3.5-3.8 8

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吗啡及纳洛酮对淋巴细胞体外增殖的作用[1]

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目的：研究吗啡对不同淋巴细胞增殖的作用及纳洛酮的影响. **方法**：观察吗啡对未成熟的、静止的及活化的脾脏淋巴细胞体外增殖影响及纳洛酮的阻断作用. **结果**：吗啡 ($1\times10^{-10}-1\times10^{-6}$ mol L^{-1})能增加 Con A 诱导的 T-细胞的增殖，1 μmol L^{-1}还能促进 LPS 诱导的 B-细胞的增殖. 同时这些增强作用都能被纳洛酮 50 μmol L^{-1}阻断. 纳洛酮单独亦能促进活化 T-细胞的增殖. 而吗啡 $1\times10^{-16}-1\times10^{-5}$ mol L^{-1}对静止的脾脏淋巴细胞及 Con A 活化的胸腺淋巴细胞的增殖都无影响. 但是吗啡 1 mmol L^{-1}能广泛抑制静止的、LPS 活化的脾脏细胞及 Con A 活化的胸腺，脾脏淋巴细胞增殖，且都不能被纳洛酮阻断. **结论**：吗啡对活化 T-和 B-细胞的促进作用是由细胞表面的阿片受体介导的，此阿片受体随着淋巴细胞的成熟和活化而变化. 而吗啡1 mmol L^{-1}对淋巴细胞增殖的抑制作用却不是由经典的阿片受体介导的.

关键词 吗啡；纳洛酮；阿片受体；T-淋巴细胞；B-淋巴细胞；刀豆蛋白 A；脂多糖；培养的细胞

T淋巴细胞

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Platelet adhesion to cultured bovine cerebral microvascular endothelial cells by stimulation of platelet activating factor and antagonism of drugs

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AIM: To study platelet activating factor (PAF) stimulating the platelets to adhere to cultured bovine cerebral microvascular endothelial cells (CMEC) and the inhibitory effect of triazelodiazepine (WEB), 1,5-bis-(3,4-dimethoxyphenyl)-tetrahydro-(4H)-pyran (DMPP), tetrandrine (Tet). **METHODS**: The platelets adhesion to CMEC and the inhibitory effect of drugs were investigated by [^{3}H]adenine labeling of rabbit blood platelet. **RESULTS**: The platelet adhesion to CMEC was increased by 36 % *vs* control after CMEC was stimulated with PAF 10 nmol L^{-1} for 25 min. WEB 0.1, 1, 10 mmol L^{-1} or DMPP 0.1, 1, 10 mmol L^{-1} or Tet 0.1, 1, 10 mmol L^{-1} inhibited the PAF stimulating platelet adhesion to CMEC by 5.4 %, 16.3 %, 20.1 %; 13.7 %, 19.4 %, 22.4 %; and 5.5 %, 23.1 %, 32.6 %, respectively. **CONCLUSION**: DMPP and Tet inhibited the PAF action in cerebral vascular system.

KEY WORDS platelet activating factor; vascular endothelium; platelet adhesiveness; triazoles; tetrandrine; pyrans

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Platelet activating factor (PAF) is a phospholipid mediator with multifunctional actions[1,2]. PAF plays an important role in cerebrovascular disorders[3]. Our previous studies showed that PAF increased the cerebrovascular permeability[4], and specific binding sites of PAF existed microvascular endothelial cells[5]. Triazelodiazepine (WEB) and 1, 5-bis-(3, 4-dimethoxyphenyl)-tetrahydro-(4*H*)-pyran (DMPP) are PAF receptor antagonist[6]. WEB, DMPP, and tetrandrine (Tet) antagonize the PAF receptors on CMEC, and inhibit the increase of cerebrovascular permeability induced by PAF[4]. The mechanism remains unclear. The stimulation of PAF on platelet adhesion to cultured bovine CMEC and antagonisms of WEB, DMPP and Tet were investigated.

MATERIALS AND METHODS

Trypsin, Triton X-100 (Sigma, USA). Minimum essential medium (MEM, Gibco Laboratories, USA). [^{3}H]Adenine (Shanghai Institute of Nuclear, Research Chinese Academy of Sciences). WEB and C_{16}-PAF were gifted by Boehringer Ingelheim KG, Germany. DMPP and Tet were synthesized by our Department.

Cultivation of bovine CMEC Cells were cultivated according to our previous method[7]. CMEC 1×10^5/well were plated to 24-well culture plates and used in adhesion assays in culture 2 d later. Monolayers were allowed to confluence before being used in the platelet adhesion studies.

Isolation and [^{3}H]adenine labeling of rabbit blood platelet Blood was collected from the heart of New Zealand white rabbits (3.1 ± 0.3 kg) with plastic syringes containing 3 % sodium citrate (1:9/vol:vol). Platelet-rich plasma (PRP) (20×10^{10} platelets L^{-1}) was prepared by centrifugation ($200\times g$, 22 °C, 10 min). [^{3}H]Adenine [370 GBq mol^{-1}, 18.5 GBq L^{-1}, 50 % ethanol solution] was added to PRP to a final concentration of 74 MBq L^{-1}, and the PRP was incubated at 37 °C for 30 min. At the end of the incubation, a portion of PRP was centrifuged at $2250\times g$ at 22 °C for 10 min to obtain PPP. The amount of radioactivity remaining in supernatant PPP samples was taken to represent nonincorporated [^{3}H]adenine. Routinely, 10 mL aliquots of PRP and PPP were added to plastic liquid scintillation vials containing scintillator and counted in a liquid scintillation spectrometer with external standardization. Samples of PRP were examined by a SPA-3 platelet aggregometer immediately after isolation.

Monolayer adhesion assay Culture medium was aspirated from confluent monolayers of CMEC into 24-well culture plates, then replaced by 1 mL of Eagle's MEM (without fetal bovine serum) at 37 °C. PAF was added to each well and incubated at 37 °C in humidified 5 % CO_2 air atmosphere for 5–90 min. The medium was aspirated and the monolayers were gently washed twice with Eagle's MEM at 37 °C, and replaced with 0.75 mL of [^{3}H]adenine-labeled PRP or PPP derived from labeled PRP. The incubation was continued for 30 min. The suspended platelet was aspirated, and the monolayers were gently washed thrice with 1.0 mL of Eagle's MEM at 37 °C. Then 0.5 mL of hot (80 °C) 1 % Triton X-100 were added to the well and agitated for 10 min. Finally, 0.2 mL of aliquots were taken to measure radioactivity by scintillation counter.

The drug was dissolved in Eagle's MEM (without fetal bovine serum) and added to each well 10 min before PAF was added in the drug test.

RESULTS

Uptake of residual plasma [^{3}H]adenine by CMEC The amount of nonincorporated [^{3}H]adenine potentially taken up from plasma by CMEC was determined by incubating CMEC in PPP derived from labeled PRP. After the 25 min incubation, CMEC incorporated little of the residual [^{3}H]adenine present in PPP (418 ± 68 dpm, means of five experiments). The amount of [^{3}H]adenine taken up by CMEC was unchanged whether PAF was added or not (Tab 1).

Time course of platelet adhesion to CMEC PAF 10 nmol L^{-1} incubated with CMEC for 5 – 90 min. The numbers of platelet adhesion to CMEC were increased

Tab 1. Uptake of residual plasma [^{3}H]adenine by CMEC. $n=5$, $\bar{x}\pm s$. $^aP>0.05$, $^cP<0.01$.

PAF/lg mol L^{-1}	PPP/dpm
—	418±68
−11	318±50^a
−10	388±24^a
−9	362±76^a
−8	478±54^a
−7	376±10^a

when CMEC was stimulated with PAF. The peak value reached a maximum at 25 min. The adherent rate of platelet was 36 % higher than that of control. On the basis of the observation, a standard incubation time of 25 min was selected (Fig 1).

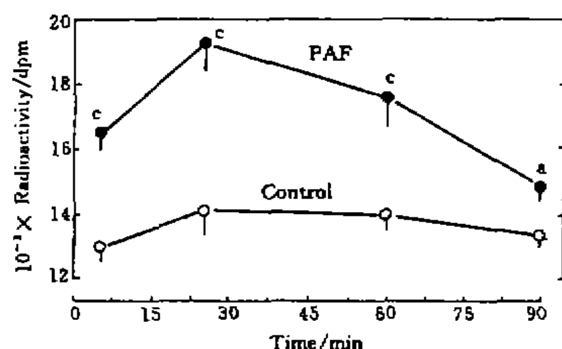

Fig 1. Platelet adhesion to CMEC stimulated by PAF. $n=5$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$.

PAF concentrations *vs* platelet adhesion to CMEC PAF $10^{-11}-10^{-7}$ mol L^{-1} stimulated CMEC for 25 min, and the numbers of platelets adherent to CMEC were increased by 16.4 %; 14.8 %; 14.5 %; 27.4 %; 29.0 %, respectively *vs* control. When the concentration of PAF was in excess of 10 nmol L^{-1}, the rate of platelet adhesion to CMEC was significantly quickened (Tab 2).

Effects of drugs on platelet adhesion to CMEC stimulated with PAF The amount of platelet adhesion to CMEC was 23 736±2552 dpm at PAF 10 nmol L^{-1} after 25 min

Tab 2. Dose course of platelet adhesion to CMEC stimulated by PAF. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$.

PAF/ lg mol L^{-1}	PRP/dpm	Increase/%
—	10 742±2 134	
−11	12 506±646^a	16.4
−10	12 334±656^a	14.8
−9	12 800±994^a	14.5
−8	13 690±840^c	27.4
−7	13 858±2 296^c	29.0

incubation with CMEC. WEB 0.1, 1, 10 mmol L^{-1} or DMPP 0.1, 1, 10 mmol L^{-1} or Tet 0.1, 1, 10 mmol L^{-1} incubated for 10 min with CMEC inhibited the platelet adhesion by 5.4 %, 16.3 %, 20.1 %, 13.7 %, 19.4 %, 22.4 %, and 5.5 %, 23.1 %, 32.6 %, respectively (Fig 2).

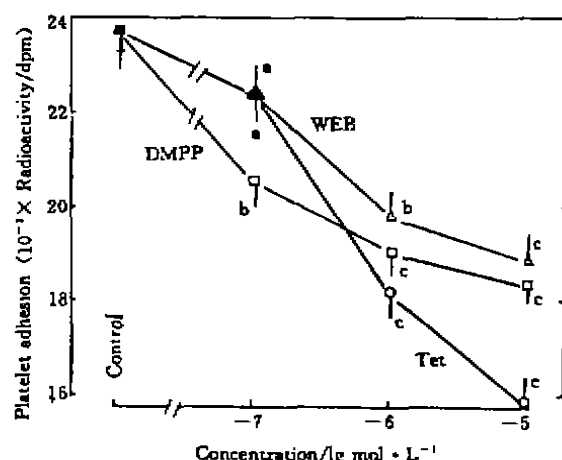

Fig 2. Effects of tetrandrine, WEB, and DMPP drugs on platelets adhesion to CMEC stimulated by PAF. $n=8$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$.

DISCUSSION

The formation of intravascular thrombi and the increases of cerebrovascular permeability were important pathological phase in the development of cerebrovascular diseases. Adherence of blood platelets to the vascular

wall is an early event in the formation of intravascular thrombi and perhaps in the development of atherosclerosis and increases of vascular permeability. The data reported here indicated for the first time the stimulation of PAF for platelet adhesion to cultured bovine CMEC. Our studies demonstrated that PAF significantly increased platelets adhesion to cultured CMEC. WEB 0.1—10 mmol L^{-1} or DMPP 0.1 — 10 mmol L^{-1} or Tet 0.1 — 10 mmol L^{-1} can inhibit the PAF stimulating platelet adhesion to CMEC. It suggests that cerebrovascular damages caused by PAF is related to stimulation of platelets adhesion to CMEC by PAF, and DMPP, Tet can inhibit the PAF action on cerebral vascular system.

The present study was carried out by assaying the adherence of platelets to CMEC *in vitro*. Rabbit platelets were radiolabeled with [^{3}H] adenine and the numbers of adherent platelets were calculated from liquid scintillation measurements. Comparative studies (Tab 1) showed that uptake of nonincorporated [^{3}H] adenine by cultured CMEC was very little. It indicates that the free [^{3}H]adenine of PRP has no influence on the platelets adhesion to CMEC stimulated by PAF.

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血小板激活因子刺激血小板在脑微血管内皮细胞上粘附及药物的阻断作用

R965.2

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目的：研究血小板激活因子（PAF）刺激脑微血管内皮细胞导致血小板在内皮细胞上粘附及WEB、DMPP和粉防己碱的抑制作用.

方法：用[^{3}H]腺嘌呤标记血小板探讨PAF导致血小板在脑微血管内皮细胞上粘附和药物的抑制作用.

结果：PAF 10～100 nmol L^{-1}显著增加血小板与脑微血管内皮细胞的粘附率，WEB、DMPP和粉防己碱抑制由PAF刺激而导致的血小板在脑微血管内皮细胞上的粘附.

结论：DMPP和粉防己碱能够抑制PAF对脑血管的损害作用.

关键词 血小板激活因子；血管内皮；血小板粘附；三唑类；粉防己碱；吡喃