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Effect of small dose hormone on TLR3 expression in peripheral blood mononuclear cells of children with sepsis and its correlation with the change of the illness

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ABSTRACT

Objective: To study the effect of small dose hormone on TLR3 expression in peripheral blood mononuclear cells of children with sepsis and its correlation with the change of the illness. **Methods:** Patients who were diagnosed with sepsis in Yan′an People′s Hospital between June 2014 and September 2017 were chosen as the research subjects and randomly divided into two groups, hormone group received small-dose glucocorticoid combined with routine therapy and control group accepted routine therapy; the TLR3 mRNA expression levels in peripheral blood as well as the contents of inflammation molecules and oxidative stress molecules in serum were measured before treatment as well as 3 d, 5 d and 7 d after treatment. **Results:** 3 d, 5 d and 7 d after treatment, TLR3 mRNA expression levels in peripheral blood as well as TNF-α, ICAM-1, sTREM1, Presepsin, HMGB1, ESM1, LPO and MDA contents in serum of both groups of children were lower than those before treatment whereas SOD and GSH-Px contents were higher than those before treatment, and TLR3 mRNA expression levels in peripheral blood as well as TNF-α, ICAM-1, sTREM1, Presepsin, HMGB1, ESM1, LPO and MDA contents in serum of hormone group were lower than those of control group whereas SOD and GSH-Px contents were higher than those of control group; the TLR3 mRNA expression of hormone group was positively correlated with TNF-α, ICAM-1, sTREM1, Presepsin, HMGB1, ESM1, LPO and MDA contents, and negatively correlated with SOD and GSH-Px contents. **Conclusion:** Small-dose hormone can inhibit the expression of TLR3 in peripheral blood mononuclear cells of children with sepsis to inhibit the inflammatory response and oxidative stress response in the course of disease.

1. Introduction

Sepsis is a systemic inflammatory response syndrome caused by infection. It is critically ill and has a high mortality rate. The incidence of multiple organ failure is high in the course of disease progression and the clinical treatment is difficult[1,2]. In recent years, the value of small-dose glucocorticoid for sepsis has received more and more attention, and the early application of glucocorticoid can fight against the excessively activated inflammatory and immune response in the course of sepsis, which can improve the condition of sepsis[3]. Although the therapeutic value of small-dose glucocorticoid is accurate, the molecular pathway that exerts therapeutic action is not yet clear. Toll-like receptor 3 (TLR3) is a member of the pattern recognition receptor family TLRs, which can identify the pathogen and activate the downstream signal transduction pathways to mediate the systemic inflammatory response in the course of sepsis[4]. In the following study, in order to determine whether glucocorticoids exert therapeutic value through TLR3 pathways, we analyzed the influence of small-dose hormone on TLR3 expression in peripheral blood mononuclear cells of children with sepsis and its correlation with illness change.

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2. Case information and research methods

2.1 General case information

Children who were diagnosed with sepsis in Yan′an People′s Hospital between June 2014 and September 2017 were chosen as the research subjects, all the children were consistent with the diagnostic criteria for sepsis, and those combined with autoimmune

diseases, congenital diseases or adrenal diseases were ruled out. A total of 58 cases were enrolled and divided into two groups by random number table method, each with 29 cases. Hormone group underwent small-dose glucocorticoid combined with routine treatment, including 15 males and 14 females who were 6-12 years old; the control group received routine treatment, including 16 males and 13 females who were 5-12 years old. There was no significant difference in the general data between the two groups (P>0.05).

2.2 Therapy

Control group received conventional treatment, such as maintaining water and electrolyte balance, anti-infection, nutrition support and protecting viscera function, hormone group received small-dose hormone treatment on the basis of conventional treatment, and the method was as follows: methylprednisolone injection 1-2 mg/kg/d, by intravenous drip, for 7 consecutive days, followed by oral administration with the dosage gradually reduced.

2.3 Peripheral blood TLR3 detection

Before treatment as well as 3 d, 5 d and 7 d after treatment, 0.5-1.0 mL of cubital venous blood was collected from two groups of children, the kits were used to separate the total RNA in peripheral blood and synthesize it into cDNA by reverse transcription, then fluorescence quantitative PCR reaction was conducted, the TLR3 was amplified and its mRNA expression was calculated.

2.4 Serum index detection

Before treatment as well as 3 d, 5 d and 7 d after treatment, 3-5 mL of cubital venous blood was collected from two groups of children respectively and centrifuged to separate serum, enzyme-linked immunosorbent assay kit was used to determine the TNF-α, ICAM-1, sTREM1, Presepsin and HMGB1 contents, and the radioimmunoprecipitation kits were used to determine the contents of ESM1, LPO, MDA, SOD and GSH-Px.

2.5 Statistical methods

Software SPSS 22.0 was used to input data, the measurement data between two groups were analyzed by t test, the correlation was analyzed by Pearson test and $P<0.05$ indicated statistical significance in differences.

3. Results

3.1 Peripheral blood TLR3 mRNA expression

Before treatment as well as 3 d, 5 d and 7 d after treatment, analysis of TLR3 mRNA expression in peripheral blood between two groups of children was as follows: before treatment, TLR3 mRNA expression levels in peripheral blood were not significantly different between two groups of children (P>0.05); 3 d, 5 d and 7 d after treatment, TLR3 mRNA expression levels in peripheral blood of both groups of children were significantly lower than those before treatment (P<0.05), and TLR3 mRNA expression levels in peripheral blood of hormone group were greatly lower than those of control group (P<0.05).

3.2 Serum inflammation molecule contents

Before treatment as well as 3 d, 5 d and 7 d after treatment, analysis of inflammation molecules TNF-α (ng/mL), ICAM-1 (pg/mL), sTREM1 (ng/mL), Presepsin (ng/mL) and HMGB1 (ng/mL) contents in serum between two groups of children was as follows: before treatment, TNF-α, ICAM-1, sTREM1, Presepsin and HMGB1 contents in serum were not significantly different between two groups of children (P>0.05); 3 days, 5 days and 7 days after treatment, TNF-α, ICAM-1, sTREM1, Presepsin and HMGB1 contents in serum of both groups of children were significantly lower than those before treatment (P<0.05), and TNF-α, ICAM-1, sTREM1, Presepsin and HMGB1 contents in serum of hormone group were significantly lower than those of control group (P<0.05).

Table 1.

Comparison of peripheral blood TLR3 before and after treatment.

Groups	n	Before treatment	3 d after treatment	5 d after treatment	7 d after treatment
Hormone group	29	1.02±0.16	0.72±0.10	0.48±0.06	0.32±0.05
Control group	29	1.03±0.14	0.87±0.12	0.69±0.08	0.52±0.08
t		0.172	7.282	8.721	8.198
P		>0.05	<0.05	<0.05	<0.05

Table 2.

Comparison of serum inflammation molecules before and after treatment.

Groups	n	Time	TNF-α	ICAM-1	sTREM1	Presepsin	HMGB1
Hormone group	29	Before treatment	26.2±3.8	2.19±0.33	17.8±2.3	2.89±0.35	53.8±7.3
		3 d after treatment	19.2±2.7*#	1.42±0.18*#	12.4±1.7*#	1.77±0.19*#	30.6±5.6*#
		5 d after treatment	14.1±1.9*#	1.08±0.15*#	10.1±1.5*#	1.48±0.17*#	23.7±3.8*#
		7 d after treatment	12.3±1.8*#	0.78±0.09*#	7.8±0.9*#	1.15±0.16*#	17.4±2.2*#
Control group	29	Before treatment	26.7±3.5	2.23±0.36	17.5±2.1	2.91±0.33	54.3±7.8
		3 d after treatment	23.1±3.5*	1.89±0.23*	15.2±1.9*	2.32±0.35*	41.7±6.4*
		5 d after treatment	19.5±2.2*	1.51±0.18*	13.7±1.6*	2.07±0.28*	34.2±5.2*
		7 d after treatment	16.7±2.1*	1.33±0.18*	11.9±1.8*	1.76±0.22*	27.4±4.8*

*: comparison between before and after treatment within group, $P<0.05$; #: comparison between groups after treatment, $P<0.05$.

Table 3.

Comparison of serum oxidative stress molecules before and after treatment.

Groups	*n*	Time	ESM1	LPO	MDA	SOD	GSH-Px
Hormone group	29	Before treatment	4.27±0.62	14.2±1.9	7.61±0.93	62.9±8.9	48.5±6.2
		3 d after treatment	2.75±0.36*#	10.2±1.8*#	4.88±0.62*#	89.4±11.4*#	67.2±8.9*#
		5 d after treatment	2.14±0.29*#	7.9±0.9*#	4.02±0.58*#	107.2±15.8*#	80.3±10.5*#
		7 d after treatment	1.67±0.22*#	6.2±0.8*#	3.25±0.42*#	119.4±17.5*#	98.3±11.8*#
Control group	29	Before treatment	4.33±0.67	14.5±1.9	7.72±0.93	63.2±8.4	49.2±6.9
		3 d after treatment	3.35±0.52*	12.8±1.6*	6.23±0.78*	72.6±8.2*	58.2±7.8*
		5 d after treatment	2.98±0.37*	10.2±1.5*	5.75±0.78*	89.3±11.4*	64.2±8.9*
		7 d after treatment	2.24±0.34*	8.2±1.0*	4.88±0.62*	105.6±11.8*	75.6±9.2*

*: comparison between before and after treatment within group, $P<0.05$; #: comparison between groups after treatment, $P<0.05$.

3.3 Serum oxidative stress molecule contents

Before treatment as well as 3 d, 5 d and 7 d after treatment, analysis of oxidative stress molecules ESM1 (ng/mL), LPO (nmol/mL), MDA (nmol/mL), SOD (U/L) and GSH-Px (U/L) contents in serum between two groups of children was as follows: before treatment, ESM1, LPO, MDA, SOD and GSH-Px contents in serum were not significantly different between two groups of children ($P>0.05$); 3 d, 5 d and 7 d after treatment, ESM1, LPO and MDA contents in serum of both groups of children were significantly lower than those before treatment whereas SOD and GSH-Px contents were significantly higher than those before treatment ($P<0.05$), and ESM1, LPO and MDA contents in serum of hormone group were significantly lower than those of control group whereas SOD and GSH-Px contents were significantly higher than those of control group ($P<0.05$).

3.4 The correlation of TLR3 with inflammation molecules and oxidative stress molecules

Pearson test analysis of the correlation of TLR3 mRNA expression in peripheral blood with inflammation molecules TNF-α, ICAM-1, sTREM1, Presepsin and HMGB1 as well as oxidative stress molecules ESM1, LPO, MDA, SOD and GSH-Px in serum of children with sepsis was as follows: the TLR3 mRNA expression of hormone group was positively correlated with TNF-α, ICAM-1, sTREM1, Presepsin, HMGB1, ESM1, LPO and MDA contents, and negatively correlated with SOD and GSH-Px contents.

4. Discussion

Sepsis is a systemic inflammatory response syndrome caused by pathogen infection, it is critical and can increase the risk of multiple organ dysfunction, and its clinical treatment is quite difficult. Glucocorticoid has the pharmacological effects of stabilizing cell membranes and lysosome membrane and resisting the excessively activated immune response and inflammatory response, and its treatment of infectious diseases can not only inhibit the excessive activation of inflammation, but can also reduce the damage to a variety of tissue viscera in the course of disease[5,6]. In recent years, the value of small-dose glucocorticoid therapy for sepsis has received more and more attention, both inflammatory response and immune response are regulated by the glucocorticoid in the course of disease, but the specific regulatory mechanism is not yet clear. TLR3 is an important pattern recognition receptor in the body and has played an important role in the development and change of sepsis, and the pathogen in children with sepsis can be identified by TLR3 as pathogen pattern molecule, and then regulate gene expression, and start inflammation and oxidative stress response through the downstream signal transduction pathways[7,8]. In the study, in order to determine whether low-dose glucocorticoid exerted therapeutic value for sepsis through TLR3 pathway, the changes of TLR3 expression in peripheral blood of children with sepsis were analyzed before and after treatment, and the results showed that TLR3 mRNA expression levels in peripheral blood of both groups of children significantly decreased after treatment, and TLR3 mRNA expression levels in peripheral blood of hormone group were significantly lower than those of control group. This means that conventional treatment can inhibit TLR3 expression in peripheral blood to a certain extent, and conventional treatment combined with small-dose hormone can further inhibit TLR3 expression and antagonize the inflammation and oxidative stress mediated by TLR3.

The excessive activation of inflammation in children with sepsis is related to the mass secretion of inflammatory mediators mediated by TLR3, and TNF-α, ICAM-1, sTREM1, Presepsin, HMGB1 and so on are the inflammatory mediators currently known to be closely related to the inflammatory reaction activation in children with sepsis. TNF-α is a pro-inflammatory cytokine secreted by mononuclear macrophages, which is massively secreted in the early stage of inflammatory response, and can mediate the cascade activation of inflammatory response[9]; ICAM-1 is a cytokine with intercellular adhesion effect, which can promote the adhesion of inflammatory cells to inflammatory site and mediate the amplification of inflammatory response[10,11]; sTREM-1 is the soluble form of TREM-1 on neutrophil and mononuclear macrophage surface, Presepsin is the soluble form of CD14 molecule that can identify lipopolysaccharide - lipopolysaccharide binding protein complex, and they can play the initiating role in the process of inflammation[12,13]; HMBG1 is a kind of late inflammatory mediator in high mobility group box, which can make sure that the inflammatory response is in a state of continuous cascade activation[14]. The analysis of the changes in above serum inflammation molecules before and after treatment showed that TNF-α, ICAM-1, sTREM1, Presepsin and HMGB1 contents in serum of both groups of children significantly decreased after treatment, and TNF-α, ICAM-1, sTREM1, Presepsin and HMGB1 contents in serum of hormone group were significantly lower than those of control group. This shows that conventional treatment can inhibit the secretion of inflammatory mediators to a certain extent and conventional treatment combined with small-dose hormone can further reduce the secretion of inflammatory mediators. Further analysis of the correlation between TLR3 expression and inflammatory mediator contents showed that TLR3 mRNA expression in peripheral blood of children with sepsis was positively

correlated with TNF- α , ICAM-1, sTREM1, Presepsin and HMGB1 levels in serum. This means that the change of TLR3 expression is correlated with the change of inflammatory mediator contents, and also indicates that small-dose glucocorticoid therapy for sepsis can inhibit the expression of TLR3 to reduce the secretion of inflammatory mediators and restrain the activation of inflammatory response.

The occurrence of multiple organ dysfunction in sepsis is related to the excessive activation of oxidative stress and the excessive generation of oxygen free radicals. ESM1, LPO and MDA are the indicators reflecting oxidative stress reaction extent, ESM1 is the soluble circulating proteoglycan secreted by endothelial cells, and the endothelial cell damage caused by excessive generation of oxygen free radicals can increase the secretion of ESM1[15]; LPO and MDA are the products of oxidative reactions between lipids and oxygen free radicals in cells, and the attack from excessively generated oxygen free radicals to the lipid in cells can cause cellular structural damage and dysfunction[16,17]. SOD and GSH-Px are catalyzing enzymes with antioxidant effect, which can catalyze reduction reaction and eliminate oxygen free radicals in the process of oxidative stress so as to protect the oxygen free radical damage to cells to some extent; however, the excessively generated oxygen free radicals will exceed the compensatory ability of antioxidant enzymes, and also cause the increased consumption and decreased contents of SOD and GSH-Px[18,19]. Analysis of the changes in above serum oxidative stress molecules before and after treatment showed that ESM1, LPO and MDA contents in serum of both groups of children significantly decreased whereas SOD and GSH-Px contents significantly increased after treatment, and ESM1, LPO and MDA contents in serum of hormone group were significantly lower than those of control group whereas SOD and GSH-Px contents were significantly higher than those of control group. This means that conventional treatment can inhibit the activation of oxidative stress reaction to a certain extent, and routine therapy combined with small-dose hormone can further inhibit the activation of oxidative stress reaction. Further analysis of the correlation between TLR3 expression and oxidative stress molecule contents showed that TLR3 mRNA expression in peripheral blood of children with sepsis was positively correlated with ESM1, LPO and MDA contents, and negatively correlated with SOD and GSH-Px contents in serum. This means that the change of TLR3 expression is correlated with the change of oxidative stress molecule contents, and also indicates that small-doses glucocorticoid therapy for sepsis can inhibit the expression of TLR3 to inhibit the activation of oxidative stress reaction.

Based on above discussion, it can be concluded that low-dose hormone therapy for children with sepsis can inhibit the expression of TLR3 in peripheral blood mononuclear cells as well as the activation of inflammation and oxidative stress, and the hormone inhibits TLR3 to exert the inhibiting effect on inflammatory response and oxidative stress response in the course of sepsis.

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