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The Study of Dynamic Expression of Nrf2 in Sepsis Rat Liver

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To investigate the expression of Nrf2 in sepsis rat liver. Methods: Wistar rats were divided into normal control group, S.aureus sepsis combined with Pseudomonas aeruginosa group. According to different phases (2 hours, 8 hours,24 hours), divided into three subgroups, to test the expression of Nrf2 mRNA and protein in liver. Results:high expression of Nrf2 mRNA in normal mouse liver; with increased expression of Nrf2 in Staphylococcus aureus septic rat liver tissue, and increased to peak after 2 hours, and then at 24 hours showed a downward trend. With upregulated expression of Nrf2 at 2 hours in Pseudomonas aeruginosa bacteria septic rat liver tissue and the remaining phases showed downward trend. Conclusion:Nrf2 directly involved in the immune response of sepsis.

Key words: Nrf2, sepsis, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

Nrf2 (nuclear factor erythroid-derived 2-like 2) as a nuclear transcription factor, is the key factor for the adjustment of oxidative stress in sepsis[1-7], it has specific regulatory function on a number of cytokines and inflammatory mediator gene[8-10]. In this experiment, Staphylococcus aureus, Pseudomonas aeruginosa infection rat sepsis model simulation was observed the expression of Nrf2 in septic rat liver.

MATERIALS AND METHODS

Animal model and specimen collection

Wister rats of 65 were supplied by Animal Department of Central South University, male and female in random, weighing 250-300 g. The rats were randomly divided into normal control group of 5, *Staphylococcus aureus* infection group (referred to infect group 1) of 30, Pseudomonas aeruginosa infection group (referred to infection group 2) of 30. Infection group 1 and 2 were infected by intraperitoneal injection of Staphylococcus aureus 1mL (the concentration of 8×10^9 CFU/mL), Pseudomonas aeruginosa 0.5 mL (the concentration of 1×10^8 CFU/mL) for the preparation of sepsis model[11], placed in metabolic cages and observed at room temperature 20-25°C, free water. The standard for septic rats is fever (the rectal temperature of 1°C above the control group). With accelerated heart rate (approximately 2 times of the control value) using stethoscope to listen, and with increased respiratory rate (approximately 2 times of the control value). Rats in the infection group2 were sacrificed by cervical at 2,8,and 24h after injury(each of 10) laparotomy, the liver was excised under aseptic; ditto handle the normal control group.

Main material

Staphylococcus aureus is the classical S6, only enterotoxigenic B, Pseudomonas aeruginosa is the standard strain ATCC27853, and all supplied by the Third Xiangya Hospital. RT-

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PCR kit (two-step), purchased from USA Promega Corporation. Nrf2 antibody was purchased from UK AbcamCompany. Primer was purchased from ShangHai Biotechnology Company. Goat anti-mouse Nrf2 antibodies were purchased from American Biolegend Company. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody and Dithiothreitol (DTT)were purchased from Gibco, USA. PCR product purification kit, thermostable DNA polymerase (Taq DNA polymerase), deoxyribonucleoside triphosphates (dNTPs), were purchased from Takara.

EXPERIMENTAL

General observation

Observe the general situation of rats in infection group 1 and 2.

RT-PCR

Extract total RNA of the samples, and quantify. Synthesis and dilute the first strand of cDNA synthesis product as a template, adding primers (upstream: 5'-accggagaattcctcccaat-3'downstream: 5'-agctcctgccaaacttgctc-3') for PCR amplification. Electrophoresis for 45 min, after ethidium bromide staining, scanning under UV light the absorbance was measured and imaging on the gel imaging system, using the absorbance ratio of target gene and internal reference β-actin to measure the expression.

Western blotting

Take about 50mg tissues sample to prepare for cell protein solution, using the bovine serum albumin as standard, Bradford method for protein quantitation. Preparation of SDS-polyacrylamide gel, and total protein sample, transferred tomembrane, the closure for 1 h, followed by adding anti-Nrf2 first antibody (1:1000), anti-rabbit IgG/HRP secondary antibody (1:4000). Put the PVDF membrane in HRP-enhanced chemiluminescence light and developed. Using Gel DoC 2000 gel imaging system for analysis, the results showed the ratio of aim protein and internal reference β-actin absorbance value.

Statistical analysis

The data shown with $\bar{x} \pm s$, using SPSS 13.0 statistical software for the F-test, independent samples t-test, two variables were analyzed using Pearson correlation analysis.

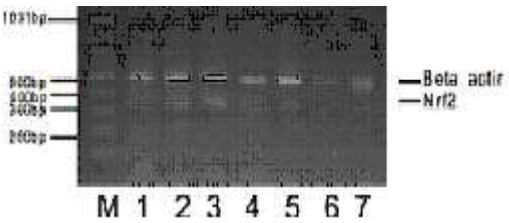
RESULTS

General situation of infection group rats infected rats

Rats in infection group 1 with reduced activity at 30 min after damage, loss of appetite; rectal temperature was (40.5 ± 0.5)°C, with significantly faster heart rate, an average of 95 beats/min, respiratory rate increased more than 2 times up to 160 beats/min, two rats died at 8h; Three rats died after infection at 24h. Rats in infection group 2 with reduced activity after infection at 30 min, decreased appetite; rectal temperature was (39.6 ± 0.8)°C, with significantly faster heart rate, an average of 85 beats/min, respiratory rate increased more than 2 times up to 150 beats/min, four rats died after infection at 8h; two rats died after infection at 24h.

The Nrf2 mRNA expression in rat liver of each group

Extract total RNA in the liver tissue of each group, with β-actin as internal control, RT-PCR amplification Nrf2 gene, PCR products were agarose gel electrophoresis, EB stained and using gel electrophoresis image analyzer for the analysis of results. The results showed that: PCR products of the corresponding rat liver tissue and normal liver tissue showed specific bands, using the light gray comparative analysis to obtain Nrf2 expression in liver tissue of each group (Fig. 1). It can be seen the expression pattern of Nrf2 mRNA in liver tissue: normal rat liver tissue slightly expression of Nrf2 mRNA; Staphylococcus bacteria sepsis group at 2h with increased expression,8-24h with decreased expression (P <0.01); Pseudomonas aeruginosa sepsis group at 2h with increased expression (P <0.01), 8h, 24h were decreased (P <0.01).

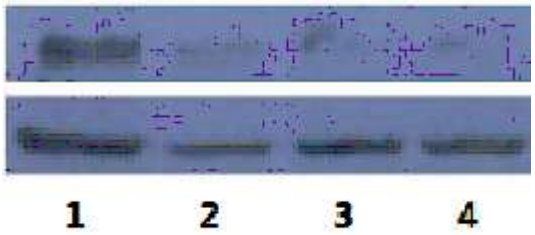


1.Normal liver; 2.Staphylococcus aureus at 2h; 3. *Pseudomonas aeruginos*a at 2h; 4.*Staphylococcus aureus* at 8h; 5. *Pseudomonas* at 8h; 6. *Staphylococcus aureus* at24 h; 7. Pseudomonas aeruginosa at 24h

Fig. 1. The expression of Nrf2 in liver tissue

The expression of Nrf2 protein in rat liver in each group

Nrf2 protein was highly expressed in rat liver in normal control group, OD value was (59.250 ± .054), Staphylococcus bacteria sepsis group at 24 hours Nrf2 protein in liver the OD value was (10.623±0.667), significantly decreased (P<0.05); Pseudomonas aeruginosa sepsis group at 24 hours Nrf2 protein in liver the OD value was (8.330 ± 0.445), compared with the control group was significantly decreased (P <0.01).



1.Normal control group; 2. *Staphylococcus aureus* group at 24h; 3/4.Paeruginosa group at 24 h.

Fig. 2. Nrf2 protein expressions in rat liver of each group

DISCUSSION

Gram-negative bacteria plays the most important role in sepsis pathogenic bacterium, the endotoxin (ET) is the major pathogenic toxin in infection, trauma, shock that occurred, in sepsis/ septic shock pathogenesis play an important role [12,13]. When the ET into the bloodstream, it quickly initiates cell response, and produce pro-inflammatory cytokines, leading to the occurrence of sepsis. Clinical data showed that in recent years, the incidence of gram-positive bacteria sepsis increased year by year[14], the incidence of sepsis has reached more than 50% and is still with increasing trend. Including the incidence of Staphylococcus aureus (refer to S. aureus) rank the first, is the important pathogenic bacteria of infection, acute liver failure and others, and the drug resistance is growing, its prevention and treatment of sepsis has become one of the thorny problems faced by modern critical medicine[]. Because after infection the toxins into the body, mainly in the liver, spleen, lungs and other tissues, and the endotoxin in the liver tissue was significantly higher than that in spleen and lungs, indicating that the liver may be the most important distribution places of endotoxin after infection, and clinical observation in Dobke clearly showed that 7-12 hours after infection the content of endotoxin reached the peak, another peak appeared in the first four days after infection [15]. So the selection of liver tissue in this study, and to select phases of 2 hours, 8 hours and 24 hours.

In normal rat liver tissues all contain small amounts of Nrf2 expression, after severe trauma the function of PMN was significantly inhibited, including chemotaxis, phagocytosis and bactericidal function, neutrophil apoptosis can be inhibited by LPS, TNF-α. In the acute phase of sepsis, neutrophils were inhibited, so with the extending life of neutrophils, show better anti-inflammatory function. However, a large number of neutrophils aggregated and activated in the inflammation part, proteinases of the dead neutrophils overflowed and macrophages activated to produce large amounts of cytokines may cause SIRS and organ damage, which significantly associated with early sepsis dysfunction[16]. The expression of Nrf2 in tissue was rarely; violent endogenous oxidative stress response can damage the endogenous protection system, decreased the content of intranuclear Nrf2, and down regulated the transcription regulator activity.

Expression of Nrf2 mRNA in liver tissue of sepsis animal caused by *Staphylococcus aureus* was up regulated in 2-8 hours, and raised to peak in 2hours, at 24 hours showed a downward trend. Its Western Blot results showed that: Nrf2 protein in the liver tissue slightly increased. It shows that the liver may be the main place for toxins accumulation, because the liver is the body's largest epithelial reticular system, with a strong phagocytosis, neutralization and inactivate toxins function, so septic liver "uptake" and gathered a lot of toxins. A large number of toxins induce expression of a variety of inflammatory mediators, in early sepsis Nrf2 involved in inflammation, increased Nrf2 out and into nuclear response; But the longer it lasts, a strong oxidative stress reaction produce large amounts of ROS, resulting in oxidation-reduction imbalance, causing further damage to tissues and cells, so that the out nuclear of Nrf2 reduced, the transcriptional activity decreased. Expression of Nrf2 mRNA in liver tissue of sepsis animal caused by Staphylococcus aureus

increased in 2 hours in liver tissue, followed by a downward trend of expression. Western Blot results showed that in their liver tissue in 2 hours was consistent with normal expression, in 8 hours, and 24 hours the expression was significantly reduced. It showed that in Pseudomonas aeruginosa sepsis group, due to a large number of exogenous toxins molecules into the body to stimulate the natural immune response and oxidative stress response, making the expression ofNrf2 mRNA increased, the longer it lasts, a large number of TNF-α produced in the body and ensued immune suppression, making Nrf2 mRNA downregulated.

Nrf2 is the most primitive antioxidant genes, can regulate the expression of multiple genes, and is a key transcription factor of endogenous antioxidant system. When the cells were subjected to oxides and other inflammatory mediators may cause damage factors to stimulate intracellular redox state changes, Nrf2 with phosphorylation changes, after dissociation with keap1 into the nucleus[17,18]. Kawachi[3] showed that Nrf2 can weaken the acute phase inflammatory response. In Nrf2 gene knockout rats, the rats increased inflammation, more susceptible to septic peritonitis and endotoxin shock, with increased mortality. Relative to anti-oxidoreductive key genes Nrf2 in the natural immune system, when cells suffer oxides, inflammatory mediators, such as damage to physical and chemical factors that stimulation leading to intracellular redox state changes, expression of Nrf2 changed, in sepsis, Nrf2 has a dual immunological effects. In this study found that in sepsis the expression of Nrf2 mRNA and protein changes, particularly in the early stage of sepsis, Nrf2 mRNA and Nrf2 protein upregulated in the liver, which showed that the increased Nrf2 in nuclear, transcription regulator activity increased, with enhanced capacity of cells against endogenous damage. In late phase of sepsis, with the amplification of large inflammatory mediators, in immunosuppression state Nrf2 decreased in nuclear, transcription regulator activity decreased, causing the destruction of antioxidant system, the formation of antioxidant system damage and oxidative stress in vicious cycle, eventually leading to severe organ dysfunction. The foregoing analysis early in the burn sepsis, expression of Nrf2 increased, may be involved in the natural immune response. While in the latter, it downregulated, may be caused by a variety of inflammation response products. But to be sure, Nrf2 in sepsis played an important role in immune, may become a target for prevention and treatment of sepsis. As for the in-depth mechanism, should be researched further.

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