferon Regulatory Factor (IRF3), the transcriptional regulator, late-phase activation of NF-B, and Mitogen-Activated Protein Kinase (MAPK) are all activated by the TRIF-dependent signalling pathway [2]. Inflammatory cytokines and Type I Interferons (IFNs) are produced by the TRIF-dependent signalling pathway. Many extreme situations can cause Acute Lung Injury (ALI), which is defined by increased permeability of the alveolar-capillary barrier, resulting in lung edoema with protein-rich fluid and, as a result, Impaired Arterial Oxygenation [3]. LPS inhalation mimics gram-negative ALI in humans, causing neutrophil recruitment, lung edoema, and eventually gas exchange impairment [4]. Streptochlorin, a yellowish amorphous compound isolated from Streptomyces sp., possesses selective cytotoxicity against several cancer cell lines. We have previously reported that streptochlorin has anti-allergic activity in RBL-2H3 cells [5]. In this study, we report the anti-inflammatory effects of streptochlorin and underlying mechanisms involved in both cellular and animal models.

Streptochlorin reduced the production of proinflammatory mediators in raw264 cells stimulated with Lipopolysaccharide (LPS).

Streptochlorin concentrations up to 100 M had no effect on RAW264.7 cell survival. For the following analyses, the maximum concentration of streptochlorin was set to 50 M. Streptochlorin suppressed Nitric Oxide (NO) production by decreasing Inducible Nitric Oxide Synthase (iNOS) protein expression and inhibited Cyclooxygenase-2 (COX-2) in RAW264.7 cells, as demonstrated in ,B. Streptochlorin also decreased pro-IL-1 and IL-6 protein and mRNA levels. Streptochlorin, on the other hand, decreased LPS-induced Tumour Necrosis Factor (TNF-) release only somewhat. Streptochlorin decreased both the protein and mRNA levels of IFN- in RAW264.7 cells, which was surprising. IRF3 and IFN-expression are activated by TRIF-dependent signals IFN- then activates signal Transduction and Transcription 1 (STAT1) signalling, resulting in the production of inducible genes including iNOS and the induction of IL-6 and pro-IL-1 [6]. In RAW264 cells, streptochlorin dramatically reduced LPS-induced IRF phosphorylation As a result, we looked at how streptochlorin affected LPS-induced STAT1 activation in RAW264.7 cells. In RAW264.7 cells,

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streptochlorin dramatically reduced LPS-induced STAT1 activation . Streptochlorin did not impact STAT1 phosphorylation in IFN-primed cells, showing that streptochlorin had no effect on IFN-receptor downstream signals. Streptochlorin inhibited FcRI-mediated tyrosine kinases such as Lyn, Fyn, and Syk in IgE/DNP-HSA-stimulated RBL-2H3 cells in a prior work [7]. The tyrosine kinase Jak/STAT pathway is well-known. Streptochlorin suppressed phosphorylation of the STAT1 tyrosine residue strongly, as shown in 1K; consequently, we investigated the effect of streptochlorin on the phosphorylation of the STAT3 tyrosine residue in LPS-stimulated RAW264.7 cells. Streptochlorin's effect on LPS-stimulated peritoneal macrophages from MyD88 mutant mice was investigated to confirm its participation in the LPS-induced TRIF-dependent signal pathway. Streptochlorin significantly decreased IL-6 production in both LPS-stimulated wild type and MyD88 deletion peritoneal macrophages, as demonstrated in . Streptochlorin, on the other hand, had no effect on TNF-production in either wild type or MyD88 deletion peritoneal macrophages. Streptochlorin inhibited LPS-mediated inflammatory responses mostly via a TRIF-dependent signal pathway, according to these findings. Furthermore, streptochlorin had no effect on LPS-driven MAPK signalling in RAW264.7 cells or matured IL-1 secretion in bone marrow-derived macrophages triggered by NLRP3 inflammasome activation. Streptochlorin reduced MAPK signalling, as we described in a recent publication. It's still unclear how streptochlorin interacts with MAPK in distinct ways. Streptochlorin suppressed the LPS-induced inflammatory responses by inhibiting TRIF-dependent signals, according to our findings.

Streptochlorin Protected Mice against LPS-Induced ALI The role of endotoxin or LPS generated from Gram-negative bacteria in the pathophysiology of ALI has long been recognised. As a result, we expanded our research to include an in vivo mouse model to back up our in vitro findings. Lung tissue H&E staining, cell counting, and an enzyme-linked immunosorbent assay were used to examine the effects of streptochlorin on cell infiltration and proinflammatory cytokine release in BALF (ELISA). Inflammatory cells were not discovered in the alveolar gaps of mice in the normal group . After LPS treatment, however, a considerable number of inflammatory cells were attracted into the alveolar gaps . Streptochlorin treatment at the prescribed levels, on the other hand, significantly reduced inflammatory cell infiltration. Furthermore, streptochlorin therapy reduced inflammatory cytokines such as TNF- and IL-6 in the BALF. With the tested levels of streptochlorin treatment, no notable side effects were noted, including body weight loss. These findings suggest that streptochlorin protects against LPS-induced ALI by decreasing inflammatory cells at the site of inflammation, such as neutrophil migration and proinflammatory cytokine production.

Reagants and antibodies

The production of streptochlorin has been previously described [8]. Gibco provided penicillin, streptomycin, Dulbecco's modified Eagle's medium (DMEM), and Foetal Bovine Serum (FBS) (Grans Island, NY, USA). Biolegend provided the IFN-ELISA kit for this study (San Diego, CA, USA). BD Biosciences provided the TNF-, IL-6 ELISA Set (BD OptEIA TMSet) and TMB Substrate Reagent Set (Franklin Lakes, SD, USA). Santa Cruz Biotechnology provided antibodies against iNOS, COX-2, IL-1, and -actin (Santa Cruz, CA, USA). Cell Signaling Technology, Inc. provided antibodies to phosphor and the total form of IRF3 (S396), as well as STAT1 (Y701) and STAT3 (Y705) (Beverly, MA, USA). PBL Interferon Source provided us with recombinant mouse IFN- (Piscataway, NJ, USA). Pierce Chemical provided Western blot Chemiluminescence reagent kits (Super Signal West Pico Stable Peroxide and Super Signal West Pico Luminol/Enhancer solutions) (Rockford, IL, USA). Millipore Corporation supplied the Polyvinylidene Fluoride (PVDF) membrane (Bedford, MA, USA). Sigma–Aldrich provided LPS (E. coli 026:B6) and other compounds (St. Louis, MO, USA). All of the other compounds we employed in our research were of the greatest possible grade.

Culture of Cells

The RAW 264.7 murine macrophage cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and kept at 37 °C in humidified 5 percent CO2 and 95 percent air in DMEM supplemented with 10% heat-inactivated FBS and antibiotics (100 U/mL penicillin, 100 g/mL streptomycin). 8-week-old C57BL/6 and MyD88 mutant mice were used to isolate peritoneal macrophages. Mice were injected with 2 mL of 4 percent thioglycollate broth and peritoneal macrophages were collected by peritoneal lavage (BD Diagnostic Systems; Sparks, MD, USA). Three days following injection, thioglycollate-elicited peritoneal macrophages were collected from the peritoneal cavity of mice.

Lps-Induced ali mouse model and animals

Orient Bio sold female BALB/c mice (22–25 g, 8 weeks old) to us (Seoul, Korea). MyD88-deficient (MyD88/) C57BL/6 mice have been characterised. They were kept in groups of five in regular settings (temperature 22 °C, humidity 55 %, 12-hour light/dark cycle), with food and water available at all times. The Konkuk University Committee on the Ethics of Animal Experiments examined and approved the study protocol.

Hematoxylin and eosin (H&E) staining

Lung tissues were taken 24 hours after LPS injection, fixed for 24 hours in 4 percent paraformaldehyde, embedded in paraffin medium, and sectioned (4 m). H&E was used to stain the tissue-sectioned samples. Light microscopy was used to examine pulmonary edoema and inflammatory cell infiltration.

Streptochlorin inhibits antigen-induced degranulation in RBL-2H3 cells

The anti-allergic action of steptochlorin was evaluated and its molecular processes were elucidated using RBL-2H3 mast cells as an in vitro model. The cytotoxicity of streptochlorin was initially assessed using an MTT-based viability assay. For 24 hours, RBL-2H3 cells were exposed to various doses of streptochlorin. The viability of RBL-2H3 cells was unaffected by streptochlorin doses up to 100 M. Mast cells secrete prepared allergic mediators in granules, such as histamine and different proteases, which is a critical step in local allergic reactions. The amount of -hexosaminidase produced from cells has been utilised as a marker for mast cell degranulation. Streptochlorin was used to see if it could stop Antigen-stimulated degranulation in RBL-2H3 cells. In RBL-2H3 cells, DNP-HSA-induced degranulation was significantly and dose-dependently inhibited.

Streptochlorin inhibits allergy and pro-inflammatory cytokine expression and production.

TNF- and IL-4 are important cytokines for generating delayed type hypersensitive allergy and inflammatory reactions. As a result, we investigated whether streptochlorin could decrease TNF- and IL-4 production in antigen-induced RBL-2H3 cells. Streptochlorin inhibited TNF- and IL-4 secretion from RBL-2H3 cells induced by DNP-HSA, as well as the expression levels of the related mRNA. Streptochlorin reduces the generation of TNF- and IL-4 in FcRI-stimulated RBL-2H3 cells by preventing their transcription, according to these findings. Streptochlorin significantly lowered TNF- protein and mRNA levels, but not IL-4 levels, which were not statistically significant. Streptochlorin strongly inhibited LPS-induced

proinflammatory mediators such as NO, pro-IL-1β, and IL-6 in RAW264.7 cells through inhibition of the TRIF-dependent signaling pathway. Streptochlorin inhibited TRIF-dependent signaling from LPS-primed TLR4, leading to reduced activation of IRF3 and STAT1. Streptochlorin also attenuated LPS-induced ALI via suppression of neutrophil infiltration and proinflammatory cytokine production, such as TNF-α and IL-6.

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