

## Ginkgolide A, B, and huperzine A inhibit nitric oxide production from rat C6 and human BT325 glioma cells

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**KEY WORDS** ginkgolide A; ginkgolide B; huperzine A; nitric oxide; cultured tumor cells; astrocytoma

### ABSTRACT

**AIM:** To study the effects of ginkgolide A, B (Gin A, Gin B) and huperzine A (Hup A) on nitric oxide (NO) production from cultured astrocytes. **METHODS:** Nitrites in supernatants were measured with Griess assay. **RESULTS:** Hup A 0.001–100  $\mu\text{mol}\cdot\text{L}^{-1}$  time- and concentration-dependently inhibited the NO production from rat C6 astrocytoma cells. The NO production from C6 cells was concentration-dependently inhibited by the treatment with Gin A or Gin B 0.001–10  $\mu\text{mol}\cdot\text{L}^{-1}$  for 24 h. The NO production from human BT325 astrocytoma cells was concentration-dependently inhibited by Hup A, Gin A, or Gin B 0.01–10  $\mu\text{mol}\cdot\text{L}^{-1}$  for 24 h. **CONCLUSION:** Gin A, Gin B, and Hup A inhibited astrocytes producing NO.

### INTRODUCTION

Recent studies showed that pre-inflammatory cytokines, complement proteins, activated glia (microglia and astrocytes) and nitric oxide (NO) play important roles in Alzheimer disease (AD)<sup>[1]</sup>. Lipopolysaccharides (LPS) and pre-inflammatory cytokines, such as interferon- $\gamma$  (IFN- $\gamma$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1, IL-2, and IL-6 can activate glia (microglia and astrocytes) to

produce neurotoxic NO<sup>[2-4]</sup>, by which exerting their neurotoxic effects<sup>[5]</sup>. Hence, modulating the NO synthesis in central nervous system may be a new method in reducing AD and other neurodegenerative diseases.

Hup A, a novel alkaloid isolated from Chinese herb *Huperzia serrata*, is a potent and selective cholinesterase inhibitor (ChEI), and exhibit memory-enhancing activities in a broad range of animal cognitive models and patients with AD<sup>[6]</sup>. Whether Hup A affects NO production from glia remains unclear. Gin A (BN52020) and Gin B (BN52021) are compounds with antagonistic effect on platelet activating factor (PAF), and are isolated from Chinese herb *Ginkgo Biloba* L. Gin A and Gin B protect neurons against glutamate neurotoxicity<sup>[7]</sup>, inhibit IL-1, TNF- $\alpha$ , and NO production from rat microglia<sup>[8]</sup>, and are effective in treating patients with multiple sclerosis and AD. Whether Gin A and Gin B affect NO production from astrocytes also remains unclear. In this study, the effects of Gin A, Gin B, and Hup A on NO production from rat C6 and human BT325 astrocytoma cells were investigated.

### MATERIALS AND METHODS

**Reagents** Gin A and Gin B (purity > 98 %), kindly provided by Prof CHEN Zhong-Liang (Shanghai Institute of Materia Medica, Chinese Academy of Sciences), and Hup A (purity > 98 %), kindly provided by Prof ZHU Da-Yuan (Shanghai Institute of Materia Medica, Chinese Academy of Sciences), were isolated in Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Sulfanilamide was from BDH. *N*-(1-Naphthyl) ethylene diamine dihydrochloride (NEDA), phosphoric acid, and sodium nitrite were domestic AR grade products. Griess reagent was composed of 1 % sulfanilamide and 0.2 %

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NEDA in 4 % phosphoric acid.

**Cell culture** Rat C6 astrocytoma cells were kindly provided by Dr WANG Shun-You (Fudan University). Human BT325 astrocytoma cells were from Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Both cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % heat-inactivated new-born bovine serum, *L*-glutamine  $2 \text{ mmol} \cdot \text{L}^{-1}$ , sodium pyruvate  $1 \text{ mmol} \cdot \text{L}^{-1}$ , benzylpenicillin  $100 \text{ kU} \cdot \text{L}^{-1}$ , and streptomycin  $100 \text{ mg} \cdot \text{L}^{-1}$ . Cells were seeded in 24-well plastic plates (Costar) at  $2 \times 10^6 \text{ cells} \cdot \text{L}^{-1}$  and incubated at  $37 \text{ }^\circ\text{C}$  in a humidified air with 5 %  $\text{CO}_2$ . After a 12-h incubation, the medium was replaced by new DMEM containing various concentrations of drugs, and cells were incubated for further 8, 24, and 48 h.

**Assay for NO production**<sup>[9]</sup> At the end of incubation,  $100 \mu\text{L}$  of supernatants were mixed with  $100 \mu\text{L}$  of Griess reagent. After chromophore was formed at  $25 \text{ }^\circ\text{C}$  for 15 min, the absorbance was determined at  $570 \text{ nm}$  with ELISA reader, and the zero point was adjusted with DMEM medium. NO levels were represented as nitrite concentration, and were determined with reference to a standard curve of sodium nitrite.

**Statistical analysis** Data were expressed as  $\bar{x} \pm s$  and compared by *t*-test.

## RESULTS

The NO production from rat C6 cells was time- and concentration-dependently inhibited by the treatment with Hup A  $0.1 - 100 \mu\text{mol} \cdot \text{L}^{-1}$  for 8, 24, and 48 h (Fig 1).

The NO production from C6 cells was concentration-dependently inhibited by the treatment with Hup A, Gin A, or Gin B  $0.001 - 10 \mu\text{mol} \cdot \text{L}^{-1}$  for 24 h (Fig 2).

The NO production from human BT325 cells was concentration-dependently inhibited with Hup A, Gin A, or Gin B  $0.01 - 10 \mu\text{mol} \cdot \text{L}^{-1}$  for 24 h (Fig 3).

## DISCUSSION

It was reported<sup>[1]</sup> that LPS and pre-inflammatory cytokines such as IL-1, IL-2, IL-6, TNF- $\alpha$ , and IFN- $\gamma$  can activate the central microglia and astrocytes.

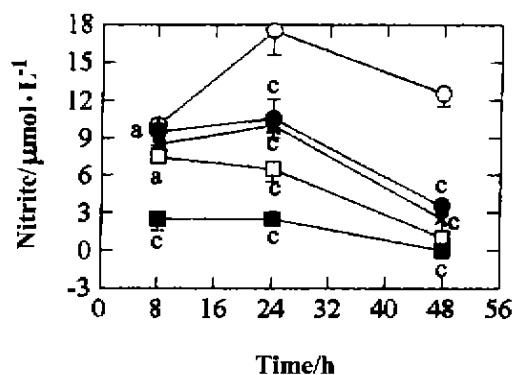


Fig 1. NO production from C6 cells untreated (○, as control) or with Hup A  $0.1$  (●),  $1$  (×),  $10$  (□), and  $100$  (■)  $\mu\text{mol} \cdot \text{L}^{-1}$  for different time.  $n = 4$  experiments.  $\bar{x} \pm s$ .  $^*P > 0.05$ ,  $^cP < 0.01$  vs control.

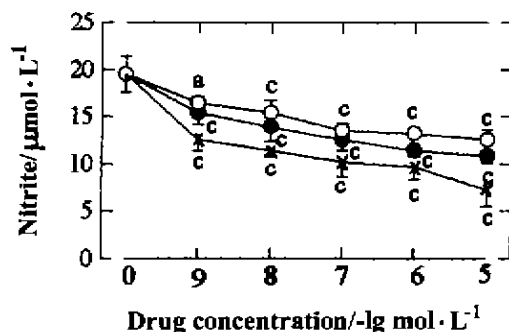


Fig 2. NO production from C6 cells treated with Hup A (○), Gin A (●), and Gin B (×) for 24 h.  $n = 4$  experiments.  $\bar{x} \pm s$ .  $^*P > 0.05$ ,  $^cP < 0.01$  vs control.

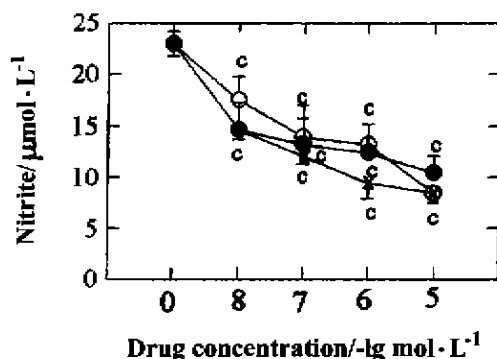


Fig 3. NO production from BT325 cells treated with Gin B (○), Hup A (●), and Gin A (×) for 24 h.  $n = 4$  experiments.  $\bar{x} \pm s$ .  $^cP < 0.01$  vs control.

Activated microglia and astrocytes may hurt the neurons by releasing NO and other neurotoxic substances, such as excitatory amino acids, reactive oxygen intermediates, and pre-inflammatory cytokines<sup>[5]</sup>. Most of these cytokines, such as IL-1, IL-6, TNF- $\alpha$ , have been shown to exert neurotoxicity through L-arginine-NO pathway<sup>[5,9-11]</sup>. The previous works in our laboratory have proved that LPS and pre-inflammatory cytokines enhanced NO production from rat microglia and astrocytes. We also found that NO inhibited the proliferation and induced the apoptosis of cultured human, SK-N-SH neuroblastoma cells (unpublished data). The above results suggested that modulating the NO synthesis in central nervous system may be a new method in reducing AD and other neurodegenerative diseases.

This study showed that rat C6 and human BT325 astrocytoma cells produced NO spontaneously, which was consistent with the results reported by Zhao<sup>[3]</sup> and Du<sup>[4]</sup>. The NO production from C6 and BT325 cells was concentration-dependently inhibited by Hup A, Gin A, and Gin B. These results were similar to those reported by Du<sup>[8]</sup>, indicating that the memory-enhancing effect of the three compounds may be partially mediated by decreasing NO level in AD brain, beside their ChE-inhibiting and PAF-antagonistic mechanisms. Further studies are needed to elucidate whether the inhibiting effects of Hup A, Gin A, and Gin B on NO production from glia are related to their ChE-inhibiting or PAF-antagonistic activities.

REFERENCES

- 1 McGeer PL, McGeer EG. Immune mechanisms in neurodegenerative disorders. *Drug Today* 1996; 32: 149-58.
- 2 Ma TC, Zhu XZ. Interleukin-6 increases the levels of cyclic GMP and nitrite in rat hippocampal slices. *Eur J Pharmacol* 1997; 321: 343-7.
- 3 Zhao HW, Li XY. Effects of interleukin 2 and tumor necrosis factor  $\alpha$  on nitric oxide production from rat C6 glioma cells. *Chin J Pharmacol Toxicol* 1998; 12: 188-91.
- 4 Du ZY, Li XY. Cytokine and nitric oxide production by rat microglia stimulated with lipopolysaccharides *in vitro*. *Acta Pharmacol Sin* 1998; 19: 257-60.
- 5 Chao CC, Hu SX, Peterson PK. Glia, cytokines, and

- neurotoxicity. *Crit Rev Neurobiol* 1995; 9: 189-205.
- 6 Tang XC. Huperzine A (Shuangyiping): a promising drug of Alzheimer disease. *Acta Pharmacol Sin* 1996; 17: 481-4.
- 7 Zhu L, Wu J, Liao H, Guo J, Zhao XN, Zhang ZX. Antagonistic effects of extract from leaves of *Ginkgo biloba* on glutamate neurotoxicity. *Acta Pharmacol Sin* 1997; 18: 344-7.
- 8 Du ZY, Li XY. Effects of ginkgolides on interleukin-1, tumor necrosis factor- $\alpha$  and nitric oxide production from rat microglia stimulated with lipopolysaccharides *in vitro*. *Arzneimittelforschung* 1998; 48: 1126-30.
- 9 Rossi F, Bianchini E. Synergistic induction of nitric oxide by  $\beta$ -amyloid and cytokines in astrocytes. *Biochem Biophys Res Commun* 1996; 225: 474-8.
- 10 Arvin B, Neville LF, Barone FC, Feuerstein GZ. The role of inflammation and cytokines in brain injury. *Neurosci Biobehav Rev* 1996; 20: 445-52.
- 11 Malek-Ahmadi P. Neuropsychiatric aspects of cytokines research: a review. *Neurosci Biobehav Rev* 1996; 20: 359-65.

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银杏内酯 A 和 B 及石杉碱甲抑制大鼠 C6 及人 BT325 胶质瘤细胞产生一氧化氮

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关键词 银杏内酯 A; 银杏内酯 B; 石杉碱甲; 一氧化氮; 培养的肿瘤细胞; 星形胶质瘤

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目的: 观察银杏内酯 A (Gin A), 银杏内酯 B (Gin B) 及石杉碱甲 (Hup A) 对体外培养的星形细胞产生一氧化氮(NO)的影响. 方法: Griess 法检测细胞培养上清中 NO<sub>2</sub><sup>-</sup> 含量. 结果: HupA 0.001 - 100  $\mu\text{mol}\cdot\text{L}^{-1}$  明显抑制大鼠 C6 细胞产生 NO, 该抑制作用呈浓度及时间依赖性, 在 0.001 - 10  $\mu\text{mol}\cdot\text{L}^{-1}$  范围作用 24 h, Gin A 和 Gin B 均浓度依赖性地抑制 C6 细胞产生 NO. 在 0.01 - 10  $\mu\text{mol}\cdot\text{L}^{-1}$  范围作用 24 h, Hup A, Gin A 和 Gin B 均浓度依赖性地抑制人 BT325 细胞产生 NO. 结论: Hup A, Gin A 和 Gin B 抑制大鼠及人星形细胞产生 NO.

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