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Ro 31-8220 inhibits release of interleukin-1 and interleukin-6 from mouse peritoneal macrophages induced by fibrin fibrinogen degradation products[1]

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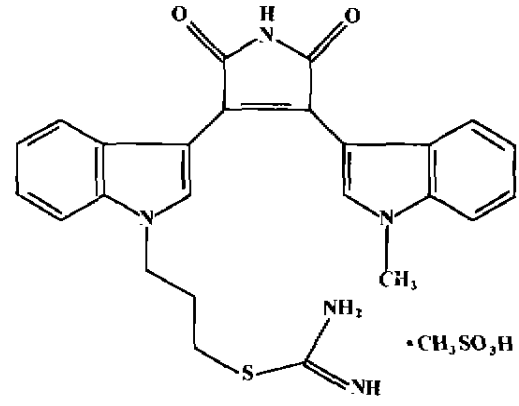
KEY WORDS Ro 31-8220; fibrin fibrinogen degradation products; protein kinase C; interleukin-1; interleukin-6; peritoneal macrophages

ABSTRACT

AIM: To study the effect of fibrin fibrinogen degradation products (FFDP) on release of interleukin-1 (IL-1) and interleukin-6 (IL-6) from mouse peritoneal macrophages, and the effect of a new selectively potent protein kinase C inhibitor Ro 31-8220 (Ro). **METHODS**: IL-1 and IL-6 activities were measured by thymocyte proliferation assay and B_9 cell proliferation methyl thiazolyl tetrazolium (MTT) colorimetric method, respectively. **RESULTS**: Ro 0.01 - 1 $\mu mol \cdot L^{-1}$ obviously inhibited FFDP-induced release of IL-1 and IL-6 from mouse peritoneal macrophages. **CONCLUSION**: Ro exerted inhibitory effects on FFDP-induced release of IL-1 and IL-6 *in vitro*.

INTRODUCTION

Fibrin fibrinogen degradation products (FFDP) have diverse effects in inflammatory processes and acute phase responses[1,2]. Interleukin-1 (IL-1) and interleukin-6 (IL-6) have been identified as chemical mediators released from monocytes/macrophages and exhibit important biologic activities in inflammatory and immunologic responses. Protein kinase C (PKC), a family of serine/threonine specific protein kinases, is an important element in signal transduction pathways in many inflammatory cell types. Inappropriate activation of PKC occurs in a number of diseases states[3]. 3-[1-[3-(Amidinothio) propyl]-3-indolyl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2, 5-dione methanesulfonate (Ro 31-8220, Ro[4]) is a new selectively potent PKC inhibitor with an IC_{50} of 10 $nmol \cdot L^{-1}$ which had been used in the therapy of some autoimmune diseases, such as rheumatoid arthritis. This study was to test whether FFDP could increase the release of IL-1 and IL-6 from peritoneal macrophages, and the effect of Ro.



Ro 31-8220
$C_{25}H_{23}N_5O_2S \cdot CH_3SO_3H$, M_r 553.65

MATERIALS AND METHODS

Reagents Concanavalin A (Con A), RPMI-1640 medium, human fibrinogen, fibrin, plasmin, and aprotinin were purchased from Sigma (USA); methyl thiazolyl tetrazolium (MTT) was obtained from Fluka; Ro was produced by Roche Research Center.

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Mice and cell line ICR mice, weighing 22 g ± *s* 1.8 g, were obtained from the Animal Center of Second Military Medical University (Grade Ⅱ, Certificate № 02-25-2).

B_9 cell line was a generous gift of Dr L A Aarden, Denmark.

Experimental protocol Peritoneal exudate cells were obtained from thioglycollate-primed ICR mice[5]. The cell suspension was adjusted to $4 \times 10^6 \cdot L^{-1}$ 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. Adherent cells were then incubated with FFDP in the absence of serum at defined intervals. Cell-free supernatant fluids were collected for cytokines assay. To test the effect of PKC inhibitor Ro on FFDP action, cells were incubated with FFDP 0.5 $g \cdot L^{-1}$ in the presence of Ro for 48 h.

Cytokines assay *IL-1 was assayed using Con* A (2.5 $mg \cdot L^{-1}$)-induced ICR thymocyte proliferation assay[6], and IL-6 was assayed using IL-6-dependent B_9 cell proliferation MTT colorimetric method [7].

Preparation of FFDP FFDP was obtained by digestion of fibrin with plasmin [10 casein unit (CU)/g (protein)]. Plasmin digestion was conducted in the presence of $CaCl_2$ 1 $mmol \cdot L^{-1}$. The reaction was terminated after 6-h digestion by addition of aprotinin 200 kU /CU plasmin[8].

Statistics Comparisons were carried out using *t* test.

RESULTS

Treating the peritoneal macrophages with FFDP (0.5 $g \cdot L^{-1}$) for 24 h caused only slight increases in the levels of IL-1 and IL-6 compared with control group [(9.4 ± 2.1) *vs* (6.8 ± 1.8); (22.8 ± 2.6) *vs* (18.1 ± 2.2) $kU \cdot L^{-1}$, $n = 4$]. But stimulating for 48 h, resulted in increases in the amounts of IL-1 and IL-6 in all 3 FFDP groups. Ro 10 – 1000 $nmol \cdot L^{-1}$ decreased the IL-1 and IL-6 releases from peritoneal macrophages induced by FFDP (0.5 $g \cdot L^{-1}$) (Tab 1).

Tab 1. Effect of Ro 31-8220 on IL-1 and IL-6 release from peritoneal macrophages stimulated with fibrin fibrinogen degradation products (FFDP) for 48 h. $n = 4$ **homogenates (each was pooled from 4 mice).** $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* **control.**

FFDP/ $g \cdot L^{-1}$	Ro/ $nmol \cdot L^{-1}$	IL-1/ $kU \cdot L^{-1}$	IL-6/ $kU \cdot L^{-1}$
0		19.6 ± 3.1	23.1 ± 2.9
0.2		40.7 ± 3.9^c	30.1 ± 3.2^b
0.5		80.1 ± 5.6^c	56.3 ± 4.8^c
1		111.7 ± 9.8^c	88.6 ± 5.8^c
0.5	0	84.3 ± 7.4	60.3 ± 4.4
0.5	10	74.1 ± 5.7^a	48.3 ± 4.9^b
0.5	100	60.5 ± 8.7^b	39.0 ± 2.9^c
0.5	1000	48.7 ± 5.4^c	30.8 ± 3.3^c

DISCUSSION

The present study demonstrates that incubating peritoneal macrophages with FFDP enhances release of biologically active IL-1 and IL-6. IL-1 and IL-6 are important mediators in inflammatory reactions. FFDP are present at higher concentrations at sites of tissue damage or inflammation. It is therefore conceivable that FFDP might play an important role in immune response through influencing the synthesis or release of cytokines. Our results also showed that Ro inhibited the FFDP-induced IL-1 and IL-6 secretion. The production of IL-1 and IL-6 is regulated by TPA and PKC is one of the pathways regulating the mRNA expression of IL-1 and IL-6. The adherence of platelets to fibrinogen is also regulated by PKC. Ro, a *new generation of bis-indolylmaleimides, derived from* the lead provided by staurosporine, shows a high degree of selectivity for PKC. Hence, PKC may be involved in the signal transduction in the interaction of FFDP with macrophage, and at least in part, inhibition of FFDP-induced cytokines release is the anti-inflammatory mechanism of Ro.

In conclusion, the present study showed the inhibitory effect of Ro against the FFDP-induced IL-1 and IL-6 release.

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Ro 31-8220 抑制纤维蛋白原降解产物诱导的小鼠腹腔巨噬细胞释放白细胞介素-1 及白细胞介素-6[1]

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关键词 Ro 31-8220; 纤维蛋白原降解产物; 蛋白激酶 C; 白细胞介素-1; 白细胞介素-6; 腹腔巨噬细胞

目的: 研究纤维蛋白原降解产物体外诱导小鼠腹腔巨噬细胞释放白细胞介素-1 (IL-1) 及白细胞介素-6 (IL-6) 的作用及一种新型蛋白激酶 C (PKC) 抑制剂 Ro 31-8220 (Ro) 的影响. **方法**: 胸腺细胞增殖法和 B_9 细胞增殖 MTT 法测定 IL-1 和 IL-6 活性. **结果**: 纤维蛋白原降解产物促进小鼠腹腔巨噬细胞释放 IL-1 及 IL-6, Ro (0.01 – 1 μmol·L^{-1}) 明显抑制 IL-1 及 IL-6 的释放. **结论**: Ro 抑制纤维蛋白原降解产物诱导的小鼠腹腔巨噬细胞释放 IL-1 及 IL-6.

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