Dexamethasone Down-Regulates the Expression of microRNA-155 in the Livers of Septic Mice

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Abstract

To investigate the expression of microRNA-155 (miRNA-155) in the livers of mice with lipopolysaccharide (LPS)-induced sepsis and to determine the role of dexamethasone (DXM) in the regulation of miRNA-155 expression, we pretreated mice with or without DXM prior to LPS exposure. Our study demonstrated that the expression of miRNA-155 and inflammatory factors increased in the liver tissues of mice with LPS-induced sepsis and that DXM down-regulated their expression in a dose-dependent manner. Moreover, DXM alone inhibited the expression of miRNA-155 to below the baseline level, but did not impact the expression of inflammatory factors, suggesting that the down-regulation of miRNA-155 by DXM may partially, but not completely, depend on the suppression of pro-inflammatory cytokines by DXM. Our data indicate that the overexpression of miRNA-155 in the livers of mice with LPS-induced sepsis may play an important role in the pathological processes of sepsis and that the down-regulation of miRNA-155 by DXM may be a novel mechanism regulating inflammation and immunity.

Introduction

Sepsis, an infection-induced systemic inflammatory response syndrome, remains as the main cause of death in critical patients and the process is complex. Several regulatory mechanisms are involved in the development of sepsis. The discovery of microRNAs (miRNAs) suggests a novel regulatory mechanism for this process.

miRNAs are a class of small non-coding RNA molecules with critical roles in cell proliferation, differentiation, and apoptosis [1]. Ample evidence suggests that miRNAs are key regulators in animal development and are involved in a variety of human diseases [2]. The transcription of primary miRNAs (pri-miRNAs) by RNA polymerase II is the first step of miRNA biogenesis. In the nucleus, pri-miRNAs are converted into hairpin precursor miRNAs (pre-miRNAs). Pre-miRNAs are transported into the cytoplasm where they are further processed into mature miRNAs consisting of 22 nucleotides; Dicer is involved in these final processing events. Mature miRNAs have the ability to bind to the 3’ untranslated region (UTR) of their target mRNAs in a sequence-specific manner [3], initiate partial or full degradation of mRNA transcripts, and regulate the expression of protein-coding genes at the post-transcriptional level. The effects of miRNAs are mediated by inhibiting the translation or degradation of the target mRNA. TNF-α, an important inflammatory factor, is inhibited indirectly by miRNA-146a, which targets TRAF6 and IRAK1 [4], and directly by miRNA-125b, which targets the 3’UTR of TNF-α mRNA[5]. More than 700 miRNAs have been identified in mammals to date. These miRNAs are associated with diverse biological processes, such as the regulation of insulin secretion, viral infection, and tumorigenesis. Previous studies have indicated that most miRNAs play a role in innate immune responses and inflammation. In response to inflammation, some miRNAs are up-regulated, while some others are down-regulated. For example, the expression of miRNA-146a, miRNA-155and miRNA-21 are up-regulated in monocytes challenged by LPS [4,6,7], whereas that of miRNA-125b is down-regulated [5].

miRNA-155 was initially discovered as a proto-oncogene in lymphoma [8]. The overexpression of miRNA-155 has been detected in B-cell lymphomas[9][10] and chronic lymphocytic leukemia[11]. It is also overexpressed in various solid tumors, including lung[12], breast[13], pancreatic[14], and thyroid cancers[15]. The roles of miRNA-155 in various physiological
and pathological processes, such as hematopoietic lineage differentiation, inflammation and immunity, have been identified recently [16]. miRNA-155 is required for the development of T cells, B cells, and dendritic cells. Previous studies have shown that miRNA-155 plays an important role in immunoglobulin class switching to IgG in B cells via the targeted repression of the transcription factor PU.1 [17] and activation-induced cytidine deaminase (AID) [18]. Other validated target genes of miRNA-155, such as interleukin-1 (IL-1), IkappaB kinase ε (IKKε), Ets-1, and Meis1, are associated with the hematopoietic and immune systems [19–21]. miRNA-155 gene knockout mice displayed severe immune response deficiencies after pathogen exposure. In antigen-specific inflammatory responses against autologous tissue, miRNA-155 has been shown to promote autoimmune inflammation [22]. Furthermore, miRNA-155 has been found to be up-regulated in macrophages following stimulation by a broad range of inflammatory mediators [6]. LPS induces the expression of miRNA-155 in the spleens of mice [5], but this increase in expression is not observed in other organs. The LPS-induced expression of miRNA-155 should be investigated in the liver because this organ is commonly damaged in mice with sepsis.

Dexamethasone (DXM), a potent synthetic member of the glucocorticoid family, has anti-inflammatory, anti-allergic, anti-shock, and anti-endotoxin effects. DXM has been widely used to treat inflammatory and autoimmune diseases, including severe sepsis, multiple sclerosis, rheumatoid arthritis, asthma, and systemic lupus erythematosus. In the cytoplasm, DXM interacts with the glucocorticoid receptor and forms a ligand-receptor complex, which subsequently translocates to the nucleus. In the nucleus, the complex binds to genomic DNA and regulates the expression of both anti-inflammatory and inflammatory genes at the transcriptional level. Moreover, DXM may impact the activities of signal-dependent transcription factors, including members of the activator protein 1 (AP-1) and nuclear factor-κB (NF-κB) families [23], to negatively regulate inflammatory responses. To elucidate the mechanism of DXM in regulating the inflammatory and immune response in mice with sepsis, we established a mouse model of sepsis and determined the effect of DXM on the expression of miRNA-155 in the liver.

Materials and Methods

Animal experiments were performed in strict accordance with the Guide for the Care and Use of Laboratory Animals of Sun Yat-sen University. The protocol was approved by the Committee on the Ethics of Animal Experiments of the First Affiliated Hospital of Sun Yat-sen University. A total of 350 female BALB/c mice (6-8 weeks of age and weighted 20-25 g) were purchased from the Experimental Animal Center of Sun Yat-sen University (Guzhangzhu, China). Mice were housed in a pathogen-free animal facility that was maintained at 24°C, 55% humidity, and had a 12 h light/dark cycle. All mice had free access to food and water, and they received humane care in accordance with the National Institutes of Health guidelines and the legal requirements of China. The mouse model of sepsis was developed by intraperitoneal injections of LPS as previously described [24].

Three hundred mice were divided randomly into five groups, with 60 mice in each group: <1> the normal saline (NS) control group received an intraperitoneal injection of 50 μl of NS; <2> the LPS group received an intraperitoneal injection of 15 mg/kg E. coli LPS (Escherichia coli O55:B5, Sigma, St. Louis, MO, USA); <3> the NS-LPS group received an intraperitoneal injection of 50 μl NS 1 hour prior to LPS exposure; <4> the DXM-LPS group received an intraperitoneal injection of 5 mg/kg DXM (Sigma, St. Louis, MO, USA) dissolved in 50 μl NS 1 hour prior to LPS exposure; and <5> the DXM group received an intraperitoneal injection of 5 mg/kg DXM dissolved in 50 μl NS. We prepared the DXM according to the manufacturer’s instruction. The LPS and DXM were dissolved in sterile saline solution under aseptic condition before injection. Ten mice from each group were killed at each indicated time point following LPS treatment (0, 2, 6, 12, 24, and 48 hours), and the liver tissue and peripheral blood were collected.

The remaining 50 mice were divided equally into five groups which received injection of 0, 0.5, 2, 5, and 10 mg/kg DXM, respectively, dissolved in 50 μl NS 1 hour prior to LPS exposure and were killed at 12 h following LPS treatment. The liver tissues were collected.

Measurements of serum ALT, TNF-α, IL-6, and IL-10 levels

The serum levels of alanine aminotransferase (ALT), TNF-α, IL-6, and IL-10 were measured. Blood samples from the NS and LPS groups were centrifuged at 1,000 ×g at 4°C for 20 minutes. Serum ALT was determined using a commercial kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Serum levels of TNF-α, IL-10, and IL-6 were quantified using an ELISA kit (R&D Systems, Inc, Minneapolis, USA). Initially, the serum from each mouse was added to 96-well plates (10 μl/well) containing capture antibodies specific to mouse TNF-α, IL-6, and IL-10. Next, 100 μl of HRP-conjugated reagent was added to each well. The plates were washed, and a substrate solution was added to each well. The plates were incubated for 15 minutes at 37°C, and subsequently, a stop solution was added to each well. Finally, the plates were read at 450 nm.

Measurement of tissue TNF-α, IL-6 and IL-10 levels

Liver tissues were homogenized in ice-cold sterile saline at a ratio of 1 ml per 100 mg tissue using an Ultra-turrax T8 homogenizer (IKA, Germany). The homogenates were centrifuged at 1,000 ×g at 4°C for 15 minutes. The supernatant was transferred to a new tube and stored at -80°C. The amount of total protein was detected using the Bradford reagent (Sigma, St. Louis, MO, USA) according to the manufacturer’s instructions. A total of 50 μg of protein was used to examine tissue TNF-α, IL-6, and IL-10 levels with an ELISA Kit (R&D Systems, Inc, Minneapolis, USA).

Histopathologic evaluation

The liver tissues were subjected to histological examination. All liver tissues were immersed in 4% formaldehyde and...
An acute inflammatory response was induced in mice with LPS-induced sepsis by 12 hours, but IL-6 could still be detected at 24 hours after LPS injection. A large number of leukocytes infiltrated in the liver was observed 2 hours after LPS exposure, indicating that TNF-α or IL-6, IL-10 significantly increased in serum (Figure 1A-C) and liver tissues of BALB/c mice (Figure 2A-C). Serum and tissue TNF-α levels simultaneously peaked at 6 hours and declined as the internal normalized reference. Real-time PCR primers were provided by RiboBio (Guangzhou, China). The expression of miRNA-155 was measured using the Roche Light Cycler 480 Real-Time PCR system. PCR reactions were performed at 95°C for 10 minutes followed by 40 cycles of denaturing at 95°C for 15 seconds and an annealing/extension at 60°C for 60 seconds. All reactions were performed in triplicate. The qRT-PCR data were analyzed and expressed as relative miRNA levels of the cycle threshold value, which was subsequently converted to fold change with 2^−ΔΔCT method[25].

Statistical analysis
Statistical changes in cytokine and ALT expression were determined with the two-tailed student T test; the changes of miRNA expression were analyzed by analysis of variance (ANOVA). Differences were considered significant at P < 0.05.

Results
An acute inflammatory response was induced in mice with LPS-induced sepsis
After LPS exposure, the expression of inflammatory cytokines TNF-α, IL-6, and IL-10 were dramatically increased in serum (Figure 1A-C) and liver tissues of BALB/c mice (Figure 2A-C). Serum and tissue TNF-α levels simultaneously peaked at approximately 2 hours after LPS exposure and quickly declined to baseline levels over 12 hours (Figure 1A and 2A). Serum and tissue IL-6 levels peaked at 6 hours and declined by 12 hours, but IL-6 could still be detected at 24 hours after LPS exposure (Figure 1B and 2B). Serum and tissue IL-10 levels as well as serum ALT level increased with time after LPS injection (Figure 1C, 2C, and 1D).

Liver tissues were observed under a microscope with HE staining to investigate the inflammatory reaction. Compare to the normal liver tissue pathology (Figure 3A). The recruitment of inflammatory cells into the liver was observed 2 hours after LPS injection. A large number of leukocytes infiltrated in the portal tracts and sinusoids. Furthermore, hepatocyte vacuolation, architectural distortion, and nodular necrosis were evident at 12 hours after LPS injection (Figure 3B).

Expression of miRNA-155 was up-regulated in the livers of mice with LPS-induced sepsis
The expression of miRNA-155 in the liver peaked at 12 hours after LPS exposure, approximately 70-fold (72.56 ± 9.34) higher than the level observed in the NS group, and returned to the baseline level after 48 hours (Figure 4A).

Effect of DXM on the expression of inflammatory cytokines and miRNA-155 in liver tissue
Compared with the increased levels of TNF-α, IL-6, and IL-10 in liver tissues in the LPS group, those levels in the NS-LPS group were similar, whereas those in the DXM-LPS group were significantly decreased (Figure 2). Moreover, pretreatment with DXM appeared to prevent most of the pathological damages induced by LPS in the livers (Figure 3C). Compared with the increased expression of miRNA-155 in the LPS and NS-LPS groups, the LPS-induced overexpression of miRNA-155 was suppressed in the DXM-LPS group at 2 hours after treatment, and the greatest reduction occurred at 12 hours (Figure 4A). The expression level of miRNA-155 in the DXM group was significantly down-regulated from 6 to 12 hours after treatment (Figure 4A). Furthermore, we found that DXM suppressed miRNA-155 expression in a dose-dependent manner (Figure 4B).

Discussion
To investigate the role of miRNA-155 during sepsis, we detected the dynamic changes in miRNA-155 expression in the liver of mice with LPS-induced sepsis and determined the regulatory effect of DXM on LPS-induced miRNA-155 overexpression. We found that the expression of inflammatory mediators TNF-α, IL-6, and IL-10 significantly increased in the serum and liver after LPS exposure, which initiated the systemic inflammatory response and tissue injury. As a biomarker of liver injury, ALT expression increased at 2 hours following LPS challenge, and hepatocyte vacuolation, architectural distortion and nodular necrosis became evident over time. The expression of miRNA-155 was also up-regulated, coincident with the up-regulation of inflammatory cytokines. These observations were consistent with our previous research [26].

The simultaneous increase in the expression of miRNA-155 and inflammatory cytokines in mice with LPS-induced sepsis may attribute to their interactions. Previous study found that TNF-α stimulated the expression of miRNA-155[27]. On the other hand, Eμ-miRNA-155 transgenic mice, which overexpress miRNA-155 in cells from the B-cell lineage, produce more TNF-α when compared with wild-type mice after LPS exposure, indicating that miRNA-155 may increase the production of inflammatory mediators [5]. Similarly, Jiang et al. [28] stimulated the breast cancer cells with IL-6 and found that expression of miRNA-155 increase, suggesting that IL-6 also induces the miRNA-155 expression. Compared with the pro-inflammatory cytokines TNF-α or IL-6, IL-10, which service as an important anti-inflammatory cytokine, prevents inflammation-mediated tissue damage by targeting various leukocytes and repressing excessive inflammatory responses.
It has been found to inhibit miRNA-155 transcription by Toll-like receptors in a STAT3-dependent manner[30,31], and miRNA-155-deficient CD4+ T cells have been shown to produce more IL-10[32]. However, we observed simultaneous up-regulation of miRNA-155 and IL-10. This may be related to the complex internal environment.

DXM can induce anti-inflammatory cytokines and inhibit pro-inflammatory cytokines at the transcriptional level, and thus could be used to treat sepsis. Consistent with previous studies, our data showed that the increased serum and tissue TNF-α and IL-6 levels in mice with early stages of sepsis were down-regulated by DXM. miRNA-155 and DXM are both regulators of inflammation and immunity, but the relationship between them requires further investigation. Moschos et al [7] have found limited effect of DXM on LPS-induced expression of miRNAs in the lungs of mice. We found that the expression level of miRNA-155 in the liver tissues from the LPS group was approximately 70 folds higher than the NS group, but this increase was impaired by DXM pretreatment. DXM may inhibit LPS-induced miRNA-155 expression indirectly by reducing the expression of pro-inflammatory cytokines. On other hand, the expression level of miRNA-155 in the DXM group was even lower than that in the NS group. DXM reduced the basal expression level of miRNA-155 in mouse liver without LPS exposure, but did not reduce the basal expression of pro-inflammatory cytokines, indicating that other mechanisms may be involved in this process. In addition, we found that DXM suppressed miRNA-155 expression in the liver tissues in a dose-dependent manner. This change in miRNA-155 expression may be related to the levels of inflammatory cytokines. LPS may induce the expression of miRNA-155 by promoting the expression of inflammatory factors. A wide variety of inflammatory cytokines, such as TNF-α and IL-6, can up-regulate miRNA-155 expression. This up-regulation can be explained by the functions of pro-inflammatory transcription factors, such as NF-kB and AP-1, which promote the transcription of BIC genes. BIC genes contain the pri-miRNA-155 transcription gene [33]. However, DXM may reduce the production of TNF-α by activating multiple signaling pathways, such as the PI3K, NF-kB, Akt/PKB, and MAPK signaling pathways [34,35]. Therefore, DXM may reduce miRNA-155 expression in the liver by inhibiting pro-inflammatory cytokine expression. DXM reduced the baseline expression of miRNA-155 but did not suppress the baseline levels of TNF-α and IL-6, suggesting that down-regulation of miRNA-155 by DXM may not depend completely on the ability.

Figure 1. Serum levels of inflammatory cytokines and alanine aminotransferase (ALT) increase in mice with sepsis induced by lipopolysaccharide (LPS). (A) tumor necrosis factor-α (TNF-α), (B) interleukin-6 (IL-6), (C) IL-10 and (D) ALT. Data are provided as the mean ± standard deviation (SD) of 10 mice in each group (*P < 0.05 versus the NS group).

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Figure 2. The levels of inflammatory cytokines in the livers of mice with LPS-induced sepsis. are suppressed by dexamethasone (DXM). Mice were divided randomly into five groups: NS group received an intraperitoneal injection of 50 μl of NS; LPS group received an intraperitoneal injection of 15 mg/kg LPS; NS-LPS group received an intraperitoneal injection of 50 μl NS 1 hour prior to LPS exposure; DXM-LPS group received an intraperitoneal injection of 5 mg/kg DXM dissolved in 50 μl NS 1 hour prior to LPS exposure; DXM group received an intraperitoneal injection of 5 mg/kg DXM dissolved in 50 μl NS. (A) TNF-α, (B) IL-6 and (C) IL-10. Data are provided as the mean ± SD of 10 mice in each group (* P < 0.05 versus the NS group; # P <0.05 for the DXM-LPS group versus the NS-LPS group).

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of DXM to suppress pro-inflammatory cytokines. It is likely that DXM may directly bind to the BIC genes to suppress miRNA-155 expression.

The regulation of miRNA-155 by DXM may be a novel mechanism regulating inflammation and the immune response. Our results may provide the theoretical support for the application of DXM in the treatment of sepsis.

Author Contributions

Conceived and designed the experiments: ZFM XCH. Performed the experiments: ZHW YBL. Analyzed the data: HT. Contributed reagents/materials/analysis tools: ZYL. Wrote the manuscript: ZBC.

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