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DNA 拓扑异构酶 I 抑制剂羟基喜树碱对小鼠腹水肝癌细胞组蛋白 H_1和 H_3磷酸化的抑制

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摘要 体外用羟基喜树碱(HCPT)处理小鼠腹水肝癌细胞，DNA 合成明显降低，组蛋白磷酸化受抑，此两种效应皆为时间依赖性. 凝胶电泳进一步发现 HCPT 选择性抑制组蛋白 H_1和 H_3的磷酸化，体内实验亦显示 HCPT 显著抑制组蛋白 H_1和 H_3磷酸化. 因此，HCPT 对肿瘤细胞的致死作用至少部分与其抑制组蛋白 H_1和 H_3的磷酸化有关.

关键词 10-羟基喜树碱；实验性肝肿瘤；培养的肿瘤细胞；DNA 解结蛋白；组蛋白磷酸化

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Effect of esculentoside H on release of tumor necrosis factor from mouse peritoneal macrophages[1]

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ABSTRACT Effect of esculentoside H (EH) on release of tumor necrosis factor (TNF) from murine peritoneal macrophage (MΦ) *in vitro* was studied. The results showed that EH (12.5—200 $\mu g \cdot ml^{-1}$) induced the thioglycolate-broth elicited peritoneal MΦ to release TNF into supernatants in a dose-dependent manner, and higher levels of TNF activity were detected in the supernatants from EH-stimulated calcimycin-primed MΦ culture. EH-induced TNF release had a different type of kinetics compared with that of lipopolysaccharides (LPS). LPS-induced release of TNF increased rapidly until 6 h after LPS stimulation, then declined gradually, while EH-induced TNF release increased gradually after EH stimulation and reached its peak at approximately 24 h later. These results suggested that the anti-tumor mechanisms of *Phytolaccaceae* may be related to the capacity of EH for TNF release.

KEY WORDS esculentoside H; tumor necrosis factor; macrophages; calcimycin

Tumor necrosis factor (TNF), a macrophage (MΦ)-derived peptide, was originally described as a mediator of lipopolysaccharides (LPS)-induced hemorrhagic necrosis of tumor in animals and as a molecule with cytostatic/cytotoxic activity for tumor cells in culture[1]. The anti-tumor potency of systemically administered TNF has been shown to be disappointing in phase I clinical trails, using a variety of recombinant TNF preparations over last 5 years, as its toxicity severely limited the dosage of TNF administered in humans[2]. Hence the triggering of endogenous TNF production may be a key strategy that lessen the side effects of high TNF dosages administered. In order to search for the inducer of endogenous TNF and investigate the anti-tumor mechanisms of *Phytolaccaceae*, we studied the effects of esculentoside H (EH), a water-soluble saponin isolated from the roots of *Phytolacca esculenta* van Houtte (*Phytolaccaceae*)[3], on release of TNF from thioglyco-

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late (TG)-broth elicited murine peritoneal MΦ primed (or not primed) with calcimycin (Cal) and compared the effects of EH with that of a TNF eliciting-agent, the lipopolysaccharides (LPS).

MATERIALS AND METHODS

Reagents EH was provided by YI Yang—Hua (Faculty of Pharmacy, Second Military Medical University). LPS derived from *E coli* 0 111:B4 and Cal were from Sigma; Dactinomycin (Dac), Xinya Pharmaceutical Co, Shanghai; TG-broth, Shanghai Academy of Biologic Products. The cell culture medium was RPMI-1640 (Sigma) with *l*-glutamine (2 mmol·L^{-1}) supplemented with $NaHCO_3$ (2 g), penicillin (30 mg·L^{-1}), streptomycin (25 mg·L^{-1}), mercaptoethanol (100 μmol·L^{-1}), and 10% heatinactivated new-born bovine serum (NBS) (completed RPMI-1640).

ICR mice ♀, weighing 28±*s* 2 g, from the Animal Center of this university.

Preparation of MΦ ICR mice were injected ip with 1 ml 3% TG-broth and peritoneal exudate cells (PEC) were harvested 4 d later by washing the peritoneal cavity with phosphate-buffered saline (PBS). PECs were washed 3 times with PBS, then adjusted to 5×10^6/ml in completed RPMI-1640. The cell suspension was dispensed into cell culture flask (the area for cell adherence was 5 cm×3 cm) (2 ml/flask). After 6 h of incubation at 37℃, 5% CO_2, the nonadherent cells were removed by washing with RPMI-1640 medium, and the adherent cells were used as TG-elicited MΦ.

Priming MΦ for TNF release In order to enhance the TNF release from MΦ in response to the stimulation of LPS, the TG-elicited PEC were incubated in the presence of priming agent, Cal. Six hours later, the cells were washed with RPMI-1640 medium to remove the agent and the nonadherent cells, and the adherent cells were used as Cal-primed MΦ.

Induction of TNF from MΦ MΦs were incubated for 24 h in the presence of EH (0—200 μg·ml^{-1}) or LPS (100 ng·ml^{-1}) and the supernatants were collected for TNF bioassay. For kinetics analysis of EH or LPS-induced TNF production, MΦs were cultured with EH (100 μg·ml^{-1}) or LPS (100 ng·ml^{-1}) for various periods (2—30 h), and the supernatants were assayed for TNF activity.

Bioassay for TNF activity TNF activity in the supernatants were monitored by crystal violet staining assay *in vitro*[6]. In brief, L929 cells (5×10^4/well) were incubated for 18 h in the presence of Dac (1 μg·ml^{-1}) and serial 1:2 dilutions of test samples in 96-well flat-bottom microtiter plates. Units of TNF were defined as the dilution required to lyse 50% of L929 target cells.

RESULTS

Priming effect of Cal for TNF release from MΦ Incubating the TG-elicited PECs for 6 h in the precence of Cal (0.1—10 μmol·L^{-1}) enhanced the TNF release from MΦ in response to the stimulation of LPS. This indicated that Cal could prime the MΦ for TNF release. Since Cal at 1 μmol·L^{-1} showed the best priming effect, this concentration was selected in the experiments (Tab 1).

Tab 1. Tumor necrosis factor (TNF) activity induced by lipopolysaccharides (LPS) (50 ng·ml^{-1}, 6 h) from the MΦ which had been incubated for 6 h in the presence of calcimycin. $n=3$, $\bar{x}\pm s$. $^*P<0.01$ vs control.

Calcimycin/ μmol·L^{-1}	TNF activity/U·ml^{-1}
0	12±4
0.1	142±19*
1	201±18*
10	54±13*

EH induced release of TNF EH (12.5—200 μg·ml^{-1}) induced the release of TNF from both TG-elicited MΦ and Cal-primed MΦ concentration-dependently. The TNF levels in the supernatants from Cal-primed MΦ cultures were higher than those from TG-elicited MΦ (Tab 2).

Comparison of LPS and EH-induced TNF production TNF levels were detectable in the supernatants 2 h after LPS-stimulation and

Tab 2. Effects of esculentoside H (EH) and lipopolysacchrides (LPS) on the release of tumor necrosis factor (TNF) from thioglycolate (TG)-broth elicited macrophage (MΦ) or calcimycin (Cal)-primed MΦ. n=3, $\bar{x}\pm s$. $^aP>0.05$ $^bP<0.05$, $^cP<0.01$ vs control.

EH/ $\mu g\cdot ml^{-1}$	LPS/ $ng\cdot ml^{-1}$	TNF ($U\cdot ml^{-1}$) induced from TG-elicited MΦ	Cal-primed MΦ
0		1.9±1.0	5.2±1.5
12.5		1.9±0.4[a]	7.2±1.4[a]
25		2.2±0.9[a]	9.8±1.8[b]
50		11±5[b]	43±8[c]
100		13.4±2.5[c]	23±4[c]
200		10.0±1.7[c]	10±4[a]
	100	14±3[c]	59±8[c]

rose rapidly until 6 h, then declined gradually, while TNF levels were not detectable until 6 h after EH-stimulation and increased gradually in 24 h (Tab 3).

Tab 3. EH (100 $\mu g\cdot ml^{-1}$) or LPS (100 $ng\cdot ml^{-1}$)-induced release of TNF from TG-elicited MΦ or Cal-primed MΦ. n=3, $\bar{x}\pm s$.

Time/ h	EH-induced release of TNF ($U\cdot ml^{-1}$) from TG-elicited MΦ	Cal-primed MΦ	LPS-induced release of TNF ($U\cdot ml^{-1}$) from Cal-primed MΦ
2	1.8±1.6	4.1±1.0	60±5
6	3.3±1.1	8.6±0.8	189±30
12	8.8±1.8	16±4	86±3
16	11.0±1.9	18.3±2.1	
24	13.9±2.4	19±4	59±8
36	11.0±0.9	18.4±1.3	

DISCUSSION

The studies reported herein demonstrated that EH could induce TNF release from murine peritoneal MΦ. Since TNF is an important cytokin involved in host immune defence, the capacity of EH for TNF release may be related to the anti-tumor mechanisms of *Phytolaccaceae*.

It would be interesting to note that the kinetics of EH-induced TNF production exhibited some differences compared with that of LPS. This suggested that LPS and EH induced the TNF release through different molecular mechanisms that need to be further investigated.

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商陆皂甙辛对小鼠腹腔巨噬细胞释出肿瘤坏死因子的影响[1]

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摘要 商陆皂甙辛(EH) (12.5-200 $\mu g\cdot ml^{-1}$)可剂量依赖性地诱导硫代乙醇酸钠培养基诱出的小鼠腹腔巨噬细胞(MΦ)以及卡西霉素启动激活的MΦ分泌肿瘤坏死因子(TNF)。时效关系研究发现：脂多糖(LPS)诱导的TNF分泌于6 h左右达峰，而EH诱导的TNF分泌随时间延长逐渐增多，于24 h左右达峰，提示EH和LPS诱导TNF分泌的机制可能不同。

关键词 商陆皂甙辛，肿瘤坏死因子，巨噬细胞，卡西霉素