Influence of *Radix Isatidis* on the Endotoxin-induced Release of TNF-α and IL-8 from HL-60 Cells*

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Summary: The effects of active antiendotoxin chemical fraction isolated from *Radix Isatidis* (fraction D) on TNF-α and IL-8 secretion in HL-60 cells induced by lipopolysaccharide (LPS) were studied. The appropriate densities of cell suspension and fraction D solution were determined by MTT colorimetric method. Fraction D and LPS were added to HL-60 cell suspension with three different methods respectively. The contents of TNF-α and IL-8 in the cultured supernatant induced by LPS were detected by using ELISA method. The results showed that the absorbance (A) was directly proportional to the number of cells and the linearity was good in the range from 0.25 × 10^5 to 2 × 10^7 cell/mL cell suspension. The fraction D significantly inhibited the oversecretion of TNF-α and IL-8 in HL-60 cells induced by LPS at the concentration of 7.812 mg/mL which had no cytotoxicity. It was indicated that the antiendotoxin mechanism of the active fraction from *Radix Isatidis* was contributed to the inhibition of the oversecretion of cytokines induced by LPS.

Key words: *Radix Isatidis*; antiendotoxin; cytokine

*Radix Isatidis* is one of the most commonly used antipyretic and detoxicant agents in traditional Chinese medicine. Its original source was identified to be the dried roots of *Isatidis indigotica* Fort. (Cruciferae). The antipyretic and detoxicant agents in traditional Chinese medicine are a type of synthetic therapeutic medicine. It can comprehensively activate the ability of anti-infection and repair of the human body. There are many pharmacological effects such as antibacteria, antivirus, antipyresis, anti-inflammation and immunoregulation, and especially the antiendotoxin activity is the important property. Endotoxins diseases are often seen in clinical practice. Especially in gram-negative microorganisms, endotoxins (LPS) constitute the main pathogenetic factor. Today there exists general agreement that the lethal action of endotoxin is mainly mediated by cytokines and inflammatory mediators. Therefore, the antiendotoxin effect of drugs can be evaluated by inspecting the release of cytokines and inflammatory mediators. Active antiendotoxin chemical fraction isolated from *Radix Isatidis* (fraction D) was acted on HL-60 cells stimulated by LPS with three methods in this experiment. The contents of TNF-α and IL-8 were examined quantitatively.

1 MATERIALS AND METHODS

1.1 Materials

1.1.1 Medical Material *Radix Isatidis* were obtained from Bozhou, Anhui province, and were appraised as dry root of crucifer plant *Isatis indigotica*.

1.1.2 Cell Line HL-60 cell lines were supplied by the Department of Biochemistry & Molecular Biology, Tongji Medical College, Huazhong University of Science and Technology. Cells were cultured in RPMI 1640 medium containing 10 % NBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere of 5 % CO₂ in air. The medium was changed two times every week. The cells in log growth phase were harvested.

1.1.3 Chemical Agents MTT, LPS, TNF-α ELISA kit and IL-8 ELISA kit were purchased from Sigma Company (USA). RPMI 1640 and NBS were from Gibco (USA).

1.2 Equipments

AEL-200 type electronic analytical balance (Japan), Model 450 type enzyme labeling device (Bio-rad, USA), XW-80A type whirlpool blender (Shanghai Medical University Instrument Company, China), CO₂-incubator for cell culture, 96-well or 24-well cell culture plates were used.

1.3 Methods

1.3.1 Preparation of the Antiendotoxin Active Fraction from *Radix Isatidis* *Radix Isatidis* was smashed into coarse powder and we percolated the powder with 95 % ethyl alcohol. Product was condensed under reducing pressure until no alcohol odor could be detected. Then we added diatomite and then set aside.

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1.3.2 Determining Suitable Concentration of Cell and Test Solution MTT method was used according to the references to select cell concentration which was linearly relative to A. The suspended HL-60 cells were diluted into 4x10^5, 2x10^5, 1x10^5, 0.5x10^5, 0.25x10^5, 0.125x10^5 cells/mL respectively. 150 μL of these cells suspension was pipetted into each well. There were 4 wells for each concentration. RPMI 1640 was used as negative control. The cells were incubated for 24 h at 37 °C in 5% CO2 condition. After centrifuging, 100 μL supernatant in each well was discarded. Then 5 mg/mL DMSO 100 μL was added into each well, mixed, re-cultured for 4 h, centrifuged and the supernatant was discarded. RPMI 1640 was used as negative control. After cultured at 37 °C with 5% CO2 for 24 h, the supernatant was collected. Then the levels of TNF-α and IL-8 were detected according to the instructions of the ELISA kits. The inhibition efficiency of the test solution was calculated as the following formula: Inhibition rate (%)=[(Levels of cytokine in group B − Levels of cytokine in groups treated with test solution)/Levels of cytokine in group B] × 100%.

1.3.3 Drawing Standard Curves of TNF-α and IL-8 By using ELISA method, the A value of the standard product in the ELISA kit was detected by MTT method. Livability of the cells was used to evaluate the toxicity of the test solution. If the livability of the cells exceeded 80%, the solution was regarded as nontoxic [Livability of the cells (%) = A value in the experimental well/A value in the control well ×100%].

1.3.4 Effect of LPS on the Production of TNF-α and IL-8 in HL-60 Cells HL-60 cells were seeded at a suitable density in the culture plate. 150 μL cell suspension was pipetted into each well. LPS was diluted into a series density of 0.2, 0.5, 1.0, 5.0, 10.0 μg/mL. They were surged on whirlpool blender and each of them was added into 4 wells with 150 μL in each well. The cell suspension without LPS was used as negative control. After cultured at 37 °C with 5% CO2 for 24 h, the supernatant was collected. By using ELISA method, the levels of TNF-α and IL-8 in cell culture supernatant.

1.3.5 Effect of Three Protocols of Adding LPS on the Production of TNF-α and IL-8 in HL-60 Cells The concentrations of test solution, LPS and HL-60 cells were adjusted to a proper one with RPMI 1640 medium. The cells were treated by the following three protocols: (1) The test solution and LPS were added into HL-60 cell suspension simultaneously (group C); (2) The suspended HL-60 cells were stimulated with LPS for 1 h; then test solution was added into the above culture cells medium (group D); (3) The suspended HL-60 cells were incubated with the test solution for 1 h; then stimulated with LPS (group E). The negative control group only containing HL-60 cell suspension (group A) and the positive control group in which the suspended HL-60 cells were stimulated with LPS but without testing sample (group B) were divided. Every treatment repeated 4 wells. After cultured at 37 °C with 5% CO2 for 24 h, the supernatant was collected. Then the levels of TNF-α and IL-8 were detected according to the instructions of the ELISA kits. The inhibition efficiency of the test solution was calculated as the following formula: Inhibition rate (%)=[(Levels of cytokine in group B − Levels of cytokine in groups treated with test solution)/Levels of cytokine in group B] × 100%.

2 RESULTS

2.1 Determining Suitable Concentrations of Cell and Test Solution When HL-60 cell suspension concentration between the range of (0, 25−2.0) ×10^6 cell/mL, it was linearly relative to A value. When the density of test solution below 7.812 mg/mL, the livability of the cells exceeded 80%.

2.2 Effect of LPS on the Production of TNF-α and IL-8 in HL-60 Cells The levels of TNF-α and IL-8 in all groups treated with LPS were significantly higher than in the negative control group (P<0.05). But the density of LPS was not linearly relative to the levels of TNF-α and IL-8 in the cell suspension. Considering the references and the ability of LPS inducing cytokines, the density of 1 μg/mL was determined.

2.3 Effect of Three Protocols of Adding LPS on the Production of TNF-α and IL-8 in HL-60 Cells Fraction D of Radix Isatidis inhibited the oversecretion of TNF-α and IL-8 in HL-60 cells induced by LPS significantly whatever the addition method. Among the three addition methods, the group C had the most significant inhibition effect (table 1).

3 DISCUSSION

The optimal concentration of cell suspension is different for different types of cells. It is crucial to choose the cells in log growth phase and adjust the cell suspension to optimal concentration. Too high cell suspension density will cause the cells overlapping mutually, resulting in the insufficient metabolism of MTT and the disproportion of absorbency and cell amount. Furthermore, in the biological response, if the concentration of traditional Chinese medicine solution is obviously unbalanced with that of cell suspension, the cells would be wra-