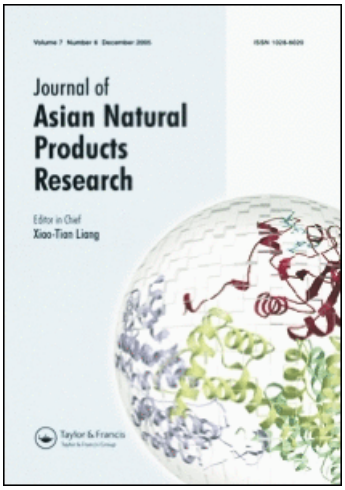

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EFFECT OF 17-β-ESTRADIOL AND GINSENOSIDE Rg_1 ON REACTIVE MICROGLIA INDUCED BY β-AMYLOID PEPTIDES

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The reactive microglias induced by 25 μmol of β-amyloid peptides (Aβ25–35) and/or IFN-γ can initiate the microglial respiratory burst and release NO, respectively. Oxidative stress and inflammatory function have been implicated in Alzheimer's disease (AD). We showed that 10 μmol 17-β-estradiol (E_2) and 1–10 μmol ginsenoside Rg_1 (Rg_1) could prevent the toxicity of Aβ25–35 and/or IFN-γ to microglias, inhibit the microglial respiratory burst activity and decrease the accumulation of NO. These results demonstrated the protectional effect of E_2 or Rg_1 on neuron from damaging by reactive microglias in AD.

Keywords: Alzheimer's disease; Microglia; 17-β-Estradiol; Ginsenoside Rg_1; Superoxide anion; Nitric oxide; Inflammation; β-amyloid; IFN-γ

INTRODUCTION

Microglial cells are the resident brain macrophages that determine the immune responses of the central nervous system (CNS). Damage of the CNS leads to activation of the resting microglia, resulting in a sequence of morphological, immunological, and functional changes [1,2]. Alzheimer's Disease (AD) is a neurodegenerative disease, characterized clinically by a progressive senile dementia, and histologically by characteristic neurofibrillary tangles and the deposition of bA4 protein in the form of amyloid plaques

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that are surrounded by reactive microglias, it has become increasingly evident that these cells play an important role in plaque pathology [3]. The reactive microglias share many features with monocytes and inflammatory tissue macrophages, and their morphology and cellular markers in AD tissue are well documented [4,5]. It has recently been demonstrated that cultured rat microglias are damaged directly by Aβ25–35 , and the reactive microglias can release NO and initiate the microglial respiratory burst [6,7]. The mechanism of Aβ25–35 toxicity in CNS is unclear but is believed to involve generation of reactive oxygen species and some cytokines. So, the development of antiinflammatory drugs designed to inhibit specifically microglial activation and/or proinflammatory function might be a promising therapeutic strategy for AD [8,9].

Ginsenoside and E_2 could scavenge oxygen free radicals and protect the function of neuron in culture [10,11]. We decided to investigate the effect of Ginsenoside and E_2 on the activition and viability of microglia by β-amyloid peptides.

RESULTS AND DISCUSSION

Effect of Rg_1 and E_2 on Cell Viability of Cortical Microglias by Aβ25–35 and IFN-γ

Consistent with Pike's observations [17], 25 μmol Aβ25–35 formed aggregates that visibly precipitated within tissue culture wells. As the concentration of Aβ25–35 was reduced, the amount of precipitate visible within tissue culture wells was also reduced. Twenty four hours following its addition to cultures, the cells had a shrunken or shriveled appearance indicative of degeneration (Fig. 1). Quantitated the viability of cultures at 24 h after treatment with Aβ25–35 peptides or fresh media, microglial cultures displayed reduced conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5,-diphenyltetrazdium bromide (MTT) and were significantly reduced to $44.3 \pm 2.7\%$ of control. When the $100\,U \cdot mL^{-1}$ IFN-γ were added in these cultures for another 48 h, the changes of morphological appearance were more obvious (Fig. 1), and the viability was lowered to $30.3 \pm 4.8\%$ (Table I). But pretreatment of cultures with 1–10 μmol Rg_1 or 10 μmol E_2 for 72 h resulted in significant protection against the toxicity of Aβ25–35 and/or IFN-γ (Fig. 1, Table I).

Since the MTT assay measures primarily oxidative mitochondral metabolism, populations of cells are detected as well as killed cells. To quantitate cell lysis after the addition of Aβ25–35 and/or IFN-γ, lactate dehydrogenase

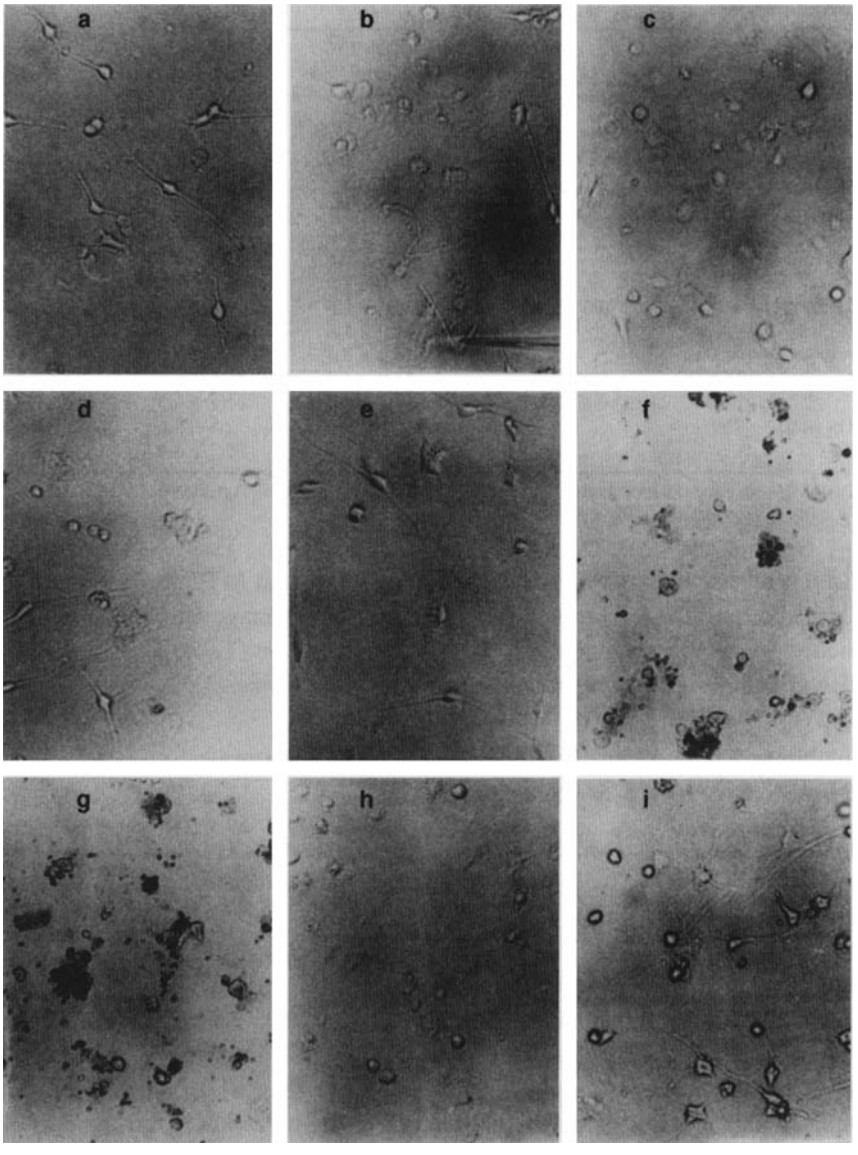

FIGURE 1 Morphological appearance of microglial cultures treated with Aβ or/and IFN-γ for 24 h after incubated concentrations of various reagents for 72 h. (a) Control, (b) 25 μmol Aβ, (c) 10 μmol Rb_1 plus 25 μmol Aβ, (d) 10 μmol E_2 plus 25 μmol Aβ, (e) 10 μmol Rg_1 plus 25 μmol Aβ, (f) 25 μmol Aβ plus 100 U · ml^{-1} IFN-γ, (g) 10 μmol Rb_1 plus 25 μmol Aβ and 100 U · ml^{-1} IFN-γ, (h) 10 μmol E_2 plus 25 μmol Aβ and 100 U · ml IFN-γ, (i) 10 μmol Rg_1 plus 25 μmol Aβ and 100 U · ml^{-1} IFN-γ.

TABLE I E_2 and Rg_1 protect microglias from the toxicity of Aβ25–35 (Aβ) and/or IFN-γ

Reagents	*Concentration* (μmol)	*MTT reduction* (%)	*LDH release* (%)	*Cell count* (%)
Control		100.0 ± 6.1	13.8 ± 3.1	100 ± 3
Aβ alone	25	44.3 ± 2.7^a	46.8 ± 6.1^a	64 ± 8^a
E_2 plus Aβ	1	49.3 ± 4.5^b	40.1 ± 4.7^c	70 ± 6
	10	60.5 ± 2.2^c	31.4 ± 3.9^c	79 ± 8^c
Rg_1 plus Aβ	1	66.1 ± 3.1^c	24.3 ± 4.3^c	85 ± 11^c
	10	63.9 ± 4.0^c	28.9 ± 6.4^c	80 ± 11^c
Rb_1 plus Aβ	1	43.9 ± 3.6	47.3 ± 3.1	67 ± 8
	10	46.0 ± 2.9	44.8 ± 3.4	65 ± 12
Aβ and IFN-γ	100 U · ml^{-1}	30.3 ± 4.8^d	79.1 ± 4.2^d	32 ± 5^d
E_2 plus Aβ/IFN-γ	1	34.0 ± 5.4	73.4 ± 5.4^e	38 ± 5^e
	10	44.0 ± 5.4^f	69.2 ± 2.4^f	42 ± 6^f
Rg_1 plus Aβ/IFN-γ	1	53.9 ± 4.7^f	55.5 ± 6.4^f	59 ± 8^f
	10	47.4 ± 3.2^f	61.1 ± 4.5^f	52 ± 10^f
Rb_1 plus Aβ/IFN-γ	1	32.4 ± 6.0	72.8 ± 5.6	33 ± 5
	10	34.2 ± 5.4	69.7 ± 4.4	35 ± 3

$\bar{X} \pm$ SD ($n = 8$); $^a p < 0.01$ vs control; $^b p < 0.05$, $^c p < 0.01$, $^d p < 0.01$ vs Aβ alone; $^e p < 0.05$, $^f p < 0.01$ vs Aβ and IFN-γ.

(LDH) release was measured. LDH-release assay and MTT assays were performed in parallel to compare the amount of cell damage after a 24 h or 48 h incubation of the cells with Aβ25–35 and/or IFN-γ (Table I). Rg_1 and E_2 also afforded significant protection against the cell lysis.

Inhibitory Effect of Rg_1 or E_2 on Microglial Respiratory Burst Activity by Aβ25–35 and PMA

The spontaneous release of O_2^- from microglial cells appeared to be unaltered after treatment with 10 μmol Aβ25–35. In contrast, the PMA-induced production of O_2^- was markedly enhanced after previous treatment with Aβ25–35 for 24 h. Quantitative data as obtained with the INTV assay are summarized in Table II. A statistically significant increase was observed in the phorbol 12-myristate 13-acetate (PMA)-induced. The production of O_2^- in the Aβ25–35-treated cultures, as compared with the release found in the absence of PMA, amounted to $426.7 \pm 65.6\%$ ($\bar{X} \pm$ SD), which is signifigcantly higher than the increase observed in the untreated controls. Similar to the viability of the cultures, 1–10 μmol Rg_1 or 10 μmol E_2 could markedly decease O_2^- release from microglia by Aβ25-35 and PMA (Table II). To exclude any confounding effects due to possible contamination of the Aβ25–35 peptide stock solution with traces of lipopolysaccharide (LPS), the control experiments were performed in the presence of polymyxin B (PMB). The 10 μmol Aβ25–35-mediated effect remained completely unaffected by concurrent administration of 10 μg · ml^{-1} of PMB.

TABLE II E_2 and Rg_1 inhibit the effect of Aβ25–35 on microglical respiratory burst activity

Treatment	*Concentration* (μmol)	*PMA-induced superoxide production (as % of unstimulated control)*
Aβ25–35	0	266.7 ± 41.0
	10	426.7 ± 65.6[a]
	0 + 10 μmol E_2	143.5 ± 12.3[a]
	10 + 1 μmol E_2	320.0 ± 28.6[b]
	10 + 10 μmol E_2	283.0 ± 16.5[b]
	0 + 10 μmol Rg_1	139.5 ± 16.5[a]
	10 + 1 μmol Rg_1	299.5 ± 32.8[b]
	10 + 10 μmol Rg_1	242.1 ± 24.6[b]
	0 + 10 μmol Rb_1	250.0 ± 51.3
	10 + 1 μmol Rb_1	361.0 ± 32.8
	10 + 10 μmol Rb_1	365.1 ± 37.0
	0 + 10 μg · ml^{-1} PMB	280.0 ± 20.0
	10 + 10 μg · ml^{-1} PMB	422.7 ± 53.3[a]
LPS	1 ng · ml^{-1}	352.8 ± 28.4[a]
	1 ng · ml^{-1} + 10 μg · ml^{-1} PMB	272.3 ± 20.5

$X \pm SD$ ($n = 8$), [a]$p < 0.01$ vs untreated Aβ25–35; [b]$p < 0.01$ vs Aβ25–35.

Effect of Rg_1 or E_2 on Microglial Nitrite Production by Aβ25–35 and IFN-γ

After 24 h, no detectable levels of nitrite could be demonstrated in the media of either untreated control or 25 μmol Aβ25–35-treated cultures. However the rat primary microglia cultures were incubated in the presence of Aβ25–35 and IFN-γ for 24 h, the NO accumulation was significantly increased, Rg_1 or E_2 could inhibit the NO accumulation (Fig. 2).

Several lines of evidence indicate that activated microglias and microglial-derived factors might play a pivotal role in the amyloid-driven pathological cascade that ultimately leads to a loss of neuronal integrity and cognitive impairment of AD patients [18,19]. Accordingly, reactive microglias might, e.g., by releasing proinflammatory cytokines and complement proteins, trigger a feedback loop resulting in the recruitment and further activation of microglia [9,20].

Our results demonstrated that cultured rat microglia exposed to Aβ25–35 peptides induced degeneration, increased LDH release, reduced the metabolic breakdown of MTT by these cells, the latter effect being associated in many cases with cell death [15]. Aβ25–35 plus IFN-γ caused a great loss of microglias (Fig. 1), 1–10 μmol Rg_1 and 10 μmol E_2 could protect the morphological signs of injury and increase the viable amount of microglias (Table I). The death of microglias may result from the activation of themselves induced by Aβ25–35 and IFN-γ. The toxicity of Aβ25–35 peptides in

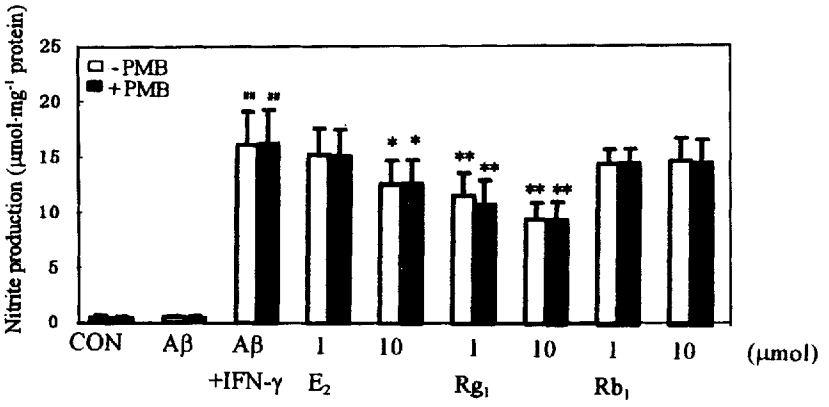

FIGURE 2 E_2 and Rg_1 inhibit the effect of Aβ25–35 (Aβ) or/and Aβ+IFN-γ on the accumulation of NO from cultured microglia. $\bar{X} \pm SD$ ($n = 8$) from three separate experiments. $^{\#\#}p < 0.01$ vs control or Aβ; $^{*}p < 0.05$, $^{**}p < 0.01$ vs Aβ+IFN-γ.

microglias perhaps depended on the state of peptide aggregation as that in neurons, suggesting that the mechanism of degeneration in both cells may be similar.

Many studies have documented the fact that cytokines induced NO production that can lead to destruction or damage of parasite, tumor cells or normal cells, microglia, activated by IFN-γ alone or in concert with Aβ, can, via NO, kill neurons or microglias [6]. In our results, Rg_1 and E_2 could inhibit the NO releasing from reactive microglias induced by Aβ25–35 and IFN-γ (Fig. 2). The inhibitory mechanism needs more experiments to be clarified.

In adult brain tissue, microglias can be found as resting ramified cells with down-regulated macrophage functional properties. But in AD, microglias induced by Aβ25–35 become reactive, which share many features with monocytes and inflammatory tissue macrophages. *Inter alia*, similar to mononuclear phages, activated microglias exhibit pronounced respiratory burst activity resulting in the release of large amount of superoxide anion (O_2^-). Although the activation of the respiratory burst may represent a beneficial physiological response in host defence, strong or chronic activation of microglias induced by Aβ25–35 may be extremely harmful that lead to an extensive release of reactive oxygen and/or nitrogen species at the sites of inflammation.

These results support that the toxin of Aβ25–35 plus PMA or IFN-γ is related to the accumulation of O_2^- or NO. Once release of these toxic

substances was inhibited, then the microglias and even neurons may be protected. Estrogen replacement therapy in older women can delay the onset and lower the risk of AD [21], and estrogen has the neuroprotective effect against oxidative stress in neuron cultures [11]. In our study, E_2 can protect the function of microglia in the presence of Aβ25–35, and Rg_1 seem to have the same function as E_2. It is important for us to make more experiments to elucidate Rg_1 or E_2 as to how to protect neuron from insulting by reactive microglia in AD.

EXPERIMENTAL SECTION

Materials

Dulbeuo's modified Eagles medium, and fetal calf serum were obtained from GIBCO Life Technologies. The synthetic Aβ25–35, p-iodonitrotetrazolium vialet (p-INTV), MTT, PMA, trypsin (1:250), interferon-γ were purchased from Sigma. LPS was purchased from Difco.

Primary Rat Microglial Cultures

Primary microglial cultures were prepared from the cerebral cortices of 2-day-old newborn rats by a combination of enzymatic and mechanical dispersion as previously described [12]. Following dissociation of the tissue, the cells were seeded in 75-cm^2 culture flasks and grown at 37°C in a humidified atmosphere of 5% CO_2/95% air. After the medium was changed on day 1, the cultures were left undisturbed for 10 consecutive days. Thereafter, to isolate nonadherent and loosely attached cells from the confluent cell layer, cultures were agitated on a rotary shaker (200 rpm) for 18 h at 37°C. From the supernatant medium, floating cells were collected, pelleted and reseeded at a density of 2.5×10^5 cells/cm^2 in uncoated flatbottom 24-well plates. After 1 h, microglial cells were removed from the cultures by gently shaking the plates before replating the medium with 900 μl of DMEM per well subsequently, cultures were treated with Aβ25–35, ginsenoside and E_2 as described below.

Treatment of Microglial Cultures

The lyophilized synthetic peptides Aβ25–35 initially dissolved at a concentration of 1 mmol in sterile double-distilled were stored at 37°C for

7 days before use, peptides were diluted in DMEM and tested in a concentration 25 μmol. Whereas control cultures were treated with equal amount of vehicle.

Measurement of Superoxide Anion Generation

The INTV reduction assay was performed according to the procedure of Turner *et al.* [13] with some modifications. In brief, the microglial cultures were previously treated with different concentrations of E_2, Rg_1 and Rb_1 for 72 h, then 10 μmol Aβ25–35 was added for 24 h, and the medium was replaced by EBSS containing 0.5 mg · ml^{-1} of INTV with or without 0.1 μg · ml^{-1} of PMA. Fortyfive min after incubation, the medium was aspirated, and the cells were solubilized by adding 1 ml of acidified dimethyl sulfoxide containing 2% HCl per well. The absorption of reduced INTV was measured in each well at a wavelength of 492 nm.

Assay of NO Production

Following treatment of microglial cultures with 25 μmol · L^{-1} Aβ25–35 and different concentrations of Rg_1 and E_2 for 24 h, and then the cultures were added 100 ng · ml^{-1} IFN-γ for 48 h, the production of NO was determined by the measurement of nitrite levels in the culture supernatants. In brief, after mixing a sample of the Griess reagent (1% (wt/vol) sulfanilamide and 0.25% (wt/vol) naphthylenediamine in 2.5% H_3PO_4), the absorbance of each well was read at a wavelength of 550 nm [14]. The amount of nitrite formed was calculated using sodium nitrite, diluted in DMEM as standard.

Assessment of Cell Viability

To assess cell viability, both the MTT colorimetric assay [15] and the LDH efflux assay were performed on cell monolayers or culture supernatants, respectively [16]. In addition, cultures were subjected to morphological examination using phase-contrast microscopy.

References

[1] Thomas, W.E. *Brain Res. Rev.* 1992, **17**, 61–74.
[2] Perry, V.H., Andersson, P.B. and Gordon, S. *Trends Neurosci.* 1993, **16**, 268–273.
[3] Selkee, D. *Neuron* 1991, **6**, 487–498.
[4] Dickson, D.W., Lee, S.C., Mattiace, L.A., Yeu, S.C. and Brosnan, C. *Glia* 1993, **7**, 75–83.

[5] Griffin, W.S.T., Sheng, J.G., Roberts, G.W. and Mark, R.E. *J. Neuropathol. Exp. Neurol.* 1995, **54**, 276–278.
[6] Meda, L., Cassatella, M.A., Seendrei, I.G., Jr., Otvos, L., Baron, P., Villalba, M., Ferrari, D. and Rossi, F. *Nature* 1995, **374**, 647–650.
[7] Van Muiswinkel, F.L., Veerhuis, R. and Eikelenboom, P. *J. Neurochem.* 1996, **66**, 2468–2476.
[8] Burger, D. and Dayer, J.M. *Neurology* 1995, **45**(suppl.), S39–S43.
[9] Eikelenboom, P., Zhan, S.S., Van Gool, W.A. and Allsop, D. *Trends Pharmacol. Sci.* 1994, **15**, 447–450.
[10] Li, J.L., Li, Z.K., Duan, H. and Zhang, J.T. *Acta Pharmacentica Sinica* 1997, **32**, 251–254.
[11] Behl, C., Skutella, T., Lezoualc'h, F., Post, A., Widmann, M., Newton, C.J. and Holsboer, F. *Mol. Pharmacol.* 1997, **51**, 535–541.
[12] Giulian, D. and Baker, T.J. *J. Neurosci.* 1986, **6**, 2163–2178.
[13] Turner, N.C., Wood, L.J., Burns, F.M., Guercmy, T. and Souness, J.E. *Br. J. Pharmacol.* 1993, **108**, 876–883.
[14] Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishwrok, J.S. and Tannenbaum, S.R. *Anal. Biochem.* 1982, **126**, 131–138.
[15] Hansen, M.B., Nielsen, S.E. and Berg, K. *J. Immunol. Methods* 1989, **119**, 203–210.
[16] Koh, J.Y. and Choi, D.W. *J. Neurosci. Methods* 1987, **20**, 83–88.
[17] Pike, C.J., Burdick, D., Walencewicy, A.J., Glabe, C.G. and Cotman, C.W. *J. Neurosci.* 1993, **13**, 1676–1687.
[18] Mackenzie, I.R.A., Hao, C.H. and Munoz, D.G. *Neurobiol. Aging* 1995, **16**, 797–804.
[19] Giulian, D., Haverkamp, L.J., Li, J., Karshin, W.L., Yu, J., Tom, D., Li, X. and Kirkpatrick, J.B. *Neurochem. Int.* 1995, **27**, 119–139.
[20] Mcgeer, P.L., Kawamata, T., Walker, D.G., Akiyama, H., Tooyama, I. and McGeer, E. *G. Glia* 1993, **7**, 84–92.
[21] Henderson, V.W., Paganini-Hill, A., Emanuel, C.K., Dunn, M.E. and Buckwalter, G. *Arch. Neurol.* 1994, **51**, 896–900.