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Effects of matrine on mouse splenocyte proliferation and release of interleukin-1 and -6 from peritoneal macrophages *in vitro*[1]

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KEY WORDS matrine; spleen; interleukin-1; interleukin-6; peritoneal macrophages; cultured cells

AIM: To investigate the mechanisms of anti-inflammatory effect of matrine (Mat), its effects on mouse splenocyte proliferation, and release of interleukin-1 (IL-1) and interleukin-6 (IL-6) from mouse peritoneal macrophages. **METHODS**: Splenocyte proliferation was assayed by [^{3}H]TdR incorporation. IL-1 and IL-6 activities were measured by thymocyte proliferation assay and B_9 cell proliferation MTT colorimetric method, respectively. **RESULTS**: Mat (125 - 500 mg·L^{-1}) obviously inhibited concanavalin A (Con A, 5 mg·L^{-1})- and lipopolysaccarides (LPS, 10 mg·L^{-1})-induced splenocyte proliferation and LPS-induced release of IL-1 and IL-6 from mouse peritoneal macrophages. **CONCLUSION**: Mat inhibited splenocyte proliferation and release of IL-1 and IL-6 *in vitro*.

Matrine (Mat), an alkaloid found in kinds of *Sophra* plants in *Leguminosae*, shows pharmacological effects such as anti-inflammation, immunoinhibition, and anti-arrhythmia, and has been used in treatment for hepatitis, chronic trachoma, and dermatopathy[1]. But its effects on cytokins such as interleukin-1 (IL-1), and interleukin-6 (IL-6) remain unknown.

Abnormal production of IL-1 and IL-6 may be involved in the pathogenesis of autoimmune diseases and chronic hepatitis[2,3]. This paper was to study the effect of Mat on mouse splenocyte proliferation and release of IL-1 and IL-6 from mouse peritoneal macrophages.

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MATERIALS AND METHODS

Reagents Mat (mp 75.5 - 77.5 ℃, purity >99 %), isolated from *Sophra alopecuroides* L, was a light yellow crystalloid powder obtained from Yanchi Pharmaceutical Factory, Ning Xia. Lipopolysaccharides (LPS, *E coli* 0111:B4), calcimycin (Cal), concanavalin A (Con A), and RPMI-1640 medium were from Sigma Co. Methyl thiazolyl tetrazolium (MTT) was obtained from Fluka. [^{3}H]TdR (814 kBq·mol^{-1}) was from Shanghai Institute of Nuclear Research, Chinese Academy of Sciences. Fetal calf serum (FCS) was purchased from Department of Pathology, Second Military Medical University.

Mice and cell line ICR mice, ♀, weighing 22 ± *s* 1.8 g, from Animal Center of Second Military Medical University.

B_9 cells were provided by Dr LA Aarden, Denmark.

Splenocyte proliferation Splenocytes prepared[4] were pipetted at 5 × 10^6/well in 0.1 mL RPMI-1640 medium containing 10 % FCS. Con A 5 mg·L^{-1} or LPS 10 mg·L^{-1} with Mat (0 - 500 mg·L^{-1}) was added and incubated at 37 ℃ in 5 % CO_2 for 72 h. [^{3}H]TdR (1850 Bq/well) was added 12 h before the end of culture. The cell radioactivity was measured with a FJ-2107 scintillation counter (Xi-an, China).

IL-1 Peritoneal exudate cells were obtained from thioglycollate-primed ICR mice[5]. The cell suspension was adjusted to 2 × 10^9·L^{-1} in RPMI-1640 medium containing 10 % FCS, and dispensed at 1 mL/well. After 2-h incubation at 37 ℃ in 5 % CO_2, the nonadherent cells were removed by washing with RPMI-1640 medium, and LPS 10 mg·L^{-1} with Mat (0 - 500 mg·L^{-1}) was added to each well in a final volume of 1 mL RPMI-1640 to induce IL-1. After 24-h culture, the supernatants were collected for IL-1 assay using Con A (2.5 mg·L^{-1})-induced ICR thymocyte proliferation assay[4]. IL-1 activity in supernatants was expressed as dpm of [^{3}H]TdR incorporated by thymocytes at 1:8 dilution. IL-6 Peritoneal exudate cells obtained from primed ICR mice were added at 2 × 10^6/well and stimulated with priming agent, Cal 1 μmol·L^{-1} at 37 ℃ in 5 % CO_2 for 6 h to activate the macrophages. The cells were washed with RPMI-1640 medium to remove the agent and nonadherent cells. LPS 100 μg·L^{-1} with Mat (0 - 500 mg·L^{-1}) were added in a final volume of 1 mL RPMI-1640 medium to

induce IL-6. The supernatants were collected after 8-h culture, and assayed for IL-6 activity using IL-6-dependent B_9 cells proliferation MTT colorimetric method[6]. B_9 cells (5×10^3/well) were incubated for 68 h in the presence of tested samples. The cell proliferation was measured using the MTT colorimetric method. IL-6 activity was expressed as A_{570} at 1:8 dilution.

RESULTS

Mat 125 - 500 mg · L^{-1} inhibited the splenocyte proliferation induced by Con A 5 mg · L^{-1} or LPS 10 mg · L^{-1} in a concentration-dependent manner (Tab 1).

Tab 1. Effect of Mat on mouse splenocyte proliferation induced by Con A 5 mg · L^{-1} or LPS 10 mg · L^{-1}. $n = 6$ homogenates (each was pooled from 4 mice and assayed in triplicate). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ vs 0.

Matrine/ mg · L^{-1}	Radioactivity/dpm	
	Concanvalin A	Lipopolysaccharides
0	36 306 ± 4 342	2 280 ± 214
62.5	30 293 ± 3 333[a]	1 736 ± 288[a]
125	26 973 ± 1 420[b]	1 710 ± 222[b]
250	21 676 ± 2 388[c]	1 416 ± 225[c]
500	17 994 ± 1 995[c]	1 140 ± 253[c]

Mat 125 - 500 mg · L^{-1} inhibited the LPS-induced release of IL-1 and IL-6 from mouse peritoneal macrophages (Tab 2).

Tab 2. Effects of Mat on LPS-induced release of IL-1 and IL-6 from mouse peritoneal macrophages. $n = 3$ homogenates (each was pooled from 8 mice and assayed in triplicate). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ vs 0.

Matrine/ mg · L^{-1}	IL-1 activity/ dpm	IL-6 activity/ A_{570}
0	9 342 ± 970	1.72 ± 0.19
62.5	8 820 ± 1 200[a]	1.48 ± 0.06[a]
125	7 189 ± 1 199[a]	1.21 ± 0.20[b]
250	6 084 ± 916[b]	0.97 ± 0.17[c]
500	4 783 ± 937[c]	0.64 ± 0.21[c]

DISCUSSION

The present study found that Mat inhibited Con A- and LPS-induced mouse splenocyte proliferation and LPS-induced release of IL-1 and IL-6 from mouse peritoneal macrophages. Con A and LPS induced the proliferation of T- and B-lymphocytes, which is an important immunological process in immune response. IL-1 and IL-6 are important mediators in inflammatory reactions and play a central role in immune response. So the inhibition of Mat on the splenocyte proliferation and the release of IL-1 and IL-6 may be involved in the mechanisms of anti-inflammatory and immune inhibitory effect of Mat.

In conclution, Mat showed inhibitory effects on mouse splenocyte proliferation and the release of IL-1 and IL-6, which might be involved in the mechanisms of its anti-inflammation and immunoinhibition.

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苦参碱对体外小鼠脾细胞增殖及腹腔巨噬细胞释放白细胞介素-1 及-6 的影响

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关键词 苦参碱；脾；白细胞介素-1；白细胞介素-6；腹腔巨噬细胞；培养的细胞

A目的：研究苦参碱对小鼠脾细胞增殖及腹腔巨噬细胞释放白细胞介素-1（IL-1）及-6（IL-6）的影响. 方法：[^{3}H]TdR 参入法测定脾细胞增殖，胸腺细

R967 R285.5

胞增殖法和 B_9 细胞增殖 MTT 法测定 IL-1 和 IL-6 活性 结果：苦参碱(125－500 mg·L^{-1})以剂量依赖方式显著抑制 Con A 及脂多糖(LPS)诱导的小鼠脾细胞增殖以及 LPS 诱导的小鼠腹腔巨噬细胞释放 IL-1 和 IL-6. 结论：苦参碱抑制体外小鼠脾细胞增殖及巨噬细胞分泌 IL-1 和 IL-6

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Inhibitory effect of quercetin on tumor necrosis factor and interleukin-1β pro-osteoclastic activities[1]

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KEY WORDS quercetin; tumor necrosis factor; interleukin-1; osteoblasts; cultured cells

AIM: To study the effects of quercetin on tumor necrosis factor (TNF) and interleukin-1β (IL-1β) pro-osteoclastic activities. **METHODS:** [^{3}H]TdR uptake by osteoblasts was used to measure cell proliferation, microspectrophotometer for cellular AlP activity using *p*-nitrophenyl phosphate as enzyme substrate, and radioimmunoassay for prostaglandin E_2. **RESULTS:** Quercetin 5 － 40 μmol·L^{-1} reduced the inhibition of cell proliferation and AlP activity induced by TNF or IL-1β in a concentration-dependent manner. PGE_2 production stimulated by either cytokines was also reduced by quercetin at 20 and 40 μmol·L^{-1}. **CONCLUSION:** quercetin exerted a marked inhibitory effect on TNF and IL-1 activities, related to their pro-osteoclastic function.

Cytokines play an important role in the bone resorption associated with inflammatory diseases. Tumor necrosis factor (TNF) and interleukin-1 (IL-1) elicited similar responses in a variety of cell types, including stimulating osteoclastic bone resorption and inhibiting osteoblastic bone formation *in vitro* and *in vivo*[1-4]. Furthermore, the elevated levels of TNF and IL-1 have been detected in the synovial fluids from the patients with inflammatory diseases[5-6]. Quercetin inhibited TNF and IL-1 production *in vitro* and *in vivo*[7]. This paper was to examine the effect of quercetin on TNF and IL-1 activities, related to their pro-osteoclastic function.

MATERIALS AND METHODS

Reagents Recombinant human TNF and IL-1β (specific activity 3 · 10^9 U·g^{-1}) was kindly provided by Dr Y Sohmura (Dainippon Pharmaceutical Co, Japan). Minimal essential medium (MEM) was purchased from Gibco. PGE_2 radioimmunoassay (RIA) kit was obtained from Chinese Academy of Medical Sciences. Quercetin and the remainder of the reagents were purchased from Sigma (USA).

Cell cultivation Osteoblasts were prepared from neonatal ICR mouse calvaria (Animal Center of this University), by a modification of the method[8]. Briefly, 100 calvariae were diggested in isotonic saline containing 0.2 % collagenase and 0.1 % trypsin. The cells were centrifuged and incubated with MEM containing 10 % fetal calf serum at 37 ℃ in humid chamber with 5 % CO_2 for 1 wk. The culture medium was refreshed until cell confluenced. These cells appeared morphologically to be osteoblasts which responded to parathyroid hormone to increase intracytoplasmic cAMP and expressed inducible high alkaline phosphatase (AlP) activity.

Cell proliferation The cells were treated with 0.1 % trypsin for 5 min, then 1 · 10^4 cells were placed in 96-well microplates for 24-h attachment and incubated with either TNF or IL-1β (100 kU·L^{-1}) in the presence or absence of quercetin for 48 h. [^{3}H]TdR 7.4 kBq was added 8 h before termination of culture. The cells were harvested onto glass fibers and its [^{3}H]TdR was counted in a liquid scintillation

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