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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counter.

AlP activity Cells (1×10^4/well) were seeded in 96-well microplates for 24 h attachment, and then incubated with either TNF or IL-1β (100 kU·L^{-1}) in the presence or absence of quercetin to induce AlP activity. After incubation, cell layers were washed twice with phosphate buffer saline and solubilized by the incubation with 10 μL of 0.1 % Triton X-100 in saline. Cellular AlP were determined by a modification of method[3] using *p*-nitrophenyl phosphate 27 mmol·L^{-1} with monoethanolamine 0.1 mol·L^{-1} (pH 10.5) as substrates. In brief, the substrate (100 μL) was added to the resultant cell lysates, which was then incubated at 37 ℃ for 30 min. The reaction was stopped by adding 100 μL NaOH 1 mol·L^{-1} and the absorbance at 410 nm was measured in a microplate reader using *p*-nitrophenol as a standard reference. AlP activity was expressed as the units (μmol·min^{-1} by 1×10^4 cells).

Measurement of prostaglandin E_2 Cells (5×10^4) in 0.5 mL medium was placed in each well and allowed 24 h for attachment, and then 0.5 mL of medium with appropriate agent was placed in each well. After 48-h incubation, the supernatant was adjusted to pH 3.5, and extracted with ethylacetate twice. The organic was evaporated and the residue was reconstituted with 0.2 mL of RIA assay buffer. The PGE_2 content was tested in the RIA assay using antiserum to PGE_2. PGE_2 content was expressed as pg/well.

Statistics Statistical analyses were carried out using *t* test.

RESULTS

Cell proliferation Quercetin 5 – 40 μmol·L^{-1} reduced the inhibition of proliferation of osteoblasts induced by TNF or IL-1β in a concentration-dependent manner (Tab 1).

Tab 1. Effects of quercetin on cytokine-induced osteoblast proliferation. $n = 3$ homogenates (each was pooled from 100 calvariae), $\bar{x} \pm s$.
$^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Quercetin/ μmol·L^{-1}	[^{3}H]TdR uptake/dpm		
	Medium	TNF	IL-1β
Control	1 286 ± 116	379 ± 68	886 ± 43
5	1 104 ± 162^a	639 ± 81^c	813 ± 72^a
10	1 284 ± 102^a	748 ± 39^c	920 ± 104^b
20	1 197 ± 152^a	800 ± 76^c	1 053 ± 104^b
40	1 189 ± 73^a	905 ± 112^c	1 251 ± 107^c

AlP activity TNF for 2 d decreased AlP activity, whereas IL-1β had no effects (Control, 1371 ± 51 mU; IL-1β-treated, 1246 ± 87 mU; $P > 0.05$). The AlP activity was inhibited by IL-1β on d 6 of the culture. Quercetin 5 – 40 μmol·L^{-1} reduced the inhibition of AlP activity induced by either TNF or IL-1β concentration-dependently (Tab 2).

Tab 2. Effects of quercetin on cytokine – induced alkaline phosphatase (AlP) activity by murine calvarial osteoblastic cells. $n = 3$ homogenates (each was pooled from 100 calvariae), $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^fP < 0.01$ *vs* medium.

Quercetin/ μmol·L^{-1}	AlP activity, mU/well			
	Medium *	TNF *	Medium +	IL-1β +
Control	1 371 ± 51	637 ± 46^f	1 696 ± 83	1 169 ± 57^f
5	1 305 ± 52^a	841 ± 92^b	1 721 ± 64^a	1 272 ± 43^a
10	1 273 ± 68^a	1 075 ± 89^c	1 689 ± 78^a	1 362 ± 95^b
20	1 238 ± 96^a	1 277 ± 58^c	1 763 ± 98^a	1 447 ± 71^c
40	1 346 ± 73^a	1 322 ± 66^c	1 745 ± 95^a	1 544 ± 58^c

*, + Cells were treated for 2 or 6 d, respectively.

PGE_2 production PGE_2 production was increased about 3.0 folds and 3.5 folds by TNF and IL-1β respectively, compared with baseline values (256 ± 28 pg/well, $P < 0.01$). Addition of quercetin to these cultures had no effects on basal production of PGE_2, However, quercetin 20 and 40 μmol·L^{-1} reduced PGE_2 production stimulated by either TNF or IL-1β (Tab 3).

Tab 3. Effects of quercetin on cytokine-induced PGE_2 production by murine calvarial osteoblastic cells. $n = 3$ homogenates (each was pooled from 100 calvariae), $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Quercetin/ μmol·L^{-1}	PGE_2, pg/well	
	TNF	IL-1β
Control	768 ± 68	886 ± 43
5	746 ± 44^a	821 ± 70^a
10	691 ± 79^a	732 ± 48^a
20	604 ± 38^b	683 ± 85^b
40	512 ± 40^c	541 ± 31^c

DISCUSSION

The data in this report demonstrated that quercetin produced a marked inhibitory effect on TNF and IL-1β activities, AlP activity and PGE_2 production. Quercetin inhibited TNF and IL-1 production *in vitro* and *in vivo*[7], these indicate

that quercetin is a potent modulator of TNF and IL-1. The precise mechanism for quercetin modulating the responsiveness of osteoblastic cells to TNF and IL-1 remains poorly understood. Further studies are needed.

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槲皮素对肿瘤坏死因子和白细胞介素-1β前破骨活性的抑制作用[1]

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关键词 槲皮素；肿瘤坏死因子；白细胞介素-1；成骨细胞；细胞培养

A **目的**：测试槲皮素对肿瘤坏死因子(TNF)和白细胞介素-1β (IL-1β)两种前破骨活性的影响。**方法**：[^{3}H]TdR 参入法观察细胞增殖。以对-硝基苯酚磷酸酯为底物用微量分光光度法测定细胞内碱性磷酸酶活性。放射免疫法测定前列腺素 E_2 (PGE_2)。**结果**：槲皮素(5－40 μmol·L^{-1})以浓度依赖方式减少 TNF 和 IL-1β 对成骨细胞增殖和碱性磷酸酶活性的抑制作用，20－40 μmol·L^{-1}时减少两种细胞因子刺激成骨细胞产生 PGE_2。**结论**：槲皮素对 TNF 和 IL-1β 两种前破骨活性有抑制作用

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