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Research Article

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Influence of extracts from *Rodgersia aesculifolia* Batal on cytokines

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ABSTRACT

Human monocytes in vitro culture were divided into lipopolysaccharide (LPS) group, different concentration of medicated groups and the blank control group. Each group were added the same amount of LPS except the control group. Collecting supermatant after 12 hours in cultural incubator with constant temperature and humidity, measuring the absorbance values of each solution by enzyme linked immunosorbentassay(ELISA)method. To compute cytokine concentration of each groups. Measuring enthalpy by microcalorimetry. Observing the effects of Rodgersia aesculifolia ingredients on 3 proinflammatory cytokines. Results showed several extracts of Rodgersia aesculifolia could inhibit interleukin-1 β (IL-1 β), interleukin-6(IL-6) and tumor necrosis factor- α (TNF-α)(P<0.05)in different degree. The optimum final concentration is 60μg·mL^{-1}.

Key words: *Rodgersia aesculifolia*; Cytokines; ELISA; Enthalpy

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INTRODUCTION

Rodgersia aesculifolia is the dried rhizomes of Saxifragaceae plants *Rodgersia aesculifolia* Batal., having functions of treating rheumatic pain, throat swelling and pain, loss of appetite, abdominal distension, diarrhea, leucorrhea, bleeding disorders and other diseases [1-3]. The main effective components are anthraquinone, bergenin, tannins, flavonoids and polysaccharides. Cytokines area class of small molecular polypeptide secreted by cells. Mononuclear cells can produce and release various cytokines after stimulated by LPS and other factors. Cytokines include IL-1, IL-6 and TNF-a and other inflammatory cytokines. The released IL-1, TNF-a can also enhance the releasing of monocytes, forming a cascade effect, thereby causing the occurrence of inflammation in the body [4-6]. To observe the effect of *Rodgersia aesculifolia* composition on proinflammatory cytokine, has important meaning for exploring the mechanism of treatment inflammatory diseases.

EXPERIMENTAL SECTION

MATERIALS

Rodgersia aesculifolia, purchased from Chinese Taibai Mountain; Human monocytic leukemia cells (THP-1), THP-1 special culture medium (Wuhan boster Biological Engineering Co. Ltd.); Lipopolysaccharide (LPS, cor Biological Engineering Co. Ltd.); Trypsin (Solon Ind. Pkwy);Human TNF-α ELISA kit, Human IL-1β ELISA kit, Human IL-6 ELISA Kit (were purchased from Wuhan boster Biological Engineering Co. Ltd); Bergenin (Shanghai RongWo Pharmaceutical Technology Development Co. Ltd.).

INSTRUMENT

AB135-S electronic balance (Sartorius Scientific Instrument Co. Ltd.); RD496-2000micro calorimeter (Mianyang Zhongwu Thermal Analysis Instrument Co. Ltd); Galaxy 170S CO_2 incubator (Germany Eppendorf company); SW-CJ-2D type clean bench (Suzhou purification equipment Co. Ltd.); Power Wave XS_2 continuous wavelength enzyme mark instrument (Bio Tek USA company); Advantage A10 ultrapure water (Merck Millipore America company).

EXTRACTING EFFECTIVE COMPONENTS FROM RODGERSIA AESCULIFOLIA

3 crushed *Rodgersia aesculifolia* were weighted each 50.0g. According to the ratio of solid to liquid 1:10 , *Rodgersia aesculifolia* were dissolved in deionized water, 80% ethanol and 95% ethanol respectively, ultrasonic extracted 30min in 45℃.Pouring out the supernatant and extracting sediment again. The filtrate was merged and separated by centrifugation. The supernatant was concentrated to extractum, the water extract was dried. The 80% ethanol extract was extracted two times with equal volume of chloroform, precipitation layer and the chloroform layer was discarded. Solution was extracted again with the same volume of methanol. Precipitation was abandoned; supernatant was vacuum concentrated and dried to obtain total anthraquinone powder. The 95% ethanol extract was extracted with same volume of ether. Separating of water layer from ether and extracting ether layer with equal volume of ethyl acetate. Precipitation was abandoned. Water layer and ethyl acetate layer were vacuum concentrated. Finally get the tannin.

THP-1 CELL CULTURE

THP-1 cells were fed in special culture medium containing 10% fetal bovine serum. Fermentation conditions were 37℃, 5% CO_2 and saturated humidity. Replacing medium every 72 hours, the 5-15 generation cells were used as experiments. THP-1 cells were inoculated into 6 well plates, about 10^{6-7} cell per hole. Adding culture medium 4.7mL in 6 well plates, in addition, adding drug 0.3mL in drug group. After 24 hours of incubation, adding LPS in every well and continue to develop 12h. The supernatant was collected for detection.

Cell processing and grouping: (1) the drug group: extract final concentrations were 30 μ g·mL^{-1},60μg·mL^{-1} and 90μg·mL^{-1},LPS final concentration was 0.1μg·mL^{-1} (2):LPS group: cells 1mL, medium 5mL, LPS final concentration was 0.1μg· mL^{-1} (3) the blank control group: cells and medium equal volume of LPS group.

DETECTION OF CYTOKINES

By using enzyme linked immunosorbent assay (ELISA) method, to operate in accordance strictly with the ELISA Kit, detecting the absorption value of wavelength 450nm with enzyme immunoassay instrument. Detection of each sample was not less than 10.

RELEVANCE DETERMINATION OF RODGERSIA AESCULIFOLIA EXTRACT AND CYTOKINE WITH MICROCALORIMETRIC METHOD

On the condition of ordinary pressure and 309.65K, taken the collected supernatant 0.1mL, dissolved it in deionized water1.0mL. Measuring thermodynamic enthalpy by using RD 496-2000 micro calorimeter. According to the experimental values, it can be seen that along with the increase of sample quality, the heat is on the increase, but the molar enthalpy remained almost unchanged. So the mole enthalpy average value, calculated under different quality, can be thought of as the molar enthalpies that sample was dissolved at infinite dilution in deionized water.

STATISTICAL TREATMENT

The data represented by $\bar{x}$ ±s, groups were compared by t test, there were significant differences in $P<0.05$.

RESULTS AND DISCUSSION

EFFECT OF DIFFERENT EXTRACTS OF RODGERSIA AESCULIFOLIA ON CYTOKINE

To observe the effect of different extracts on cytokines, the results in table1,2,3,4.

Table1 Effect of aqueous extract from *Rodgersia aesculifolia* on cytokines($\bar{X}$ ±s)

group	sample number	IL-1β/pg·mL^{-1}	IL-6/pg·mL^{-1}	TNF-α/pg·mL^{-1}
blank control	10	11.19± 1.3 1 Δ	9.02± 0.77ΔΔ	15.45± 1.17Δ
LPS	10	75.48± 21.89	57.57± 13.71	112.88± 33.23
30μg·mL^{-1}	10	16.69± 1.49Δ	17.87± 0.24Δ	32.97± 3.34Δ
60μg·mL^{-1}	10	20.66± 1.51Δ	17.48± 4.17Δ	24.11± 4.22Δ
90μg·mL^{-1}	10	22.13± 1.73Δ	11.88± 1.71Δ	23.7± 3.79Δ

compared with LPS, ΔP<0.05 ΔΔP<0.01

Table2 Effect of anthraquinone from *Rodgersia aesculifolia*on cytokines($\bar{X}$ ±s)

group	sample number	IL-1β/pg·mL^{-1}	IL-6/pg·mL^{-1}	TNF-α/pg·mL^{-1}
blank control	10	11.19± 1.3 1 Δ	9.02± 0.77ΔΔ	1 54 5 ±1.17Δ
LPS	10	75.48± 21.89	57.57± 13.71	112.88± 33.23
30μg·mL^{-1}	10	32.93± 3.38	33.31± 4.35	11.01± 1.18Δ
60μg·mL^{-1}	10	13.99± 1.5 5 Δ	14.24± 3 9 4 Δ	10.45± 1.23Δ
90μg·mL^{-1}	10	16.08± 1.99Δ	19.39± 4 0 5 Δ	15.26± 1.19Δ

compared with LPS, ΔP<0.05 ΔΔP<0.01

Table3 Influence of tannins from *Rodgersia aesculifolia* on cytokines($\bar{X}$ ±s)

group	sample number	IL-1β/pg·mL^{-1}	IL-6/pg·mL^{-1}	TNF-α/pg·mL^{-1}
blank control	10	—	—	15.45± 1.17Δ
LPS	10	—	—	112.88± 33.23
30μg·mL^{-1}	10	—	—	30.37± 0.26Δ
60μg·mL^{-1}	10	—	—	11.46± 0.88Δ
90μg·mL^{-1}	10	—	—	12.19± 2.01Δ

compared with LPS, Δ P<0.05

Table4 Influence of bergenin on cytokines($\bar{X}$ ±s)

group	sample number	IL-1β/pg·mL^{-1}	IL-6/pg·mL^{-1}	TNF-α/pg·mL^{-1}
blank control	10	11.19± 1.31Δ	—	—
LPS	10	75.48± 21.89	—	—
30μg·mL^{-1}	10	20.00± 3.46Δ	—	—
60μg·mL^{-1}	10	13.72± 1.18Δ	—	—
90μg·mL^{-1}	10	12.73± 1.56Δ	—	—

compared with LPS, Δ P<0.05

MOLAR ENTHALPY OF RODGERSIA AESCULIFOLIA EXTRACTS

Determining enthalpy that different extracts on cytokine by microcalorimetry. From results it can be seen that there is a great difference of $\Delta_{sol}H$ between the various samples of the same concentration, the order is: water extract>anthraquinone> tannin>LPS>bergenin.

Table5 Effect of extract from *Rodgersia aesculifolia*on on cytokines by microcalorimetry

group(60μg·mL^{-1})	Q/mJ
blank control	5.4
LPS	20.2
water extract	121.8
anthraquinone	78.1
tannin	58.4
bergenin.	12.2

From table 1,it can be seen that water extracts reduced three kinds of cytokines significantly. But with the increase of the water extract concentration,IL-1β elevated slightly, IL-6 was almost constant, TNF-α decreased. When the water extract at the concentration of 90μg · mL^{-1}, the expression of IL-6 and TNF-α decreased. It can be seen from table 2, with the increase of the concentration of total anthraquinone, the concentrations of IL-1β, IL-6 and TNF-α decreased, when the concentrations of anthraquinone at 90μg·mL^{-1}, three kinds of cytokines slight increased. As shown in table 3, *Rodgersia aesculifolia* tannin had no obvious effect on IL-1β and IL-6. But with the increase of the concentration of tannin, the expression of TNF-α decreased, when the tannin concentration was 90μg·mL^{-1}, expression of TNF-α reversed. Table 4 showed, with the concentration of bergenin increased, concentrations of IL-1βlowered, when the concentration at 90μg·mL^{-1},IL-1β increased. While the bergenin almost no effect on IL-6 and TNF- α.

In general, with the chemical reaction, system can appear enthalpy. The enthalpy increases with the violent chemical reaction. The order of the enthalpy of 4 systems as: $\triangle H_{water\ extract} > \triangle H_{anthraquinone} > \triangle H_{tannin} > \triangle H_{bergenin}$ (showed as table 5). Clearly, the changes are consistent with the basic law of chemical reaction.

Interesting thing is that 4 systems all reversed at concentration increased to 90μg·mL^{-1}. That indicate that in the use of drugs to suppress inflammation, the drugs is not the more the better, the correct approach is to find the most suitable dosage. The optimal concentration of each system of *Rodgersia aesculifolia* are final concentration at 60μg ·mL^{-1}.The microcalorimetric technique is used for the study of anti-inflammatory, to find the best drugs and dosage in chaotic systems, is an ideal method.

Several extracts of *Rodgersia aesculifolia* could inhibit 3 proinflammatory cytokines in different degree. It follows that treatment of *Rodgersia aesculifolia* on inflammatory diseases may be related to reduced proinflammatory cytokines. The enthalpy value displayed that extracts consumed cytokines by reacting with them released from monocytes, this process may be an important part of *Rodgersia aesculifolia* anti-inflammatory.

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