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考福新或咪唑二酮增强内皮素-1升高大鼠主动脉腺苷环一磷酸

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摘要 硝苯啶(100 nmol·L^{-1})或无细胞外钙抑制内皮素-1(ET-1)收缩大鼠胸主动脉80%以上. ET-1(20 nmol·L^{-1}; 15、25 min)增加血管环腺苷酸(cAMP)含量, 该作用被咪唑二酮(100 μmol·L^{-1})增强. ET-1还能增强福斯科林的增加cAMP作用. 本文证明ET-1收缩大鼠胸主动脉至少涉及两种信息转导机制, 即开放硝苯啶敏感性钙通道和增加cAMP.

关键词 内皮素; 胸主动脉; 血管平滑肌; 钙; 硝苯啶; 腺苷环一磷酸; 福斯科林; 咪唑类

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Three drugs inhibit phospholipase A_2-induced high permeability of endothelial monolayers[1]

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ABSTRACT The permeability of aortic endothelial monolayers to fluid and albumin increased 13.5 and 16.1 times respectively after pretreatment with phospholipase A_2 (PLA_2, 100 U·ml^{-1}) for 30 min. 1-(*p*-Chlorobenzoyl)-5-methylindole-3-acetic acid (1.16 mmol·L^{-1}), SRI 63-441 (30 nmol·L^{-1}), and 1,25-dihydroxycholecalciferol (0.1 μmol·L^{-1}) decreased PLA_2-induced high permeability. PLA_2 did not damage the endothelial cells significantly. Our results indicate that the action of PLA_2 to increase the permeability of endothelial monolayers is mainly due to PLA_2-induced lipid mediators released from endothelial cells.

KEY WORDS phospholipases A; capillary permeability; vascular endothelium; prostaglandins; platelet activating factor; cholecalciferols

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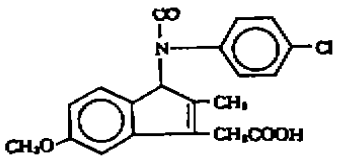
Phospholipase A_2 (PLA_2) increased the pulmonary vascular permeability and caused pulmonary injury in isolated perfused guinea pig lungs[1]. Extracellular PLA_2 is associated with many inflammatory diseases[1,2]. It causes the damages by generating proinflammatory products such as platelet activating factor (PAF), arachidonate and their derivatives[1,3]. PLA_2 also increased the membrane permeability of endothelial cells (EC) and the releases of lactate dehydrogenase (LDH), kininase II and malondialdehyde from EC in high concentrations[4]. Pulmonary responses in-

duced by intratracheal administration of PLA_2 were attenuated by a cyclooxygenase inhibitor and a PAF receptor antagonist[3]. 1,25-Dihydroxycholecalciferol (DHCC) is a differentiation inducer which has profound influence on the cell shape[5]. Cultured bovine aortic EC and human dermal capillaries can express the DHCC receptor[6]. DHCC decreased burn-induced high vasopermeability of rat pads and PAF-induced high vasopermeability of lungs *in vivo* and in isolated perfused lungs (our unpublished data). This experiment is to determine whether 1-(*p*-chlorobenzoyl)-5-methyl-indole-3-acetic acid (CMMAA, a cyclooxygen- ase inhibitor), SRI 63-441 (a PAF receptor antagonist) and DHCC attenuate PLA_2-induced high permeability of endothelial monolayers in a leukocyte-independent manner.



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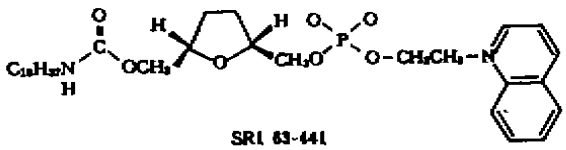
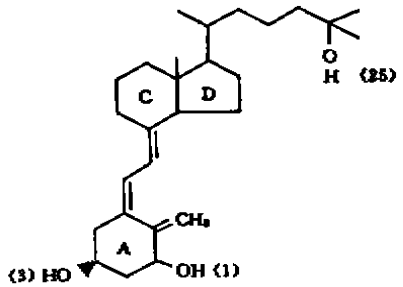


SRI 63-441



1,25(OH)$_2$D3

MATERIALS AND METHODS

PLA_2, Dulbecco's modified Eagle's medium (DMEM), DHCC, and CMMAA (Lot 19890704) were purchased from Sigma, Gibco, Roche Inc, and Shanghai 17th Pharmaceutical Factory, respectively. SRI 63-441, *DL*-erythro-hexitol, 2, 5-anhydro-3, 4-dideoxyoctadecyl-carbamate-2-quinolinioethyl hydrogen phosphate, hydroxide, inner salt, was a gift from Dr D A Handley, Sandoz Research Institute, USA.

Endothelial cells Sprague-Dawley rats of either sex weighing 190 ± *s* 74 g were anesthetized, heparinized, and exsanguinated. The thoracic aorta was isolated and cut into rings of 1 mm. Ten rings were placed into a flask with a bottom of 45 cm^2 and cultured in DMEM supplemented with 20 % fetal bovine-serum. After 60 h of culture, the tissue was discarded and the medium was changed partially. The flask contained only EC and blood cells. The latter were cleared out after the cells were subcultured 1 — 2 times. The EC gave regular confluent cobblestone appearance and positive reaction to the antibody against von Willebrand factor.

Permeability of endothelial monolayers Fluid filtration coefficient (K_f) and albumin clearance rate were used to evaluate the permeability to fluid and albumin. Cells 2×10^6 in 1 ml were seeded onto the culture dish containing 2 gelatinized nitrocellulose microporous (0.8 μm pore size) filters. Twelve days after seeding, the monolayers were used for measuring the permeability by mounting the monolayers on modified Boydon chambers. The prepared filters were perfused at 37 ℃ under 2.65 kPa with Hanks balanced salt solution (HBSS) containing albumin 5 $g\cdot L^{-1}$. The fluid filtering through the monolayer and filter (out-fluid) was collected for 20 min. The collecting tubes were changed every 5 min. The weight and albumin concentration in each tube were measured. The K_f and albumin clearance rate (ACR) were calculated as follows. K_f ($ml\cdot min^{-1}\cdot kPa^{-1}/m^2$) = the volume of the out-fluid/(efficient area of the filter × time of collection × perfusion pressure). ACR ($g\cdot min^{-1}\cdot kPa^{-1}/m^2$) = albumin concentration × K_f.

Kininase II and LDH measurement Kininase II and LDH were measured by colorimetric methods. One unit of kininase II activity was defined as 1 ml sample produced 1 nmol hippuric acid per minute at

37 ℃.

Experimental protocol The monolayers were divided into 5 groups with 5 filters in each group: control, PLA_2 100 U/ml medium, PLA_2 + CMMAA 1.16 mmol·L^{-1}, PLA_2 + SRI 63-441 30 nmol·L^{-1}, and PLA_2 + DHCC 0.1 μmol·L^{-1}. The permeability of the monolayers was measured after 30-min pretreatment with these agents.

Analysis of data Results were evaluated using t test.

RESULTS

Permeability of endothelial monolayers

PLA_2 increased K_f and ACR more than 5.38 and 3.75 times, respectively, within 20 min of observation. The effects of PLA_2 were inhibited by CMMAA, SRI 63-441, and DHCC (Tab 1). The changes of albumin concentration of out-fluid were non-significant (Tab 1).

PLA_2-induced EC injury The releases of kininase II and LDH were not significantly different among the 5 groups except the decreased LDH activity from DHCC-treated monolayers (Tab 1). The detactment of EC from culture well in PLA_2-treated group and control group were 19 987±11 367 and 9 225 ±6 642 cells/well, respectively ($P>0.05$).

DISCUSSION

PLA_2 increased the permeability of cultured EC monolayers suggests that PLA_2 has direct effect on vascular permeability. It was also found that PLA_2 did not increase the kininase II and LDH activities in the cultured

Tab 1. Effects of CMMAA, SRI 63-441 (SRI), and DHCC on PLA_2-induced high permeability to fluid and albumin of endothelial monolayers and to injury of endothelial cells. Five monolayers in each gruop, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ vs PLA_2. $^dP<0.05$, $^eP<0.01$ vs control.

	Control	PLA_2 100 U·ml^{-1}	PLA_2 100 U·ml^{-1} + CMMA 1.16 mmol·L^{-1}	PLA_2 100 U·ml^{-1} + SRI 30 nmol·L^{-1}	PLA_2 100 U·ml^{-1} + DHCC 0.1 μmol·L
Filtration coefficient, ml·min^{-1}·kPa^{-1}/m^2					
0—5 min	259±242^a	3 757±2 692	163±594^b	1 249±1 341^c	595±102
6—10 min	140±108^b	2 487±1 756	212±177^b	251±78^b	351±257^b
11—15 min	314±601^c	2 007±562	173±171^c	98±30^c	295±319^c
16—20 min	141±242^c	1 090±501	134±66	98±30^c	261±83^b
Albumin clearance rate, ml·min^{-1}·kPa^{-1}/m^2					
0—5 min	1.07±1.06^b	17.20±14.42	0.58±0.59^b	5.19±6.18	3.17±0.77
6—10 min	0.60±0.25^b	10.40±7.31	0.97±0.76^b	1.11±0.38^c	1.41±1.08^c
11—15 min	1.77±3.04^b	8.43±2.17	0.68±0.70	1.31±1.21^c	2.12±1.63^c
16—20 min	0.84±1.07^b	1.19±2.11	0.41±0.31	0.39±0.16^c	1.02±0.22^c
Albumin concentration, g·L^{-1}					
0—5 min	1.39±0.30^e	4.15±0.36	4.10±0.43^e	1.02±0.56	1.11±0.01
6—10 min	1.28±0.35^e	4.22±0.26	1.32±0.11^e	1.02±0.62	1.25±0.15
11—15 min	1.14±0.26^e	4.33±0.30	3.61±0.69^e	3.79±0.72	1.26±0.38^e
16—20 min	4.13±0.28^e	4.18±0.21	3.11±1.26^e	3.62±0.77	1.01±0.10^e
The injury of endothelial cells					
Kininase (U)	19.3±5.3	15.3±2.4^d	11.0±3.0^d	23.7±6.9	16.2±2.2
Lactate dehydrogenase (U)	514±117	305±36^d	354±29^d	379±39	17±13

monolayers very significantly. SRI 63-441 and CMMAA prevented the PLA_2-induced high permeability. The above changes suggests that the actions of PLA_2 is mainly mediated by hydrolysis of membrane lipids or the activation of the intrinsic arachidonic acid metabolism with subsequent generation of lipid mediators including platelet activating factor and prostaglandins.

The present study showed that DHCC decreased the PLA_2-induced high permeability of aortic endothelial monolayers. But the mechanisms by which DHCC decreases endothelial monolayer permeability need further investigation.

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三种药物对磷脂酶 A_2 所致内皮细胞单层通透性升高的抑制作用

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摘要 磷脂酶 A_2 (PLA_2, 100 $U \cdot ml^{-1}$) 预处理血管内皮细胞单层30 min，可使单层对液体和白蛋白的通透性分别升高14.5和16.1倍。1-(对-氯苯甲酰)-5-甲氧-2-甲基吲哚-3-乙酸(1.16 $mmol \cdot L^{-1}$)，SRI 63-441 (30 $nmol \cdot L^{-1}$) 和1,25-二羟胆骨化醇(0.1 $\mu mol \cdot L^{-1}$)可降低 PLA_2 所致的通透性升高。PLA_2 对内皮细胞无明显损伤作用。提示 PLA_2 升高内皮细胞单层通透性的作用主要由内皮细胞产生的脂质介质介导。

关键词 磷脂酶 A；毛细血管通透性；血管内皮细胞；前列腺素；血小板激活因子；胆骨化醇