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Effects of Ro 31-8220 on lipopolysaccharides-induced hepatotoxicity and release of tumor necrosis factor from rat Kupffer cells[1]

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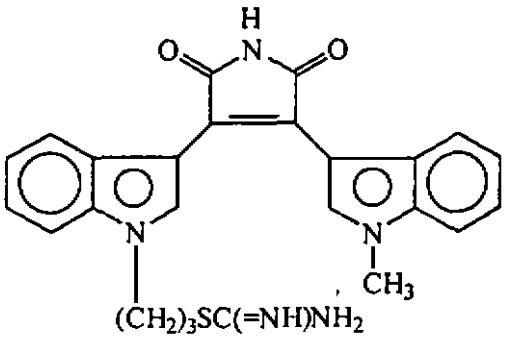
KEY WORDS Ro 31-8220; Kupffer cells; liver; cultured cells; tumor necrosis factor; lipopolysaccharides; alanine aminotransferase

AIM: To investigate protein kinase C (PKC) functions on lipopolysaccharide (LPS)-induced hepatotoxicity, a new potent PKC inhibitor Ro 31-8220 (Ro) was used to detect its effect on LPS-induced hepatotoxicity in rat hepatocytes and tumor necrosis factor (TNF) release from rat Kupffer cells (KC). **METHODS:** Hepatocytes (containing KC) were incubated with LPS (10 mg $\cdot L^{-1}$) and Ro ($0.1 - 10$ $\mu mol \cdot L^{-1}$) for 24 h, alanine aminotransferase (AlaA) leakage in the culture as indication of hepatotoxicity. The TNF activity in the supernatant of rat KC culture with LPS in the presence of Ro ($0.1 - 10$ $\mu mol \cdot L^{-1}$) was monitored by the L929 target cell lytic assay. **RESULTS:** Ro ($0.1 - 10$ $\mu mol \cdot L^{-1}$) reduced AlaA leakage in the hepatocyte culture. Ro inhibited dose-dependently the LPS-induced TNF production from rat KC. **CONCLUSION:** PKC inhibitor Ro protects the hepatocytes from LPS-induced cytotoxicity and inhibits the LPS-induced TNF production from rat KC.

Tumor necrosis factor (TNF) produced by Kupffer cells (KC) is a prominent mediator in lipopolysaccharide (LPS)-induced hepatotoxicity[1]. One of the major cellular sources for the production of TNF appears to be macrophages and KC which play a pivotal role in mediating LPS-induced hepatotoxicity[2].

TNF production by KC requires protein kinase C (PKC) activation[3]. Ro 31-8220 (Ro) is a new potent PKC inhibitor showing high selectivity for PKC[4]. This paper reports the effect of Ro on LPS-induced hepatotoxicity in rat liver cells and TNF release from KC.



3-[3-[2,2-dihydro-4-(1-methyl-1H-indol-3-yl)-2,5-dioxo-1H-pyrrol-3yl]-1H-indol-1-yl]propylisothiourea (Ro 31-8220)

MATERIALS AND METHODS

Reagents LPS isolated from *E coli* 0111: B4, calcimycin (Cal), and collagenase (Type Ⅳ) were purchased from Sigma (USA). Dulbecco's modified EagLe medium (DMEM) was purchased from Gibco BRL. Ro was a generous gift from Dr Gerald LAWTON (Roche Products Ltd, UK).

Rats Wistar rats, ♀, aging $8 \pm s$ 1 wk, weighing 250 $\pm s$ 20 g, were obtained from the Animal Center of Second Military Medical University (Certificate number 02-25-4).

Cell preparation Rat liver perfusion was carried out with collagenase[5,6]; a cannula was inserted into abdominal aorta instead of portal vein. This modification shortened the perfusion time so that cell viability was improved. Liver nonparenchymal cells were enriched for KC by adherence, and 95 % viable by exclusion of trypan-blue. The isolated KC were suspended in RPMI 1640 tissue culture (10 % fetal calf serum, FCS) in 24 well plates. The proportion of nonparenchymal cells to parenchymal cells was increased by altering the centrifugation scheme ($100 \times g$ instead of the usual $50 \times g$). Cells were allowed to adhere on collagen coated 24-well plates (rat-tail collagen, prepared as Grinnell[7]) for 2 h with 3×10^6 hepatocytes/well. The mixed liver cells, containing more than 3 % KC, were suspended in DMEM culture (10 % FCS), and used in

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hepatotoxicity assay.

Assessment of *in vitro* hepatotoxicity The mixed liver cells (containing KC) were triggered with LPS (10 mg·L^{-1}) for 24 h, while the supernatants were assayed for AlaA activity as indication of hepatotoxicity[8]. For investigating the effects of Ro on the hepatocyte injury, Ro (0.10 – 10 μmol·L^{-1}) was added together with LPS.

Induction of TNF from KC[3] KC were plated in 24-well tissue culture overnight and triggered with Cal (1 μmol·L^{-1}). After 6-h incubation, the cultures were washed with RPMI 1640 to remove the agent. The Cal-primed KC were incubated for another 6 h in the presence of LPS (100 μg·L^{-1}) and the supernatants were collected for TNF bioassay. To determine the effect of Ro on LPS-induced TNF release, Ro (0.10 – 10 μmol·L^{-1}) was added together with LPS.

RESULTS

The release of AlaA in supernatant of mixed liver cells (containing KC), was elevated 24 h after the challenging with LPS 10 mg·L^{-1}, while the AlaA activity of hepatocyte culture (without KC) showed no increase after LPS challenge (Tab 1).

Tab 1. AlaA leakage of cultured liver cells within 24 h in the presence of LPS (10 mg·L^{-1}) under various preparation and incubation conditions. n = 3 homogenates (each was pooled from 4 rats), $\bar{x} \pm s$.
$^{b}P < 0.05$ *vs* hepatocyte group.

Centrifugation force/× g	AlaA leakage/kU·L^{-1} 0	LPS
50 (Hepatocytes)	170 ± 10	220 ± 40
100 (Mixed liver cells)	150 ± 20	410 ± 80^{b}

The AlaA leakage in supernatant was reduced by Ro. The LPS-induced release of TNF from KC was markedly inhibited by Ro in a concentration-dependent manner (Tab 2).

Tab 2. Effects of Ro on LPS-induced AlaA leakage in liver cell cultures and TNF activity in supernatants of Cal-primed KC stimulated with LPS (100 μg·L^{-1}) for 6 h. Ro was added to the cultures in the presence of LPS (10 mg·L^{-1}). n = 3 homogenates (each was pooled from 4 rats), $\bar{x} \pm s$.
$^{b}P < 0.05$, $^{c}P < 0.01$ *vs* control.

Ro/μmol·L^{-1}	AlaA activity/ kU·L^{-1}	TNF activity/ kU·L^{-1}
Control	410 ± 80	11.5 ± 4.3
0.1	210 ± 20^{b}	8.8 ± 2.1^{b}
1	240 ± 20^{b}	3.1 ± 0.8^{c}
10	280 ± 40^{b}	2.0 ± 0.4^{c}

DISCUSSION

The present study indicates that KC, the resident macrophages, are indispensable in LPS-induced hepatotoxicity, which is in accordance with our results.

Ro decreased the LPS-induced cytotoxicity towards hepatocytes and suppressed the LPS-induced TNF release from KC. TNF has been proved to be a prominent mediator in LPS-induced hepatic injury *in vivo* or *in vitro*[9], so the inhibitory effect of Ro on TNF production from KC may be involved in the mechanism of its protection against LPS-triggered hepatotoxicity.

In conclusion, our study demonstrates the protective effect of Ro against the LPS-induced hepatotoxicity and its inhibitory effect on the LPS-induced TNF release, a cytokine playing a pivotal role in the LPS reaction.

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Ro 31-8220 对脂多糖诱导的肝细胞毒性及大鼠库普弗细胞释放肿瘤坏死因子的影响[1]

R975.5

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KC 肝细胞毒性

关键词 Ro 31-8220；库普弗细胞；肝；培养的细胞；肿瘤坏死因子；脂多糖；丙氨酸转氨酶类

目的：为探讨蛋白激酶 C 在脂多糖（LPS）诱导的肝细胞毒性中的作用，研究蛋白激酶 C 抑制剂 Ro 31-8220（Ro）对脂多糖诱导的大鼠肝细胞毒性以及对库普弗细胞（Kupffer Cell，KC）释放肿瘤坏死因子（TNF）的影响。方法：大鼠肝细胞（含 KC）以 LPS 刺激 24 h，以肝细胞培养上清丙氨酸转氨酶（AlaA）活性评价肝细胞损伤。大鼠 KC 培养上清的 TNF 活性以杀伤 L929 细胞结晶紫染色法测定。结果：Ro 降低 LPS 诱导的大鼠肝细胞培养上清 AlaA 活性升高并抑制 LPS 诱导的库普弗细胞释放 TNF。结论：Ro 拮抗 LPS 诱导的肝细胞毒性，并抑制 LPS 诱导的 TNF 释放。

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87-89 蜂毒素 离体心房 心脏作用

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蜂毒素对大鼠离体心房的作用

R977.6

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Effects of melitten on isolated rat atrium

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KEY WORDS melitten; verapamil; calcium; myocardial contraction; heart rate

AIM: To study the effects of melitten (Mel) on rat heart. **METHODS**: The isolated rat atria were adopted. Before and after giving Mel, the heart rate, contraction of atrium, post-rest contraction and staircase were measured. **RESULTS**: Verapamil (Ver) 0.1 μmol·L^{-1} was found to inhibit heart rate in the right atria of rats increased by Mel 20.0 mg·L^{-1}. In left atria of rats, the *post-rest contraction was inhibited and typical* positive staircase was markedly attenuated by Mel 2.5 mg·L^{-1}. Mel possessed negative inotropic effects on both the left and right atria of rats. **CONCLUSION**: Mel possessed negative inotropic and positive chronotropic actions on rat heart.

关键词 蜂毒素；维拉帕米；钙；心肌收缩；心率

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目的：研究蜂毒素（Mel）对大鼠心脏的作用。方法：采用大鼠离体心房，测定给药前后心房率，心收缩力，左房休息后加强及阶梯现象。结果：Mel 20.0 mg·L^{-1}对大鼠右心房产生正性频率作用，可被 Ver 0.1 μmol·L^{-1}拮抗。Mel 2.5 mg·L^{-1}抑制左心房休息后加强，并使正性阶梯现象产生翻转。Mel 抑制左右心房收缩力。结论：Mel 具正性频率作用和负性肌力作用。

Melitten (Mel)是蜂毒中的多肽之一，占蜂毒干重 50 %，具抗炎、抗辐射，抗关节炎及心血管药理作用。Marsh[1]发现其抑制心脏收缩，在体动物、其心脏毒性作用几乎消失、EKG 不变，但作用机制未作进一步研究。国内未见文献报道 Mel 的心脏作用。本文旨在用大鼠离体心房，进一步研究其对心脏的作用，并探讨其作用机制。

MATERIALS AND METHODS

药品 Mel（福建农学院提取纯化，HPLC 纯化，经 C18 反相柱层析为单峰）；Mel 标准品（Sigma）；卡托普利（captopril, Cap; Sigma）；西米替丁（cimetidin, Cim; 扬州制药厂）；维拉帕米（verapamil, Ver; Orion-yhtyma Oy ORION PHARMACEUTICA Helsinki-Finland）。