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Cytokines enhance nitric oxide production from human BT325 astrocytoma cells

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KEY WORDS astrocytoma; cultured tumor cells; interleukin-1; interleukin-2; tumor necrosis factor; interferon type Ⅱ; lipopolysaccharides; nitric oxide

ABSTRACT

AIM: To study the effects of lipopolysaccharides (LPS) and pre-inflammatory cytokines on nitric oxide (NO) production from cultured astrocytes. **METHODS**: Nitrites in supernatants were measured with Griess assay. **RESULTS**: The NO production from cultured human BT325 astrocytoma cells started when cultured for 4 h, reached the peak concentration (15.0 $\mu mol \cdot L^{-1}$) at 12 h, maintained a high level (15.0-17.5 $\mu mol \cdot L^{-1}$) up to 72 h, and was enhanced by LPS 1 $mg \cdot L^{-1}$, interferon-γ (IFN-γ) 100 $kU \cdot L^{-1}$, tumor necrosis factor-α (TNF-α) 100 $kU \cdot L^{-1}$, interleukin (IL)-1 100 $kU \cdot L^{-1}$, or IL-2 100 $kU \cdot L^{-1}$. The enhancements of TNF-α, IL-1, IL-2, or the mixture of the above four cytokines were higher. **CONCLUSION**: Stimulants and pre-inflammatory cytokines enhance astrocytes producing NO.

INTRODUCTION

While the complex reasons causing Alzheimer disease (AD) remain unclear, the immune-inflammatory mechanism in AD has captured attention of more and more investigators. According to the immune-inflammatory hypothesis, pre-inflammatory cytokines, complement proteins, activated glia (microglia and astrocytes), and nitric oxide (NO) play important roles in AD[1]. Concerning the facts that the nitric-oxide synthase (NOS) has a wide spread in the central nervous system[2] and that NO released from cerebrovascular endothelia, neurons, and glia caused neurodegeneration[3], NO is considered to be one of the key factors in AD mechanisms. Lipopolysaccharides (LPS) and pre-inflammatory cytokines, such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-2, and IL-6 may exert their neurotoxic effects by increasing the NO level in brain. LPS and the above cytokines enhance the NO production from rat microglia[4] and rat C6 astrocytoma cells[5]. In this paper, the effects of LPS, IFN-γ, TNF-α, IL-1, and IL-2 on NO production from human BT325 astrocytoma cells were investigated.

MATERIALS AND METHODS

Reagents LPS (*E coli* O55:B5), N^{ω}-nitro-*L*-arginine methyl ester (*L*-NAME) were purchased from Sigma. IFN-γ and TNF-α were from Shanghai Clone Biotech Co. IL-1 was from Beijing Biotinge Biomedicine Co. IL-2 was from Shanghai Institute of Biochemistry, 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 % NEDA in 4 % phosphoric acid.

Cell culture Human BT325 astrocytoma cells were from Shanghai Institute of Cell Biology, Chinese Academy of Sciences, and were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % heat-inactived newborn bovine serum, *L*-glutamine 2 $mmol \cdot L^{-1}$, sodium pyruvate 1 $mmol \cdot L^{-1}$, benzylpenicillin 100 $kU \cdot L^{-1}$, and streptomycin 100 $mg \cdot L^{-1}$. Cells were seeded in 24-well plastic plates

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(Costar) at 2×10^8 cells·L^{-1} and incubated at 37 ℃ in a humidified air with 5 % CO_2. After a 12-h incubation, the medium was replaced by new DMEM containing various concentrations of drugs, and cells were incubated for another 4, 8, 12, 24, 48, and 72 h.

Assay for NO production[6] At the end of incubation, 100 μL of supernatants were mixed with 100 μL of Griess reagent. After chromophore was formed at 25 ℃ for 15 min, absorbance was determined at 570 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 $x \pm s$ and compared by t-test.

RESULTS

When cultured without stimulants for 4 h, human BT325 cells started producing NO spontaneously. The peak concentration (15.0 μmol·L^{-1}) of NO appeared at 12 h, kept at 15.0-17.5 μmol·L^{-1} up to 48 h, and maintained a high level up to 72 h of the incubation. The NO production from human BT325 astrocytoma cells was inhibited by the NOS inhibitor *L*-NAME 0.25 mmol·L^{-1} (Fig 1).

When cultured with LPS 1 mg·L^{-1}, IFN-γ 100 kU·L^{-1}, TNF-α 100 kU·L^{-1}, IL-1 100 kU·L^{-1}, or IL-2 100 kU·L^{-1}, BT325 cells produced more NO than the resting BT325 cells. The enhancement was higher when cultured for 4, 8, 24 h, especially when treated with TNF-α, IL-1, or IL-2. The NO production was also enhanced when treated with the mixture of the above four cytokines (Fig 1).

DISCUSSION

This study showed that human BT325 astrocytoma cells produced NO, which was enhanced by LPS and pre-inflammatory cytokines such as IFN-γ, TNF-α, IL-1, and IL-2. The results are similar to those reported by Du[4], Zhao[5], and Mollace[7] *et al*.

It was reported[8] that mononuclear cells in blood of advanced Alzheimer patients released more IL-2 and IFN-γ. They enter the brain through the patient's brain-blood-barrier with high permission, and accompany with other inflammatory mediators to activate the central microglia and astrocytes. Activated microglia and astrocytes may hurt neurons through releasing NO and other neurotoxic substances, such as excitatory amino acids, reactive oxygen intermediates, and pre-inflammatory cytokines[9]. Most of these cytokines, such as IL-1, IL-6, TNF-α, have been shown to exert neurotoxicity through *L*-arginine-NO pathway[9-12]. The previous works in our laboratory have proved that LPS and pre-inflammatory cytokines enhanced NO production from rat microglia[4] and astocytes[5]. We demonstrated in this paper that human astrocytes were also capable of producing NO, which were enhanced by treating with LPS and pre-inflammatory cytokines. Recently, we also found that NO inhibited the proliferation and induced the apoptosis of cultured human SK-N-SH neuroblastoma cells (unpublished data). Hence, modulating NO synthesis in central nervous system may be a new strategy in reducing AD and other neurodegenerative diseases.

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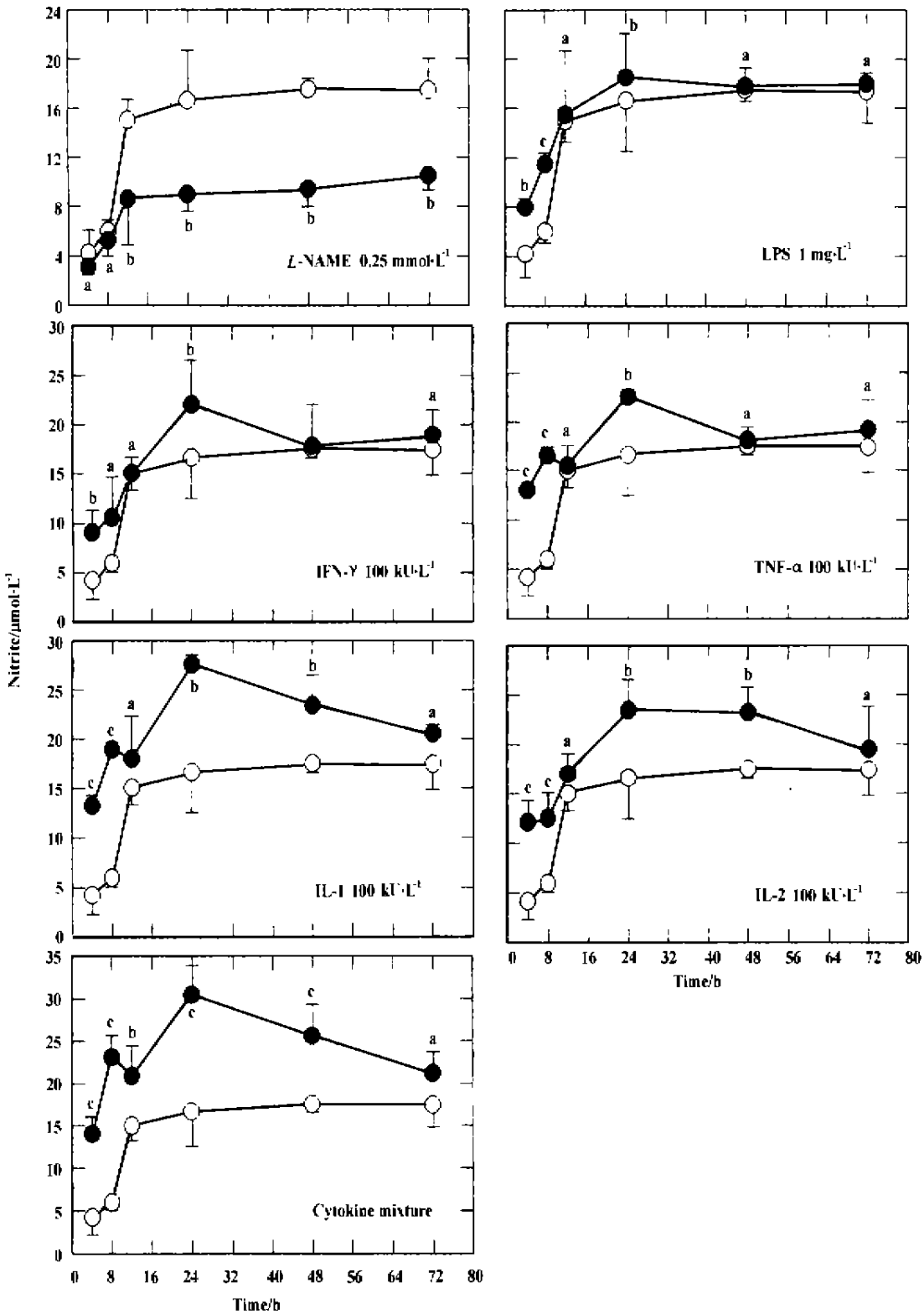

Fig 1. NO production from BT325 cells untreated (○) and treated (●) with *L*-NAME, LPS, IFN-γ, TNF-α, IL-1, IL-2, or cytokine mixture (IFN-γ 100 kU·L^{-1} + TNF-α 100 kU·L^{-1} + IL-1 100 kU·L^{-1} + IL-2 100kU·L^{-1}). $n = 4$ experiments. $\bar{x} \pm s$. $^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* untreated cells.

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细胞因子促进人 BT325 胶质瘤细胞产生一氧化氮

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关键词 星形胶质细胞; 培养的肿瘤细胞; 白细胞介素-1; 白细胞介素-2; 肿瘤坏死因子; 干扰素类Ⅱ型; 脂多糖; 一氧化氮

目的: 观察脂多糖及一些前炎性细胞因子对体外培养的星形胶质细胞产生一氧化氮(NO)的影响. **方法**: Griess 法检测细胞培养上清中 NO_2^- 含量. **结果**: 体外培养 4 h 时人 BT325 星形胶质瘤细胞开始产生 NO, 12 h 达高峰(15.0-17.5 $\mu mol \cdot L^{-1}$)并持续至 72 h. LPS 1 $mg \cdot L^{-1}$, IFN-γ 100 $kU \cdot L^{-1}$, TNF-α 100 $kU \cdot L^{-1}$, IL-1 100 $kU \cdot L^{-1}$及 IL-2 100 $kU \cdot L^{-1}$可增强体外培养的 BT325 细胞产生 NO, 以 TNF-α, IL-1 和 IL-2 作用较为明显, 四种细胞因子合用则作用更强. **结论**: 炎症刺激或前炎性细胞因子促进神经胶质细胞产生 NO.

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