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Antagonistic effects of *Ginkgo biloba* extract on adhesion of monocytes and neutrophils to cultured cerebral microvascular endothelial cells[1]

XU Jiang-Ping, RUI Yao-Cheng[2], LI Tie-Jun (*Department of Pharmacology, College of Pharmacy, Second Military Medical University, Shanghai 200433, China*)

KEY WORDS vascular endothelium; *Ginkgo biloba*; cell adhesion; monocytes; neutrophils; monoclonal antibodies; E-selectin; tumor necrosis factor

ABSTRACT

AIM: To study the action of *Ginkgo biloba* extract (*Gb*E) on tumor necrosis factor (TNF-α)-induced adhesion of monocytes (Mon) and neutrophils (Neu) to cultured cerebral microvascular endothelial cells. **METHODS**: TNF-α-induced endothelial adhesivity toward Mon and Neu was studied using bovine cerebral microvascular endothelial cells (BCMEC) *in vitro*. The number of Mon and Neu adhering to the BCMEC monolayers was determined by flow cytometry. **RESULTS**: Pretreatment of BCMEC with TNF-α increased Mon and Neu adhesion to BCMEC from 12.5 % ± 0.2 % to 31.3 % ± 0.5 % and from 13.8 % ± 0.4 % to 32.1 % ± 0.5 %, respectively. *Gb*E (1 - 100 mg·L^{-1}) inhibited the effect of TNF-α in a concentration-dependent manner. E-selectin mAb (1 mg·L^{-1}) blocked Mon and Neu adhesion to BCMEC induced by TNF-α. **CONCLUSION**: The inhibition of *Gb*E on Mon and Neu adhesion to BCMEC was mediated through the suppression of E-selection expression.

INTRODUCTION

The *Ginkgo biloba* extract (*Gb*E) contained 24 % of flavonoid glycosides, of which the aglycon was flaconols (including quercetin, kaempferol, and isorhamnetin), 6 % of terpene lactones (including ginkgolides A, B, C, J, and bilobalide), and 70 % of other substances (pranthocyanidins, organic acids, sugars, *etc*)[1]. *Gb*E had protective effects on cardiocytes and vascular endothelial cells[2]. It reduced experimental cerebral edema, and showed antagonism on cerebral ischemia[3,4]. To furthur understand the mechanism of *Gb*E on cerebral microvascular endothelial cell, the present study was to investigate the effects of *Gb*E on adhesion of Mon and Neu to cultured bovine cerebral microvascular endothelial cells (BCMEC).

MATERIALS AND METHODS

Reagents TNF-α was gifted by Dr Kitaura M SOHMURA (Dainippon Pharmaceutical Co, Japan). Anti-E-selectin mAb 68-5H11 (AEmAb) was purchased from Pharmingen (USA). *Gb*E (lot No 961027) produced by Shanghai Lüyuan Industry Company Ltd was analytically controlled to ensure consistency of its composition and standardized to cantain 24 % flavonoid glycosides and 6 % terpene lactones[5]. Other reagents were of AR grade. All solutions were prepared with redistilled water.

Cell cultures BCMEC was isolated and cultured[6] in Eagle's minimum essential medium containing 20 % bovine serum, benzylpenicillin 100 kU·L^{-1}, and streptomycin sulfate 100 mg·L^{-1} at 37 ℃ in humidified 95 % O_2 + 5 % CO_2. Primary BCMEC were obtained after 13 - 15 d, and passaged every 3 - 5 d.

Preparation of Mon and Neu Blood was collected from adult Sprague-Dawley rats (♂, weighing 230 g ± *s* 20 g, from Animal Center of

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[2] Correspondence to Prof RUI Yao-Cheng.
Phn 86-21-2507-0341. Fax 86-21-2507-0340.
E-mail ruiyc@ecmu.org.cn
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Second Military Medical University, Grade Ⅱ, Certificate № D02-25-3) in heparin-saline. Mon and Neu were isolated and resuspended[5] in PBS containing 0.1 % bovine serum albumin (BSA). Mon and Neu concentration was adjusted to $5 \times 10^9 \cdot L^{-1}$.

Cell adhesion assays[7] BCMEC growth to confluence in 96-well plates were treated with *Gb*E, TNF-α, and AEmAb. Mon or Neu (5×10^5 cells/well) were incubated with BCMEC monolayer at 37 ℃ for 60 min. Nonadherent cells were removed by washing twice with Hanks' solution. The Mon or Neu adhering to BCMEC monolayer were detached in the presence of PBS containing edetic acid 10 mmol·L^{-1} and their numbers were counted by flow cytometry (FACS Ⅲ, Becton-Dickinson, USA). Mon or Neu adherence to BCMEC was expressed as % of total cell added.

Experimental protocol Cultured confluent BCMEC were washed twice with PBS containing 0.1 % BSA. *Gb*E was dissolved in redistilled water. The cells were incubated with the indicated concentration of *Gb*E at 37 ℃ for 2 h, then the TNF-α 2 μg·L^{-1} was added for 4 h. In the mAb assay, BCMEC were incubated together with AEmAb 1 mg·L^{-1} and TNF-α 2 μg·L^{-1} for 4 h.

Statistics Data were expressed as $x \pm s$ and compared by t-test.

RESULTS

Treatment of BCMEC with TNF-α 2 μg·L^{-1} for 4 h resulted in an increase in the number of Mon or Neu bound. Pretreatment of BCMEC monolayer with *Gb*E (1 – 100 mg·L^{-1}) reduced adherence of Mon to TNF-α-treated BCMEC. Preincubation of AEmAb 1 mg·L^{-1} with TNF-α 2 μg·L^{-1} for 4 h, strikingly reduced the adherence (Tab 1).

Tab 1. Effect of *Gb*E and anti-E-selectin mAb (AEmAb) on adherence of Mon and Neu to BCMEC. $n = 4$ **experiments.** $\bar{x} \pm s$. $^cP < 0.01$ *vs* **TNF-α 2 μg·L^{-1}.**

Treatment	Mon adhesion rate/%	Neu adhesion rate/%
Control	12.5 ± 0.2^c	13.8 ± 0.4^c
TNF-α 2 μg·L^{-1}	31.3 ± 0.5	32.1 ± 0.5^c
*Gb*E 1 mg·L^{-1} + TNF-α	27.2 ± 0.4^c	27.4 ± 0.4^c
*Gb*E 10 mg·L^{-1} + TNF-α	20.3 ± 0.4^c	21.5 ± 0.4^c
*Gb*E 100 mg·L^{-1} + TNF-α	15.4 ± 0.5^c	16.1 ± 0.3^t
TNF-α + AEmAb 1 mg·L^{-1}	18.1 ± 0.6^t	19.3 ± 0.5^t

DISCUSSION

The ability of leukocytes to adhere to cytokines-induced endothelial cells monolayers was used to assess cell adhesion molecules[8] (eg, E-selectin, *etc*), because there was expression of the ligands for cell adhesion molecules in leukocytes. The results showed that pretreatment of BCMEC with *Gb*E decreased adherence of Mon and Neu to TNF-α-treated BCMEC. E-selectin mAb 68-5H11 may abolish the effect of TNF-α, demonstrating that *Gb*E may inhibit the expression of endothelial cell-leukocyte adhesion molecules, and inhibited adhesion of Mon or Neu to TNF-α-activated endothelial cell; further demonstrating pharmacological activity of *Gb*E in BCMEC. Our studies also suggest that *Gb*E might inhibit TNF-α-induced E-selectin expression. This inhibitory effort is probably one of *Gb*E anti-inflammation and anti-atherogenic mechanisms.

In conclusion, the result showed that the antagonism of *Gb*E on cerebral ischemia was related to inhibit adhesion of leukocytes to endothelial cells; E-selectin may be important in mediating leukocyte-endothelial interactions in central nervous system.

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银杏提取物对培养的脑微血管内皮细胞与单核细胞和中性粒细胞粘附的拮抗作用[1]

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徐江平，芮耀诚[2]，李铁军 （第二军医大学药学院药理学教研室，上海 200433，中国）

关键词 血管内皮；银杏；细胞粘附；单核细胞；中性粒细胞；单克隆抗体；E-选择素；肿瘤坏死因子

目的：探讨银杏叶提取物（*Gb*E）对培养的新生牛脑微血管细胞（BCMEC）和大鼠血单核细胞（Mon），中性粒细胞（Neu）粘附的影响及可能机制. **方法**：用流式细胞仪测定粘附到肿瘤坏死因子（TNF-α）诱导培养的 BCMEC 上的单核细胞，中性粒细胞数目. **结果**：BCMEC 经 TNF-α 处理后，使 Mon 粘附到 BCMEC 的百分率从 12.5 % ± 0.2 % 增加到 31.3 % ± 0.5 %，Neu 粘附率也增加到32.1 % ± 0.5 %（对照组为 13.8 % ± 0.4 %）. 用 *Gb*E（1-100 mg·L^{-1}）预处理 BCMEC 后，再用 TNF-α 诱导，则 *Gb*E 剂量依赖性地抑制 TNF-α 的作用. 用抗 E-选择素单克隆抗体与 TNF-α 共孵育 BCMEC 后，TNF-α 的诱导作用被明显阻断. **结论**：*Gb*E 使脑微血管内皮细胞减少 E-选择素表达，进而抑制 Mon，Neu 与 BCMEC 的粘附.

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