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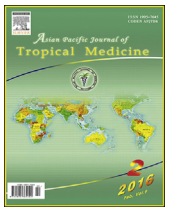

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Preventive and therapeutic effect of N-Acetyl-L-cysteine on infection-associated preterm labor in mice

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ABSTRACT

Objective: To study the preventive and therapeutic effect of N-Acetyl-L-cysteine on infection-associated preterm labor in mice.

Methods: A total of 66 C57BL/6 inbred strain pregnant mice were selected and randomly divided into groups A, B and C, with 22 cases in each group. Group A, B and C were regarded as model group, prevention group and treatment group, respectively. The model of infection-associated preterm labor was built by intraperitoneal injection of *Escherichia coli*. Ten mice of each group were taken and observed the preterm birth rates and live birth rates, respectively. Three mice of each group were killed at 3 h, 6 h, 12 h and 24 h after building the model. Their uterus tissues were collected and the expressions of the AP-1 and MCP-1 in those tissues were assayed with immunohistochemical method and the expressions of NF-κBp65 and TNF-α protein in the placenta tissues of those mice were also detected with immunohistochemical method.

Results: The preterm birth rates of mice in groups B and C were significantly lower than that in group A, while their live birth rates were distinctly higher than that in group A ($P < 0.05$); the expressions of the AP-1 and MCP-1 in the uterus tissues and NF-κBp65 and TNF-α protein in the placenta tissues of mice in groups B and C were evidently lower than those in group A ($P < 0.05$); the comparison of the expressions of the NF-κBp65 and TNF-α between group B and C showed no statistical differences ($P > 0.05$).

Conclusions: N-Acetyl-L-cysteine can lower the incidence rate of infection-associated preterm labor by prohibiting the activation of the protein AP-1/MCP-1 and decreasing the expression of NF-κBp65 and TNF-α in the pregnant tissues of premature mice to reduce the inflammatory reactions.

1. Introduction

Preterm birth is a significant cause of the neonatal death. In recent years, the preterm birth rate is increasing globally [1]. According to the statistics [2,3], infants of preterm birth has accounted for 11% of the total number of new-born infants. There are many factors inducing preterm birth in clinic. Infections play a very important role in the incidence of preterm birth and it is also one of the most common causes inducing preterm birth and the neonatal death [4]. The placenta is the intermediary organ maintaining the mother and the fetus. Before the full development of the placenta, any tiny pathogenic factor invading the placenta can cause pathological inflammatory responses. Hence, inflammatory signaling pathways play a vital role in the incidence of preterm birth [5–7]. N-Acetyl-L-cysteine (NAC) is a classic apophlegmatisant with rather strong anti-inflammatory and antioxidant properties, which has good curative effect on the treatment of various diseases [8]. It is reported that NAC processes certain preventive and therapeutic effect on infection-associated preterm labor [9]. In this study, in order to study the preventive and therapeutic effect of NAC and explore its related mechanism on infection-associated preterm labor, a number of C57BL/6 inbred strain pregnant mice were selected and an infection-associated preterm labor was established. Then, NAC was used for treatment and prevention, and the preterm birth and live birth rates were observed. Now the results are reported as follows.

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2. Materials and methods

2.1. Animals

A total of 66 sexually mature C57BL/6 inbred strain pregnant mice of clean grade weighing from 17 g to 23 g were selected. They were provided by the Experimental Animal Center of Taishan Medical University and raised at (23 ± 3) °C. They could take food and water freely. The management of the experimental animals in this study was carried out by following the Laboratory Animal Administration Rules strictly and approved by the Ethical Committee of the medical college.

2.2. Instruments and reagents

The Anke TDL-40B centrifuge was produced by Shanghai Anting Scientific Instrument Factory; the SANYO light microscope, OLYMPUS high-speed centrifuge with low temperature (Japan), constant temperature bath oscillator and spectrophotometry were all manufactured by Chongqing Tested Instrument Factory; the FOTODYNE gel image analysis system was from PE (USA); NAC was provided by Minsheng Pharmaceutical Group Co. Ltd. (approved by the state, No. H20051788); rabbit-anti-rat TNF-α polyclonal antibody, mouse anti-human NF-κBp65 monoclonal antibody and DAB color kits were purchased from Beijing Zhongshan Jinqiao Biotechnology Co. Ltd. The bacterial liquid of *Escherichia coli* (*E. coli*) was made. Standard strains of *E. coli* were collected a day before the experiment and inoculated in the culture medium at 37 °C overnight. Afterwards, they were diluted to a suspension with a concentration of 10^4 cfu/mL for the standby application at the day of experiment.

2.3. Modeling methods and animal groups

A total of 66 C57BL/6 inbred strain pregnant mice were selected and randomly divided into groups A, B and C, with 22 cases in each. Group A, B and C were regarded as the model group, prevention group and treatment group, respectively. The model of infection-associated preterm labor was built by intraperitoneal injection of *E. coli*. The experimental mice were given intraperitoneal injection of 100 μL *E. coli* suspension at day 16 after the pregnancy to make an intrauterine infection-associated preterm labor model. Mice in group A received intraperitoneal injection of equivalent normal saline treatment after the model was built; mice in group B were given intraperitoneal injection of 100 mg/kg of NAC preventive treatment at 1 h before building the model; mice in group C were treated with intraperitoneal injection of 100 mg/kg of NAC at 2 h after completing the mode.

2.4. Observation methods

Ten mice of each group were taken and observed the preterm birth rates and live birth rates for 48 h, respectively. Three mice of each group of the rest were killed at 3 h (T_1), 6 h (T_2), 12 h (T_3) and 24 h (T_4) after an intervention treatment. The uterus tissues were collected and the expressions of the AP-1 and MCP-1 in those tissues were assayed with immunohistochemical method, and the expressions of NF-κBp65 and TNF-α protein in the placenta tissues of those mice which were sacrificed at the last minute were also detected with immunohistochemical method.

2.5. Statistical methods

SPSS13.0 was applied for data analysis. The comparison of the measurement data was analyzed with One-way ANOVA and represented by mean ± SD, while the enumeration data were tested by Chi-square test and expressed as proportions. $P < 0.05$ meant that differences had statistically significance.

3. Results

3.1. Comparison of preterm birth rates and live birth rates of three groups

The preterm birth rates of groups B and C within 48 h were significantly lower than that in group A, while the live birth rates of groups B and C were observably higher than that in group A. There were significant differences between two groups ($P < 0.05$) (Table 1).

3.2. Protein expressions of AP-1 and MCP-1 in uterus tissues of mice in three groups at different time periods

The protein expressions of the AP-1 and MCP-1 in the uterus tissues of mice in groups B and C at T_1, T_2, T_3 and T_4 were all significantly lower than those in group A, and the inter-group differences had statistical significances ($P < 0.05$) (Table 2).

3.3. Expressions of NF-κBp65 and TNF-α protein in placenta tissues of mice in three groups

The expressions of NF-κBp65 and TNF-α protein in the placenta tissues of mice in groups B and C at T_4 were all significantly lower than those in group A, and the inter-group differences had statistical significances ($P < 0.05$) (Table 3).

Table 1
Comparison of preterm birth rates and live birth rates of three groups (%).

Group	*n*	Preterm birth rate	Live birth rate
A	10	9 (90.0)	24/61 (39.3)
B	10	3 (30.0)*	42/58 (72.4)*
C	10	5 (50.0)*	56/64 (87.5)*

Compared with group A, *$P < 0.05$.

Table 2
Protein expressions of AP-1 and MCP-1 in uterus tissues of mice in three groups at different time periods.

Group		*n*	AP-1 (c-fos)	MCP-1 m RNA
A	T_1	3	335.1 ± 14.5	8.1 ± 1.5
	T_2	3	298.2 ± 12.7	15.2 ± 3.5
	T_3	3	216.1 ± 13.9	12.0 ± 1.7
	T_4	3	139.4 ± 12.4	11.1 ± 1.3
B	T_1	3	245.8 ± 13.7*	6.0 ± 1.6*
	T_2	3	198.1 ± 14.0*	11.8 ± 2.2*
	T_3	3	123.1 ± 12.7*	6.9 ± 1.7*
	T_4	3	97.4 ± 6.4*	6.1 ± 3.0*
C	T_1	3	250.1 ± 18.5*	7.1 ± 1.5*
	T_2	3	238.2 ± 12.6*	12.2 ± 2.4*
	T_3	3	132.0 ± 17.5*	7.0 ± 1.4*
	T_4	3	108.4 ± 15.2*	6.4 ± 5.4*

Compared with group A, *$P < 0.05$.

Table 3
Expressions of NF-κBp65 and TNF-α protein in placenta tissues of mice in three groups.

Group	*n*	NF-κBp65	TNF-α
A	3	0.81 ± 0.29	0.61 ± 0.22
B	3	0.51 ± 0.21*	0.40 ± 0.13*
C	3	0.61 ± 0.20*	0.48 ± 0.21*

4. Discussion

Preterm birth means that women deliver babies after 28 weeks but less than 37 weeks of the pregnancy, which accounts for about 5%–10% of the total delivery number. Preterm birth becomes an important reason for the newborn deaths [10–13]. According to the statistics [14], the number of premature death accounts for 70% of the total number of newborn death, and those preterm babies who survive fortunately can be prone to suffer from different degrees of sequelae. There are researchers claiming that intrauterine and yeast infections were the vital factors of preterm birth so that if they could be controlled timely and effectively, the incidence rate of preterm birth could be reduced efficaciously [15–17].

The placenta is the intermediary organ maintaining the mother and the fetus. Before the fully development of the placenta, any tiny pathogenic factor invades the placenta can cause pathological inflammatory responses [18]. There are researches showing that inflammatory signaling pathways play a vital role in the incidence of preterm birth [16,19–22]. NF-κB is an important infectious regulating factor with the effect of regulating inflammatory response, chemokines and cytokines which may play an important part in the incidence of preterm birth [23]. NAC, a kind of N-acetylated derivative of cysteine, is a classic apophlegmatisant which can significantly lower the viscosity of mucin. Some studies have shown that NAC can lower the incidence of infection-associated preterm labor by prohibiting the expressions of NF-κBp65 and TNF-α protein in the placenta tissues of preterm mice to reduce the inflammatory reaction [24]. In this study, the expressions of NF-κBp65 and TNF-α protein in the placenta tissues of mice in groups B and C were all significantly lower than those in group A, which was consistent with the related literature [24]. It was concluded that NAC did decease the expressions of NF-κBp65 and TNF-α protein in the placenta tissues in mice of infection associated preterm labor and relieve the inflammatory reactions. In addition, the preterm birth rates of groups B and C were observably lower than those in group A, while the live birth rates were significantly higher than those in group A ($P < 0.05$), which also proved that NAC was responsible for the prevention and treatment of infection-associated preterm labor. There are researches finding that AP-1/MCP-1 signal pathways in the uterus tissue of patients with uterine cavity amniotic infection showed high expression of the state [8,25,26]. It is speculated that the abnormality of AP-1/MCP-1 signal pathways may be an important link of the incidence of infection-associated preterm labor [27]. The results of this study showed that the protein expressions of the AP-1 and MCP-1 in the uterus tissues of mice in groups B and C at T_1, T_2, T_3 and T_4 were all significantly lower than those in group A ($P < 0.05$), which indicated that the abnormality of AP-1/MCP-1 signal pathways took part in the incidence of infection-associated preterm labor. Moreover, NAC could decrease the preterm birth rate by interdicting the activating pathway of AP-1 and relieving the inflammation damage for those pregnant mice.

The findings of this study suggest that NAC can lower the incidence rate of infection-associated preterm labor by prohibiting the activation of the protein AP-1/MCP-1 and decreasing the expressions of NF-κBp65 and TNF-α in the pregnant tissues of preterm mice to reduce the inflammatory reactions.

Conflict of interest statement

We declare that we have no conflict of interest.

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