

Antagonistic effects of *Ginkgo biloba* extract on adhesion of monocytes and neutrophils to cultured cerebral microvascular endothelial cells¹

XU Jiang-Ping, RUI Yao-Cheng², 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 (*GbE*) 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% \pm 0.2% to 31.3% \pm 0.5% and from 13.8% \pm 0.4% to 32.1% \pm 0.5%, respectively. *GbE* (1-100 mg \cdot L⁻¹) inhibited the effect of TNF- α in a concentration-dependent manner. E-selectin mAb (1 mg \cdot L⁻¹) blocked Mon and Neu adhesion to BCMEC induced by TNF- α . **CONCLUSION:** The inhibition of *GbE* on Mon and Neu adhesion to BCMEC was mediated through the suppression of E-selectin expression.

INTRODUCTION

The *Ginkgo biloba* extract (*GbE*) contained

¹ Project supported by the National Natural Science Foundation of China, No 39670832.

² Correspondence to Prof RUI Yao-Cheng.
Phn 86-21-2507-0341. Fax 86-21-2507-0340.
E-mail ruiyc@ecmu.org.cn

Received 1998-03-02 Accepted 1998-11-10

24% of flavonoid glycosides, of which the aglycon was flavonols (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]. *GbE* had protective effects on cardiocytes and vascular endothelial cells^[2]. It reduced experimental cerebral edema, and showed antagonism on cerebral ischemia^[3,4]. To further understand the mechanism of *GbE* on cerebral microvascular endothelial cell, the present study was to investigate the effects of *GbE* 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). *GbE* (lot No 961027) produced by Shanghai Lüyuan Industry Company Ltd was analytically controlled to ensure consistency of its composition and standardized to contain 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 \cdot L⁻¹, and streptomycin sulfate 100 mg \cdot L⁻¹ at 37 $^{\circ}$ C in humidified 95% O₂ + 5% CO₂. 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 (δ , weighing 230 g \pm s 20 g, from Animal Center of

Second Military Medical University, Grade II, Certificate No 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 *GbE*, $TNF-\alpha$, and AEmAb. Mon or Neu (5×10^5 cells/well) were incubated with BCMEC monolayer at 37 °C 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 $10 \text{ mmol} \cdot L^{-1}$ 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. *GbE* was dissolved in redistilled water. The cells were incubated with the indicated concentration of *GbE* at 37 °C for 2 h, then the $TNF-\alpha$ $2 \mu\text{g} \cdot L^{-1}$ was added for 4 h. In the mAb assay, BCMEC were incubated together with AEmAb $1 \text{ mg} \cdot L^{-1}$ and $TNF-\alpha$ $2 \mu\text{g} \cdot L^{-1}$ for 4 h.

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

RESULTS

Treatment of BCMEC with $TNF-\alpha$ $2 \mu\text{g} \cdot L^{-1}$ for 4 h resulted in an increase in the number of Mon or Neu bound. Pretreatment of BCMEC monolayer with *GbE* ($1 - 100 \text{ mg} \cdot L^{-1}$) reduced adherence of Mon to $TNF-\alpha$ -treated BCMEC. Preincubation of AEmAb $1 \text{ mg} \cdot L^{-1}$ with $TNF-\alpha$ $2 \mu\text{g} \cdot L^{-1}$ for 4 h, strikingly reduced the adherence (Tab 1).

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 *GbE* decreased adherence of Mon and Neu to $TNF-\alpha$ -treated BCMEC.

Tab 1. Effect of *GbE* and anti-E-selectin mAb (AEmAb) on adherence of Mon and Neu to BCMEC. $n = 4$ experiments. $\bar{x} \pm s$. $^*P < 0.01$ vs $TNF-\alpha$ $2 \mu\text{g} \cdot L^{-1}$.

Treatment	Mon adhesion rate/%	Neu adhesion rate/%
Control	12.5 ± 0.2^c	13.8 ± 0.4^c
$TNF-\alpha$ $2 \mu\text{g} \cdot L^{-1}$	31.3 ± 0.5	32.1 ± 0.5^c
<i>GbE</i> $1 \text{ mg} \cdot L^{-1}$ + $TNF-\alpha$	27.2 ± 0.4^c	27.4 ± 0.4^c
<i>GbE</i> $10 \text{ mg} \cdot L^{-1}$ + $TNF-\alpha$	20.3 ± 0.4^c	21.5 ± 0.4^c
<i>GbE</i> $100 \text{ mg} \cdot L^{-1}$ + $TNF-\alpha$	15.4 ± 0.5^c	16.1 ± 0.3^c
$TNF-\alpha$ + AEmAb $1 \text{ mg} \cdot L^{-1}$	18.1 ± 0.6^c	19.3 ± 0.5^c

E-selectin mAb 68-5H11 may abolish the effect of $TNF-\alpha$, demonstrating that *GbE* may inhibit the expression of endothelial cell-leukocyte adhesion molecules, and inhibited adhesion of Mon or Neu to $TNF-\alpha$ -activated endothelial cell; further demonstrating pharmacological activity of *GbE* in BCMEC. Our studies also suggest that *GbE* might inhibit $TNF-\alpha$ -induced E-selectin expression. This inhibitory effort is probably one of *GbE* anti-inflammation and anti-atherogenic mechanisms.

In conclusion, the result showed that the antagonism of *GbE* 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.

REFERENCES

- 1 DeFeudis FV. *Ginkgo biloba* extract (EGb 761): pharmacological activities and clinical applications. Paris: Elsevier; 1991. p 9-94.
- 2 Guillon JM, Rochette L, Baranès J. Effects of *Ginkgo biloba* extract on two models of experimental myocardial ischemia. In: Fünfgeld EW, editor. *Rökan (Ginkgo biloba)*. Recent results in pharmacology and clinic. Berlin: Springer-Verlag; 1988. p 153-61.
- 3 Spinnewyn B, Blavet N, Clostre F. Effects of *Ginkgo biloba* extract on a cerebral ischemia model in gerbils. In: Fünfgeld EW, editor. *Rökan (Ginkgo biloba)*. Recent results in pharmacology and clinic. Berlin: Springer-Verlag; 1988. p 143-52.
- 4 Etienne A, Hecquet F, Clostre F. Mechanisms of effect of *Ginkgo biloba* extract on experimental cerebral edema. In: Fünfgeld EW, editor. *Rökan (Ginkgo biloba)*. Recent

results in pharmacology and clinic.

Berlin: Springer-Verlag; 1988. p 133-42.

- 5 Chen JX, Chen WZ, Huang HL, Chen LX, Xie ZZ, Zhu BY.

Protective effects of *Ginkgo biloba* extract against lysophosphatidylcholine-induced vascular endothelial cell damage.

Acta Pharmacol Sin 1998; 19: 359-63.

- 6 Sun DX, Zeng GQ, Shen YA, Rui YC. Culture of bovine cerebral microvascular endothelial cells *in vitro*.

Acad J Sec Milit Med Univ 1992; 13: 178-80.

- 7 Hoover RL, Robinson JM, Karnovsky MJ. Adhesion of polymorphonuclear leukocytes to endothelium enhances the efficiency of detoxification of oxygen-free radicals.

Am J Pathol 1987; 126: 258-68.

- 8 Vadas MA, Gamble JR.

Regulation of the adhesion of neutrophils to endothelium.

Biochem Pharmacol 1990; 40: 1683-7.

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(GbE)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% \pm 0.2% 增加到 31.3% \pm 0.5%, Neu 粘附率也增加到 32.1% \pm 0.5% (对照组为 13.8% \pm 0.4%). 用 GbE (1-100 mg·L⁻¹) 预处理 BCMEC 后, 再用 TNF- α 诱导, 则 GbE 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:** GbE 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.

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

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)

(责任编辑 杨雪芳)

中国煤炭工业医学杂志

欢迎订阅 欢迎投稿

《中国煤炭工业医学杂志》为国家煤炭工业局主管, 华北煤炭医学院主办的国家级技术类医学科期刊, 国内外公开发刊, 刊号: ISSN 1007-9564, CN13-1221/R. 本杂志以各级临床医生为主要读者对象, 侧重报道医学临床科研新成果、新技术和诊疗经验. 报道的核心学科为神经内科、心血管内科、神经外科、骨科、心脏外科、妇产科、肿瘤科、急诊医学、影像医学和预防医学等. 设专家评述、论著与经验、综述与讲座、临床用药、临床病例讨论、短篇与个例、预防医学、急诊急救等栏目. 欢迎广大医务人员投稿和订阅. 杂志定价 8.00 元/期, 48.00 元/年, 邮发代号 18-284, 全国各地邮局订购.

地址: 河北省唐山市建设南路 57 号

邮编: 063000 电话(传真): 0315-3725999

E-mail mtyx@public.tsptt.he.cn