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# 흰쥐의 뇌 astrocyte에서 amyloid-β25-35로 유발된 지질의 과산화와 항산화 효소계 및 NO 생성에 미치는 백강잠의 효과

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## Effects of Bombycis corpus on Amyloid- -induced Lipid Peroxidation, Antioxidative Enzymes and NO Synthesis in Rat Astrocytes

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**목적** : 본 연구는 熄風解痙, 疏散風熱, 化痰散結 효능이 있는 백강잠이 치매에 미치는 영향을 알아보기 위하여 실험을 행하였다.

**방법** : 치매 유발물질인 *amyloidβ(Aβ)* 25-35를 흰쥐의 뇌 astrocyte에 처리한 후 대표적인 항산화 효소인 catalase, superoxide dismutase, glutathione peroxidase 및 glutathione-S-transferase의 활성 변화와 NO 생성 변화를 관찰하였다.

**결과** : Aβ 25-35 처리로 catalase와 superoxide dismutase 활성이 현저히 감소하였으나 백강잠을 처리한 경우는 이들 효소 활성이 크게 증가하였다. 그리고, Aβ 25-35의 농도에 의존적으로 증가된 NO 생성은 백강잠의 농도에 의존적으로 유의성 있게 억제되는 것으로 나타났다.

**결론** : 백강잠은 항산화계 효소의 활성화 및 *Aβ* 처리와 같은 치매유발 물질의 독성에 대한 적응능력 향상을 통하여 astrocyte를 보호하는 효능을 가지는 것으로 사료되며, 아울러 노인성 치매 등 임상적 응용에 그 효과가 기대된다.

***중심단어*** : 백강잠, 치매, 항산화효소, NO

## I. INTRODUCTION

Alzheimer's disease (AD) is a neurodegerative disorder characterized by a progressive cognitive decline resulting from selective neuronal dysfunction, synaptic loss, and neuronal cell death[1]. It has been suggested that glial cells in AD may play in the neurodegenerative cascade and leads to Alzheimer dimentia. Neurodegerative disorders usually involves the activation of astrocytes and gliosis (microglia). Glial activation involves morphological changes (more spherical cell soma, hypertrophy of nuclei, appearance of extensive cellular processes) and changes in expression of a large number of proteins[2]. In AD, activated astrocytes surround the neuritic shell of the amyloid plaque, and activated microglia are near the center of the neuritic shell adjacent to the amyloid core[3]. There are a number of stimuli that cause glial activation. One of inducers of glial activation is neuronal dysfunction or injury. Although the role of glial activation in AD is uncertain, it was known that reactive glia are associated with amyloid plaques[4]. In addition, some cytokines and inflammatory mediators produced by activated glia have the potential to initiate of excerbate the progression of neuropathology[4,5].

The factors responsible for inducing and maintaining the glial activation state in AD are unknown. However, it was suggested that Aβis involved in the neurodegenerative process. Aβis 39-43 amino acids long and proteolytically derived from an integral membrane protein termed

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amyloid precursor protein (APP)[6], although mechanism for APP processing is still unknown. There are many in vitro studies demonstrating that Aβis directly neurotoxic and increase neuronal susceptibility to other toxic agents[4,6]. The toxic effect of Aβis correlated with its ability to form aggregates[7]. Both oxygen species[8] and excessive $Ca^{2+}$ influx[9] are also implicated in the mechanism of Aneurotoxicity. In contrast, it was also reported that Apromotes neurite outgrowth under certain culture conditions instead of toxic action[10].

On the other hand, the action of Aβ that affect glia, and the glial responses to Aexposure are not well understood. When the effects of synthetis Aβ peptides(Aβ 1-42, Aβ 17-42, and scrambled Aβ 1-42) on cultured astrocytes were examined, Aβ1-42 induced a robust astrocyte activation, as evidenced by morphological changes, upregulation of the interleukin-1β mRNA, and stimulation of inducible nitric oxide synthase (iNOS) mRNA and nitric oxide (NO) release[11].

AD could be induced by sorrounded free radicals. These free radicals can be produced by: 1) the absorption of radiant energy such as UV, 2) endogenous oxidative reactions that occur during normal metabolic processes or 3) enzymatic metabolism of exogenous chemicals such as $CCl_4$, *t*-BHP and ethanol[12]. The reactivity of different free radicals varies and some cause severe damage to biological molecules, especially to DNA, lipids and proteins. In the presence of oxygen, free radicals can react with polyunsaturated fatty acids, resulting in highly reactive peroxyl free radicals. Peroxyl free radicals can further propagate the peroxidation of lipids or compromise the integrity of cell membranes, therefore this is thought to be involved toxic actions of some chemicals[13]. However, living systems are protected from activated oxygen species by enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-S-transferase (GST): they also could be protected by non-enzymatic, antioxidative nutrients, so called vitamins (A, C and E),-carotene and antioxidative materials including methionine and glutathione[14,15].

According to recent reports that these antioxidants scavenge and minimize the formation of oxygen-derived species, and inhibit oxidative damage induced by free radicals. They also recover the level of intracellular antioxidants (vitamins, methionine, glutathione and glutathione-related minerals)[16]. Hence, these antioxidants may be particularly important in diminishing cumulative oxidative damage.

Recently, several reports presented that natural dietary plants may play an antioxidative role in the prevention of aging and carcinogenesis and may offer effective protection from lipid peroxidative damage *in vitro* and *in vivo*[17,18]. Therefore, much attention has been focused on natural antioxidants, in paticular it was reported that the extract of *Bombycis corpus* may exert an anti-aging and sexual-reinforcing actions in experimental *in vivo* system[19], although little is yet known about the pharmacological effects or active ingredients. *Bombycis corpus* (BC), white-stiff silkworm, is a drug consisting of the dried larva of silkworm, *Mobyz mori L.*, dead and stiffened due to the infection of *Beauveria (Bals.) Vuill.(family Moniliaceae)*. It is used to subdue the endogenous wind and convulsions for the treatment of headache, vertigo, tic and skin prurigo; to resolve nodulation for the treatment of scrofula, tonsillitis, parotitis and purpura[20].

On the other hand, nitric oxide (NO) is a multi-functional mediator which is implicated in a wide range of biological functions such as neurotransmission, non-specific immune defense and vasodilation[21]. Endogenous NO is synthesized from L-arginine by isoforms of nitric oxide synthase (NOS) of constitutive (cNOS) andinducible (iNOS)[22], and NO permeates cell membranes, contained an unpaired electron and readily reacts with iron atoms. nNOS is the type of cNOS and is constitutively expressed in some neural cells. Modulation of the activity of eNOS or nNOS by crude drugs is an important target of investigation in the study of pharmacological effects of oriental medicine in nervous systems.

Modification of guanylate cyclase heme iron by NO activates the enzyme, stimulates cGMP accumulation, and facilitates vasorelaxation and synaptic transmission[23]. Mitochondrial iron-containing enzymes (e.g. aconitase) are inactivated by NO, and this accounts for the reduced cellular respiration and death of target cells exposed to macrophages[23].

Nitric oxide (NO) is a multi-functional mediator which plays an important role in the regulation of various biological functions *in vivo*. Dysregulation of NO production is therefore causatively related to the pathogenesis of various disease which include inflammation, cancer and immunological disorders. The inhibitory effect on NO production from cultured astrocyte cells, which was treated with A$\beta$25-35, was examined using crude drugs of BC. Also, the present study was done to investigate the effect of BC on cultured rat astrocytes, lipid peroxidation and antioxidative enzyme activities in A$\beta$25-35-treated conditions.

As an approach to know the interactions between A$\beta$ and glia, I have further examined the effects of synthetis A$\beta$ peptides on cultured astrocytes. I tested A$\beta$25-35 peptides aggregated under various conditions. I report here that A$\beta$25-35 isresponsible for the activaion of the astrocytes. This study also reports the effect of BC on cytotoxicity of cultured astrocytes and lipid peroxidation in A$\beta$-treated conditions. A$\beta$ which can produce intracellular free radical was used for inducer of the peroxidation of cellular lipids.

## II. MATERIALS and METHODS

### *Materials*

The A$\beta$25-35 peptide was synthesized by Applied Biosystem's Protein Synthesizer Model 470A (Peptron Co., LTD, Taejon, Korea). Fetal bovine serum (FBS), penicillin-streptomycin were obtained from GIBCO-BRL (Grand Island, New York, USA). Dulbecco's Modified Eagle's Medium (DMEM), glutamine, dimethyl sulphoxide (DMSO), 2-thiobabituric acid (TBA), 1,1,3,3-tetraethoxypropane (TEP), ethylene-diamine tetraacetic acid (EDTA), superoxide dismutase (SOD: from bovine liver), nitro blue tetrazolium (NBT), catalase (from bovine liver), diethylene triamine pentaacetic acid (DETAPAC), reduced glutathione (GSH), oxidized glutathione-reductase (GSSG-reductase),- nicotinamide adenine dinucleotide phosphate ( -NADPH) were purchased from Sigma Chem. Co. (St. Louis, USA). 1-chloro-2,4-dinitrobenzene (CDNB), NaN3 were obtain from Aldrich Chem. Co. (Milwaukee, WI).

### *Extract preparation from Bombycis corpus*

*Bombycis corpus*(BC, 300 g) was obtained from Oriental Medical Hospital, Dongguk University College of Oriental Medicine and extracted with boiling water for 3 hr. Then, the extract was evaporated to under reduced pressure by 75%, 85%, 95% ethanol solution. The last extracts diluted by 0.9% NaCl and filterated. The extract solution was stored at 4℃.

### *Cell culture and treatment of Bombycis corpus*

Cortical astrocyte cultures were prepared from neonatal rat (1-2 day old) pups by the method of Levision and McCarthy[24]. Cerebral cortex was dissected from neonatal day 1-2 Sprague-Dawley rat and dissociated by gentle trituration. Cells were plated in 6-well culture plates coated with polyethylenimine (0.2 mg/ml in sodium borate buffer, pH 8.3) at a density of 40,000 cells per well. After overnight incubation in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 20% fetal bovine serum, the medium was changed to serum-free defined medium for neurons [DMEM supplemented with 2 mM glutamine, 1 mM pyruvate, penicillin-streptomycin-amphotericin B mixture (Gibco), 5 mM HEPES, 0.5% glucose, 10 $\mu$g/ml insulin, 30 nM sodium selenite, 20 nM progesterone, 100 $\mu$M putrescine, and 20 $\mu$g/ml transferrin]. The cultures were incubated at 37℃ in an atmosphere of 5% CO2/95% room air, and the medium was replaced every other

day. Experiments were performed in 6-7-day-old culture.

Depending upon the experimental group, BC ($10^{-4}$ - $10^{-7}$ g/ml) was added (at 2% volume in culture medium) to or omitted from flasks. After 16~18 hr, cells were washed twice with warm phosphate-buffered saline (PBS) and serum-free medium added to the flask. Then the cells were treated with 1 mM Aβ25-35 for 2 hr and enzyme activities measured. Aβ25-35 was diluted in serum-free medium and added to the cultures. We describe it BC+Aβ25-35 group that astrocytes was treated with BC before Aβ25-35 treatment.

### *Treatment of astrocytes with Apeptide*

Confluent astrocytes were trypsinized and plated into T-75 tissue culture flasks at a density of 5 x $10^6$ cells/flasks (for antioxidative enzyme activity). After 24 h, cells were washed with PBS to remove serum, and cultures were incubated in DMEM free FBS for an additional 12 h before addition of Aβ peptides or control buffer.

### *Assay of catalase activity*

Catalase activity was measured as the decrease in hydrogen peroxide absorbance at 240 nm on an Gilford Response spectrophotometer using 30 mM hydrogen peroxide according to Aebi[25].

### *Assay of superoxide dismutase activity*

Superoxide dismutase (SOD) were assayed by recording the inhibition of ferricytochrome c reduction with xanthine and xanthine oxidase, EDTA being replaced by DETAPAC (1 mM)[26,27]. One unit of SOD activity was defined as the amount of enzyme needed to obtain 50% inhibition of cytochrome c reduction at pH 7.8 (25 ℃) in a 3.0 ml reaction volume on 560 nm using Gilford Response spectrophotometer.

### *Assay of glutathione peroxidase activity*

Glutathione peroxidase (GPx) activity was determined by the modified coupled assay developed by Paglia and Lawrence[28,29]. The reaction was started by addition of 2.2 mM hydrogen peroxide as substrate. The change in absorbance at 340 nm was measured for 1 min on Gilford Response spectrophotometer. The activity was expressed as μmol of NADPH oxidized/min-1 · mg protein.

### *Assay of glutathione-S-transferase activity*

*Glutathione-S-transferase* (GST) activity was assayed with CDNB as substrate and enzyme activity of GST towards the glutathione conjugation of CDNB[30]. Briefly, prepared TCA supernatant added to 1 ml of a solution containing 0.1 M phosphate buffer (pH 6.5), 1 mM GSH and 1 mM CDNB. The formation of the CDNB-conjugate was followed at 340 nm (25℃) with a Gilford Response spectrophotometer.

### *Protein determination*

Protein was determined on each sample by the method of Smith *et al*[31] (using bicinchoninic acid), using bovine serum albumine as the standard.

### *Nitrite determination*

Astrocyte cells (approximately 200 cells/well) were incubated (30 min, 37℃, under 95% air/5% $CO_2$) in KRB medium with or without ST (0 - 10 mM). Medium nitrite content was measured after conversion of nitrate to nitrite with *Aspergillus* nitrate reductase (Sigma)[32]. Nitrite was then measured spectrophotometrically (540 nm) after mixing medium (0.1 ml) with Griess reagent (0.1 ml of a solution of 1 part of 1.32% sulfanilamide in 60% acetic acid and 1 part of 0.1% naphthyl-ethylene diamine HCl) and incubation for 10 min (room temperature)[33]. Nitrite concentrations (n=5) were then interpreted from a $NaNO_3$ (Sigma) standard curve (1μM - 1 M).

### *Statistical analysis*

Data are expressed as mean±S.E. Intergroup comparisions of data were made by Duncan's multiple range test using SAS statistical analysis package (SAS Institute, Cary, NC). $P<0.05$ was considered as significant.

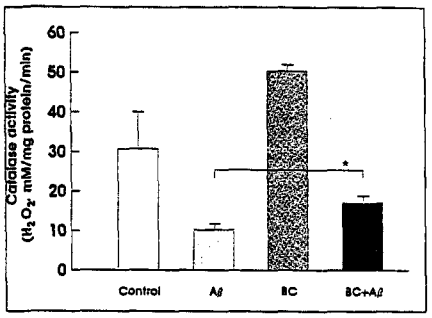


**Fig. 1.** Effect of Bombycis corpus extract on catalase activity of the Aβ25-35 treated in cultured astrocytes.
Culture cell were prepared and incubated with BC (2 x 10-6 g/ml at 2% volume in culture medium). After 16~18 hr, cells placed in DMED medium without serum, and then incubated with 1 mM A 25-35 for 2 hr.1) Mean±S.D. (N=3). *, P<0.05.

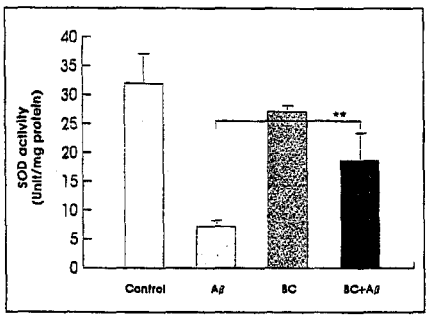


**Fig. 2.** Effect of Bombycis corpus extract on superoxide dismutase activity of the Aβ25-35 treated cultured astrocytes.
Culture cell were prepared and incubated with BC (2 x 10-6 g/ml at 2% volume in culture medium). After 16~18 hr, cells placed in DMED medium without serum, and then incubated with 1 mM A 25-35 for 2 hr. 1) Mean±S.D. (N=3). **, P<0.01.

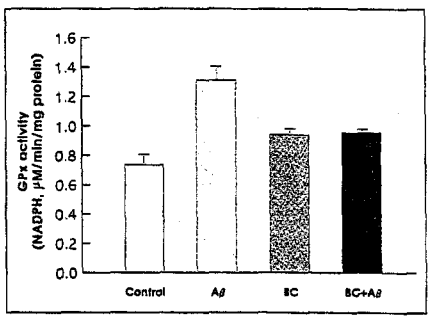


**Fig. 3.** Effect of Bombycis corpus extract on glutathione peroxidase activity of the Aβ25-35 treated cultured astrocytes.
Culture cell were prepared and incubated with BC (2 x 10-6 g/ml at 2% volume in culture medium). After 16~18 hr, cells placed in DMED medium without serum, and then incubated with 1 mM A 25-35 for 2 hr. 1) Mean±S.D. (N=3).

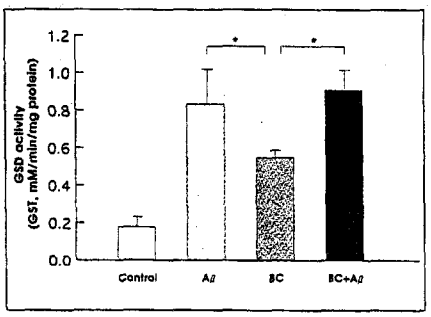


**Fig. 4** Effect of Bombycis corpus extract on glutathione-S-transferase activity of the Aβ25-35 treated cultured astrocytes.
Culture cell were prepared and incubated with BC (2 x 10-6 g/ml at 2% volume in culture medium). After 16~18 hr, cells placed in DMED medium without serum, and then incubated with 1 mM Aβ25-35 for 2 hr. 1) Mean±S.D. (N=3). *, P<0.05.

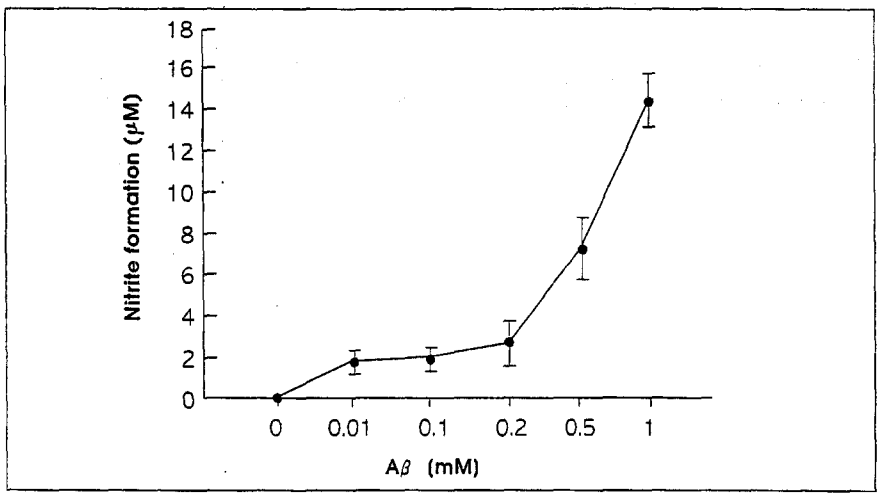


**Fig. 5** A 25-35 induced nitrite formation.
After nitrate was formed by Aβ25-35 treatment, Bombycis corpus extract was directly treated to the cells. Cells (200/condition) were incubated for 30 min at 37℃ under 95% air/5% $CO_2$ with or without Aβ25-35 and then medium nitrite content was measured as described in Materials and Methods.

## III. RESULTS

### 1. Effect of BC on catalase activities in Aβ 25-35 peptide-treated astrocytes

The effect of BC on catalase activity by Aβ25-35 treatment are shown in Fig. 1. The Aβ25-35 treated group resulted in a great decrease of catalase activity. The catalase activity by Aβ25-35 itself was significantly decreased by about 3.0-fold compared with normal group. In contrast, BC treated group markedly increased compared to those of untreated group. Whereas BC treated group showed significantly enhanced catalase activity above that of normal group (1.7-fold) and also BC+Aβ25-

**Table 1.** Inhibitory Effect of Bombycis corpus Extract on NO Production in Cultured Rat Cortical Astrocytes at 48 h after Treatment with Indicated Concentrations of A 25-35.

| Concentration (μM) | Nitrite formation | |
|---|---|---|
| | Aß25-35 | Aß25-35+BC |
| 0.1 | 2.23±0.14 | 1.76±0.21 |
| 0.5 | 6.67±0.76 | 4.87±0.40 |
| 1.0 | 10.23±2.40 | 6.04±1.00 |

For effects of Bombycis corpus extract treatment, cells were treated with A 25-35 for 2 h and further treated with 2 x 10-6 g/ml BC extract (2×10-6g/ml). NO production in the cultured rat cortical astrocytes were assayed at 48 h after treatment with BC.

35 group was slightly increased compare to Aβ 25-35 treated group, althought this increase was not significant.

### 2. Effect of BC on superoxide dismutase activities in Aβ 25-35 peptide-treated astrocytes

Fig. 2 shows that Aβ 25-35 treatment for 2 h decreased the SOD activity in the cultured cell. After 2 h of Aβ 25-35 treatment, SOD activity was significantly decreased about 4.0-fold of the normal group activity. However, these decrease was markedly recovered by BC treatment compared to Aβ 25-35 treatment group. Also, BC treatment with Aβ 25-35 had a significantly enhanced effect on SOD activity compared to only Aβ 25-35 treatment.

### 3. Effect of BC on glutathione peroxidase activities in Aβ 25-35 peptide-treated astrocytes

As shown in Fig. 3, GPx activity in Aβ 25-35 treatment group was 1.7-fold higher than those of normal group. However, BC treated group did not affected to the enzyme activity. The activity was significantly decreased in BC treatment with Aβ 25-35 group compared to that of Aβ 25-35 treated group, indicating inhibitory activities.

### 4. Effect of BC on glutathione-S-transferase activities in Aβ 25-35 peptide-treated astrocytes

The effect of GST activity by Aβ 25-35 treatment and BC treatment are documented in Fig. 4. The GST activity by Aβ 25-35 itself was highly increased by 4.0-fold as compared to those of normal group. But, only BC-treated group slightly enhanced GST activity less than Aβ 25-35 treatment group (2.5-fold) and BC+Aβ 25-35 group was not shown special changes compare to Aβ 25-35 treatment group. Hence, increased activity was also observed in BC+Aβ 25-35 group with a significant difference compare to BC treatment.

### 5. Inhibitory effects of BC on A β 25-35-induced NO formation

Incubation of isolated cells with A β 25-35 induced a concentration-dependent accumulation of nitrite (after enzymatic conversion of nitrate to nitrite) which significantly exceeded that of cells incubated with vehicle alone (Fig. 5). NO was formed during the incubation of cells with Aβ 25-35. To examine the possibility further, additional biochemical evidence of NO production in Aβ 25-35-treated islets was observed. As shown in Table 1, Aβ 25-35-induced nitrite formation was significantly inhibited by BC treatment in dose-dependent manner. When nitrate was formed by 0.5 mM Aβ 25-35 treatment, BC was directly treated to the cells. Concentrations of 0.1 and 1.0 mg/ml BC showed the suppressed nitrite production in asctrocytes (data not shown).

## IV. DISCUSSION

The present study was done to investigate the effects of BC on cultured astrocyte cell system, Aβ 25-35-induced cytotoxicity, antioxidative enzyme activities and NO synthesis in Aβ 25-35 treatment conditions.

Lipid peroxidation was prevented or greatly reduced by addition of antioxidants (Vit E, Vit C, DPPD or deferoxamine)[34]. For example, addition of antioxidants in cell culture medium significantly reduced cell killing and content of intracellular antioxidants. In case of GSH, when the cells were exposed to *t*-BHP promptly decreased, however, the preincubation of the cells with antioxidants (DPPD or deferoxamine) did not lead to change in the level of intracellular GSH or the accumulation

of GSSG in the medium after exposure to t-BHP[35]. Recently, it was reported that repeated oral administration of deer antler extract showed inhibitory effect on monoamine oxidase activity, one of the senescence-marker enzymes, reduced malondialdehyde (MDA) level and increased SOD activity in the liver and brain tissues of aged mice[36,37]. Therfore, it is suggested that changes in MDA level are related to the alterations of antioxidative enzyme activity and these biochemical changes were recovered to normal levels by the addition of antioxidants[38].

In the present study, we have examined several enzymes to see the effects of Aβ 25-35 treatment and treatment of BC; other had previously either examined only a enzyme or did not uses antioxidants. In our study, paticular importance was placed on catalase, SOD, GPx and GST for well-known as an antioxidative enzyme. Our results showed a decrease in the activity of catalase in cultured cell by Aβ 25-35 treatment. However, catalase activity by BC treatment was markedly increased compare to other groups. SOD activity by Aβ 25-35 treatment was significantly decreased about 4 fold of the normal group and decreased activity was significantly reduced by BC treatment. BC treatment had significant effect on SOD activity compare with normal group.

And our results showed elevation in the activity of GPx in Aβ 25-35 treated cultured cell. It has been shown that exposed to oxidant drug or materials resulted in the activation or inactivation of GPx and these changes of activation was recovered by addition of antioxidant. GST activity by Aβ 25-35 treatment was markedly increased compare to Aβ 25-35 untreatment groups. And, BC treatment slightly enhanced GST activity above that of normal group less than Aβ 25-35 treated group.

At the present assay, antioxidative enzyme activities by Aβ 25-35 treatment were increased in a similar fashion, except for catalase and SOD. These results possibly due to adaptive phenomenon by the direct toxic effect of Aβ 25-35 or its metabolites may cause these effects. Furthermore, enhanced activities by BC treatment may be resulted from the potentiation of antioxidative ability.

Besides its function as a physiological mediator, evidence is accumulating that NO participates in inflammatory- and autoimmune-mediated tissue destruction. NO oxidizes to nitrite (NO2-) and nitrate (NO3-) in aqueous solution, and NO generation in biological systems is reflected by measurement of nitrite production39 after enzymatic conversion of nitrate to nitrite40. The effects of oriental medicines on the activity and expression of cNOS is important. The effects of crude drugs and their constituent ingredients on signal transduction and gene expression are now important target in the investigation of the pharmacological effects of crude drugs. Interestingly, BC has strongly inhibited the NO production from cultured astrocyte cells, which was induced by Aβ 25-35, indicating anti-inflammatory property of BC. Thus, inhibiting effect on NO production was suggested that the action of BC is associated with its anti-inflammatory or immunostimulatory effects. This effect may contribute to the pharmacological effect of oriental medicine on homeostasis and defense mechanisms in human body.

From the our study, it was concluded that Aβ 25-35 is not only potent lipid peroxide inducer, but also BC cause protection of neurodegeneration induced by Aβ 25-35. It can be concluded that the activation of antioxidative enzymes may be related to the inhibition of lipid peroxidative reactions. We cannot fully explain to effects of BC at present; however, the ability of BC to reduce cell killing and MDA level induced by Aβ 25-35 suggest that BC may be a protective agent for free radical generating compounds such as Aβ 25-35.

## V. CONCLUSION

*Bombycis corpus* (BC) is a drug consisting of the dried larva of silkworm, *Mobyz mori L.*, dead and stiffened due to the infection of *Beauveria (Bals.) Vuill.(family*

*Moniliaceae).* In oriental medical, it is used to subdue the endogenous wind and convulsions for the treatment of headache, vertigo, tic and skin prurigo; to resolve nodulation for the treatment of scrofula, tonsillitis parotitis and purpura.

We studied the protective effect of the BC extracts on amyloid-β(Aβ)25-35 peptide-induced oxidation and antioxidative enzymes activities on cultured neuronal cell. When antioxidative enzyme activities such as catalase, superoxide dismutase, glutathione peroxidase and glutathione-S-transferase were assayed after Aβ25-35 treatment, the activities of enzymes were decreased in a similar fashion only in catalase and superoxide dismutase. However, those activities were enhanced by BC treatment and this may be resulted from the potentiation of antioxidative ability of BC. Reduction activity of BC on cellular cytotoxicity induced by Aβ25-35 strongly indicated that BC could be a protective agent for free radical generating compounds, and that Aβ25-35 is not only potent lipid peroxide inducer, but also cause change in antioxidative enzymes.

On the other hand, the inhibitory effect on NO production from cultured astrocyte cells, which was treated with Aβ25-35, was demonstrated in crude drugs of BC. Modulatory effect on NO production have suggested to be associated with its anti-inflammatory or immunostimulatory effects.

From the results, it was concluded that BC has a protective effect of Aβ-induced cell death in cultured astrocyte cells through the inhibition of lipid peroxidation and potentiation of antioxidative enzymes.

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