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Effects of 10-hydroxy-2-decenoic acid on phagocytosis and cytokines production of peritoneal macrophages *in vitro*

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KEY WORDS 10-hydroxy-2-decenoic acid; peritoneal macrophages; tumor necrosis factor; interleukin-1; cultured cells

AIM: To study the effect of 10-hydroxy-2-decenoic acid (HDA) on the macrophages activity of rats *in vitro*. **METHODS:** To measure the effects of HDA on phagocytosis, the production of antitumor cytokines tumor necrosis factor (TNF) and interleukin-1 (IL-1) *in vitro*. **RESULTS:** HDA 50, 100 mg·L^{-1} promoted phagocytic activity from 0.353 ± 0.017 to 0.39 ± 0.04 and 0.382 ± 0.017 *A*, increased TNF and IL-1 production from 0.23 ± 0.07 % to 0.43 ± 0.04 %, 0.47 ± 0.04 % and from 2384 ± 180 to 2943 ± 295, 3825 ± 450 dpm, respectively. **CONCLUSION:** The upregulating effects of HDA on phagocytosis of PMØ and production of TNF, IL-1 contribute to the host antitumor and immunomodulating mechanism.

10-Hydroxy-2-decenoic acid (HDA) is an unsaturated fatty acid as an active component, extracted from royal jelly. HDA increased phagocytic activity of peritoneal macrophage (PMØ) in normal mice[1] and antagonized the decrease of clearance rate of charcoal particles in cortisone-immunosuppressed mice[2]. *In vitro* test, HDA had a weak inhibitory effect on tumor cells[3]. This study was to determine whether HDA had any effects on phagocytic activity of PMØ and production of interleukin1 (IL-1) and tumor necrosis factor (TNF) from PMØ.

$$HO\text{-}CH_2(CH_2)_6\text{-}CH=CHCOOH$$

10-Hydroxy-2-decenoic acid

$C_{10}H_{18}O_3$ $M_r = 186.25$

MATERIALS AND METHODS

Chemicals and animals HDA was gifted from Prof XU Jing-Yue, Bee Institute, Chinese Academy of Agricultural Sciences. Lipopolysaccharides (LPS), dactinomycin, concanavalin A (Con A), neutral red, and RPMI-1640 medium were purchased from Sigma Chemical Co, USA. [^{3}H] TdR (850 GBq·mol^{-1}) obtained from Institute of Atomic Energy Sciences, China. L929 cell line was obtained from Department of Immunology, Beijing Medical University. SD rats and C_{57} BL/6J mice (♂ and ♀, 6-8 wk old) were purchased from Animal Center, Beijing Medical University. C_{57} BL/6J mice were used as a source of thymocytes for IL-1 assay.

Isolation of peritoneal macrophages Rat peritoneal exudate cells (PEC) were harvested by peritoneal lavage using cold Hanks', solution containing 5 % fetal calf serum (FCS). PEC were washed twice and resuspended in RPMI-1640 medium containing 10 % FCS. PMØ monolayers were prepared by plating 2×10^9 PEC·L^{-1} suspensions in 24-well plate, and incubated at 37 ℃ in a humidified atmosphere of 5 % CO_2 for 2 h to allow for PMØ adherence. The non-adherent cells were removed by washing 3 times with warm RPMI-1640 medium. About 85 % - 95 % adherent cell population was macrophages, as determined by morphology and esterase staining.

Induction of TNF and IL-1 release by PMØ The prepared PMØ were seeded in a 24-well plate at a density of $2 \cdot 10^6$/well and incubated at 37 ℃ for 2 h. Adherent MØ were treated with serial dilution of HDA + LPS 10 mg·L^{-1}, and incubated at 37 ℃ in a humidified atmosphere of 5 % CO_2 for 12 h and 24 h. At the end of this incubation, the cultured PMØ supernatants were harvested, sterilized by passage through 0.2 μm filters, and the former (12 h) was used for TNF assay, the latter (24 h) for IL-1 assay. All samples were stored at -20 ℃.

TNF assay The TNF activities were determined by cytotoxicity assay against L929 cells[4]. The density of L929 was 2×5^5 cells·L^{-1}, the culture supernatant was diluted by 1:16.

IL-1 assay IL-1 activity determined by enhancement of thymocyte proliferation in the classical costimulation assay[5]. Thymocytes from C_{57} BL/6J mice were used. The density of thymocytes was 1×10^{10}·L^{-1}. IL-1 activitiy was expressed as dpm of [^{3}H]TdR incorporation by thymocytes at the culture supernatant of 1:8 dilution.

Phagocytic activity determination Phagocytosis was determined employing the method of neutral red[6] with minor

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modifications SD rat PMØ were separated by adherence on the plates. Various concentrations of HDA were added to the adherent MØ (2×10^5/well) in 0.1 mL RPMI-1640 containing 10 % FCS. After 12-h incubation, the media was discarded and 0.072 % neutral red 0.1 mL was added to each well. The cells were incubated at 37 ℃ for 30 min. The neutral red was removed and the cells were washed twice by PBS. Extraction solution 0.1 mL (EtOH:HAc = 1:1) was added to each well to extract the dye taken by macrophages and absorbance (A) of the color was read on a microplate spectrophotometer at 540 nm. The phagocytic activity was expressed as A.

Statistical analysis All data were expressed as $\bar{x} \pm s$ and analyzed by t test.

RESULTS

Effect of HDA on neutral red phagocytosis by PMØ HDA 50, 100 mg·L^{-1} augmented neutral red phagocytosis (Tab 1).

Tab 1. Effect of 10-hydroxy-2-decenoic acid (HDA) on the phagocytosis of PMØ *in vitro*. n = 4 wells for 1 homogenate (pooled from 3 rats). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* 0.

HDA/mg·L^{-1}	Phagocytic activity/A
0	0.353 ± 0.017
10	0.343 ± 0.015^a
50	0.39 ± 0.04^b
100	0.382 ± 0.017^c
200	0.37 ± 0.03^c

Effect of HDA on TNF production by PMØ TNF production from PMØ was enhanced by HDA 50, 100, 200 mg·L^{-1} (Tab 2).

Tab 2. Effect of HDA on production of TNF and IL-1 by PMØ *in vitro*. n = 3 wells for 1 homogenate (pooled from 3 rats). $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* 0.

HDA/mg·L^{-1}	TNF Activity/%	IL-1 activity/dpm
0	0.23 ± 0.07	$2\,384 \pm 180$
50	0.43 ± 0.04^c	$2\,943 \pm 295^b$
100	0.47 ± 0.04^c	$3\,825 \pm 450^c$
200	0.41 ± 0.04^c	$4\,593 \pm 1\,264^b$

Effect of HDA on IL-1 production by PMØ HDA (50, 100, 200 mg·L^{-1}) clearly augmented the production of IL-1. IL-1 activity was gradually increased with the dose of HDA from 50 mg·L^{-1} to 200 mg·L^{-1} (Tab 2).

DISCUSSION

Phagocytic activity is one of the important characteristics of macrophages. Phagocytosis takes part in the beginning of various immunological response[6]. Our work demonstrated that neutral red phagocytosis by rat PMØ was greatly increased following treatment with HDA *in vitro*. It indicated that HDA could activate peritoneal macrophage and promote its phagocytic activity. The activated macrophages can synthesize and release a wide variety of biologically active molecules[7], such as IL-1, TNF, hydrogen peroxide, and others. Furthermore, a number of recent investigations have suggested that IL-1 and TNF play a very important role in the immunomodulation and antitumor activity. In this study, it was also demonstrated that HDA, as a potent IL-1 and TNF inducer, enhanced the production of these cytokines. These results may partially explain the immunomodulating effect and antitumor activity of HDA or royal jelly *in vitro*.

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10-羟基-2-癸烯酸对腹腔巨噬细胞的吞噬活性及其产生细胞因子的影响

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关键词 10-羟基-2-癸烯酸；腹腔巨噬细胞；肿瘤坏死因子；白细胞介素-1；培养的细胞

目的：研究 10-羟基-2-癸烯酸(HDA)在体外对大鼠腹腔巨噬细胞活性的影响. 方法：测定 HDA 对吞噬活性，抗癌细胞因子 TNF 和 IL-1 产生的影响. 结果：HDA (50, 100 mg·L^{-1})能增强巨噬细胞吞噬活性，HDA 在 50, 100, 200 mg·L^{-1} 时能促进 TNF 和 IL-1 产生. 结论：HDA 上调巨噬细胞的吞噬活性，促进 TNF 和 IL-1 产生，在抗肿瘤和免疫调节中起一定作用.

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Artificial reconstituted pulmonary surfactant in prevention and treatment of respiratory distress syndrome in neonates[1]

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KEY WORDS respiratory distress syndrome; pulmonary surfactants; newborn infant

AIM: To test an artificial reconstituted pulmonary surfactant (APS) for prevention and treatment of respiratory distress syndrome (RDS). **METHODS:** A membrane-formed method combined with supersonic dispersing was used to prepare APS. A pulsating bubble surface tension measurement was established to compare surface properties of APS with natural pulmonary surfactant (NPS). A preliminary clinical trial was made for prevention and treatment of RDS. **RESULTS:** The APS reduced surface tension from 44.0 mN/m to <1.0 mN/m *in vitro*. The changes of APS lipid contents were <5 % of labeled content at 37 ℃. Clinical trial showed that the APS prevented RDS in 20/20 and cured RDS in 2/2 premature neonates. **CONCLUSION:** The APS had good surface properties similar to NPS.

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Respiratory Distress Syndrome (RDS) is often formed in neonates, especially in low-birth-weight premature infants. The pulmonary immaturity is associated with deficiency of the pulmonary surfactant (PS). Replacement therapy used the exogenous PS (natural or artificial reconstituted) is very satisfactory[1-3]. Through analyzing the components of natural PS (NPS), we prepared an artificial reconstituted PS (APS) preparation containing mainly dipalmitolphosphatidylcholine (DPPC) and phosphatidylglycerol (PG). This paper was to compare the surface properties and effects of APS and NPS (calf PS).

MATERIALS AND METHODS

DPPC (TLC pure, 99.0 %, Sigma USA); PG (TLC pure, 99.5 %, made in our Lab); NPS (extracted from neonatal calf lung lavage); other chemicals were of AR; Coulter Counter (England); a pulsating bubble surface tensionor (modified by our Lab from a static air bubble surface tensionor[4]); ZFG81 Rotating Evaporator (Shanghai Medical Appatatus and Instruments Factory, China); CPS-1A Supersonic Micronizer (Shanghai Supersonic Apparatus Factory, China); mice (bred by Shanghai Medical University).

Lipid suspension[5] DPPC and PG were dissolved in a