Inhibitory Effect of Citrate on Alzheimer's β-Amyloid Fibrils *in vitro*

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DEDICATION

I dedicate this dissertation to my family, especially to my parents for instilling the inspiration to set high goals and the confidence to achieve them. This is also dedicated to my loving wife, my two wonderful children Jung Woo and Hyeong Taek. My wife, Il Yean, has been proud and supportive of my work and who has shared the many uncertainties, challenges and sacrifices for completing this dissertation. I wish to thank my committee members who were more than generous with their expertise and precious time. A special thanks to Professor Sung Soo Lee, my thesis supervisor for his countless hours of reflecting, reading, encouraging, and most of all patience throughout the entire process. I would like to acknowledge and thank Inam Neuroscience Research Center and Professor Il Hong Son, the director of research center for allowing me to conduct my research and providing any assistance requested. Special thank goes to Yong Hoon Park, Ph.D. for stimulating discussions and invaluable help. Finally, I would like to thank my colleagues for cooperation in the experiments. Their excitement and willingness to provide feedback made the completion of this research an enjoyable experience.

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ABSTRACT

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Formation of β-amyloid (Aβ) fibrils has been identified as one of the major characteristics of Alzheimer's disease (AD). Inhibition of Aβ fibril formation in the CNS would be attractive therapeutic targets for the treatment of AD. Several small compounds that inhibit amyloid formation or amyloid neurotoxicity *in vitro* have been known. Citrate has surfactant function effect because of its molecular structure having high anionic charge density, in addition to the well-known antibacterial and antioxidant properties. Therefore, we hypothesized that citrate might have the inhibitory effect against Aβ fibril formation *in vitro* and have the protective effect against Aβ-induced neurotoxicity in PC12 cells. We examined the effect of citrate against the formation of Aβ fibrils by measuring the intensity of fluorescence in thioflavin-T(Th-T) assay of between $A\beta_{25-35}$ groups treated with citrate and the control with $A\beta_{25-35}$ alone. The neuroprotective effect of citrate against

Aβ-induced toxicity in PC12 cells was investigated using cell morphology and WST-1 assay. Fluorescence spectroscopy showed that citrate inhibited the formation of Aβ fibrils from β-amyloid peptides. The inhibition percentages of Aβ fibril formation by citrate (1, 2.5, and 5 mM) were 31 %, 60 %, and 68 % at 7days, respectively in thioflavin-T(Th-T) assay. Evaluation of cell morphology showed that PC12 cells were rescued and remained relatively normal in appearance by citrate. WST-1 assay revealed that the toxic effect of $A\beta_{25-35}$ was reduced, in a dose-dependent manner to citrate. The percentages of neuroprotection by citrate (1, 2.5, and 5 mM) against Aβ-induced toxicity were 19 %, 31 %, and 34 %, respectively. We report that citrate inhibits the formation of Aβ fibrils *in vitro* and has neuroprotective effect against Aβ-induced toxicity in PC12 cells. Neuroprotective effects of citrate against Aβ might be, to some extent, attributable to its inhibition of Aβ fibril formation. Although the mechanism of anti-amyloidogenic activity is not clear, the possible mechanism is that citrate might have two effects, salting-in and surfactant effects. These results suggest that citrate could be of potential therapeutic value in Alzheimer's disease.

Key words : Citrate, Alzheimer's disease, β-amyloid, Fibril

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I. INTRODUCTION

Alzheimer's disease (AD) is the most common form of amyloidogenic diseases which are characterized by conformational changes that are followed by aggregation of proteins inside or outside cells. Amyloid-associated disorders include other human diseases such as Parkinson's disease, Huntington's disease, prion diseases, familial amyloidosis, and type II diabetes.[1] Over the years, evidence from biochemical, genetic, and pathologic investigations support the idea that amyloidogenesis is a pivotal and early event in AD pathogenesis.[2] The major neuropathological changes in the brains of AD patients include the accumulation of ß-amyloid (Aß) peptides in senile plaques, and the presence of hyperphosphorylated tau in neurofibrillary

tangles. Aß is generated by the sequential proteolytic processings of the type I membrane protein, termed amyloid precursor protein (APP), by ß- and γ-secretases.[3,4] Aβ promotes pro-inflammatory responses and activates neurotoxic pathways, leading to dysfunction and death of brain cells.[5] Studies have demonstrated that when aggregated in fibrillar form, Aβ has neurotoxic effects in cell culture and *in vivo*.[6-10] Thus, finding molecules to inhibit the formation of Aβ fibrils could be of therapeutic value in AD.

The delineation of the molecular biology of AD has yielded a number of therapeutic targets whose successful manipulation may result in disease modification.[11-13] Three general classes of therapies are anticipated: 1) antiamyloid agents, which affect primarily the production, accumulation, removal, or blockade of toxicity associated with the Aß; 2) neuroprotective agents that reduce the injury associated with abnormal amyloid protein processing, including antioxidants, anti-inflammatory agents, tau-related therapies, myelin preservation agents, and nerve growth factors and promoters; and 3) neurorestorative approaches, including neurotrophic and nerve growth factor strategies, transplantations, and stem-cell related interventions.[14,15] Other therapeutic strategies such as cholinesterase inhibitors, muscarinic, glutamatergic or serotonergic agonists, anti-inflammatory drugs, estrogen-replacement therapy and anti-oxidant drugs are directed towards palliating existing cognitive symptoms or retarding the disease course. The

advantage of an amyloid-related therapy for AD is that it is targeted at correcting or preventing the pathology believed to cause the disease; therefore, it might change the course of the disease or even prevent its onset. Preventing amyloid deposition will also allow evaluation of the actual role of amyloid in AD.

Small molecules have been shown to prevent Aß fibrillogenesis or inhibit Aß toxicity *in vitro*. Sulfonated dyes, such as Congo red, small sulfonated anions and benzofuran-based compounds have also been described as inhibitors of amyloid formation or neurotoxicity associated with amyloid fibrils.[10,16,17] In a similar way, melatonin, nicotine, estrogen and anthracycline 49-iodo-49-deoxydoxorubicin were identified as inhibitors of Aß aggregation or Aß neurotoxicity.[18-21] The usefulness of these small molecules as amyloid inhibitors is compromised by their lack of specificity and their unknown mechanism of action, which makes it difficult to improve them. In addition, many of these compounds are highly toxic.

ß-Sheet breaker peptides (iAß) were demonstrated to inhibit Aß fibrillogenesis, disassemble pre-formed Aß fibrils *in vitro* and to prevent neuronal death induced by Aß fibrils in cell culture.[22] In addition, $iAß_5$ (LPFFD) significantly reduced Aß deposition *in vivo* and completely blocked the formation of Aß fibril in a rat brain model of amyloidosis.[23].

A large number of studies indicate that oxidative injury may play a role in

the development of AD. Many antioxidant compounds, such as vitamin E, NDGA, nicotine, vitamin A, and coenzyme Q10 have been shown to protect the brain from Aß toxicity.[24-28]

In addition, several surfactants have been found to delay or prevent fibril formation, possibly by binding to hydrophobic region that is necessary for self-assembly.[29-32] The cationic surfactant hexadecyl-N-methylpiperidinium bromide was found in this way to be a specific inhibitor of Aß aggregation.[33] Among the various groups of biomaterials having surfactant molecular structure, citrate is particularly interesting component having the high anionic charge density which may give Aβ more hydrophilicity.

In this study, we described an *in vitro* assay to search for an inhibitory effect of citrate on Aβ fibril formation by using fluorescence spectrophotometer (Thioflavin-T assay). We used the $A\beta_{25-35}$ amino-acid peptide that preserves the properties of neurotoxicity and aggregation of the full-length sequence. Moreover, the $A\beta_{25-35}$ fragment has the experimental advantage of rapidly aggregating upon solubilisation in aqueous buffers.[34] We also investigated the protective effect on PC12 cells from Aβ-induced toxicity by evaluating cell morphology and measuring WST-1 activity which is an indicator of the activity of living cells.

II. MATERIALS AND METHODS

2.1. Synthetic peptides and citrate

Peptides $A\beta_{25–35}$ and citrate were purchased from Sigma (Saint Louis, MO, USA) and were used without further purification. The sequence of $A\beta_{25–35}$ peptide is GSNKGAIIGLM, corresponding to residues of the human wild type sequence $A\beta_{1–40}$.

2.2. Preparation of solutions

Stock solution of 1 mM was prepared by solubilizing the lyophilized $A\beta_{25–35}$ peptide by briefly vortexing in sterile water at 4°C, then by sonication for 1 min. The sterilized peptide stock solution was aliquoted and stored at -20°C. The citrate stock solution was neutralized to get pH 7.2 and filtered with 0.2 micromiter syringe filter. The citrate stock solution was aliquoted and stored at -20°C.

2.3. Thioflavin T fluorescence (ThT) assay of $A\beta_{25–35}$ fibrillization

Experiments were carried out by using the reaction mixtures containing 80 μL phosphate buffer (10 mM final concentration) with 10 μL citrate (1, 2.5, and 5 mM final concentration) and 10 μL $A\beta_{25–35}$ (100 μM final concentration), pH 7.2. Moreover, one sample containing $A\beta_{25–35}$ alone and

another containing several concentrations of citrate alone were used in parallel as control in the same experimental conditions. All reactions were carried out at 37°C. for 7 days. The fluorescence intensities of Th-T dye of fibril formation in several experiments were measure by PerkinElmer LS 55 Fluorescence Spectrometer (Waltham, Massachusetts, USA) as described elsewhere[35]. 100 μL of Th-T dye solution (1 mM in phosphate buffered saline, pH 7.4, Sigma, Saint Louis, MO, USA) was added to 20 μL of the $A\beta_{25-35}$ reaction solutions. Then the fluorescence intensities of the reaction mixture were measured at the excitation and emission wavelengths of 450 and 485 nm, respectively. For clear estimation of inhibitory effect of citrate on $A\beta_{25-35}$ fibrillization, the $A\beta_{25-35}$ absorption spectra of different concentrations of citrate were subtracted with the fluorescence spectra of citrate alone, respectively. At least three independent measurements were made for all cases. All results are presented with means and standard deviation.

2.4. PC12 cell cultures and Aβ-induced toxicity

Rat PC12 cells were sustained in Dulbecco's modified Eagle's medium (Gibco, NY, USA) with 4.5 g/L D-glucose, 2 mM L-Glutamine and 110 mg/L sodium pyruvate supplemented with 10% fetal bovine serum (FBS) and antibiotic-antimycotic (10,000 u/mL penicillin G sodium, 10,000 μg/mL streptomycin sulfate and 25 μg/mL amphotericin B as Fungizone® in 0.85 %

saline) (Gibco, NY, USA) in a humidified 5% CO_2/95% O_2 atmosphere at 37°C. The culture medium was changed every two days. For cell morphology and WST-1 assay, the cells were plated at a density of 3 x 10^4 cells/well in 48-well plates and incubated for 24 h. At the end of incubation period, cells were treated with $A\beta_{25–35}$ (50 μM final concentration) preincubated in the absence or presence of citrate at 37°C for 7 days. Controls were not treated with both citrate and $A\beta_{25–35}$.

2.5. Cell morphology

Photomicrographs of selected regions of the culture were taken with a Spot digital camera (Diagnostic Instruments, Sterling Heights, MI, USA) on a Nikon Diaphot 300 inverted microscope with a 200X objective (Nikon USA).

2.6. Measurement of cell viability

Cell viability was evaluated 24 h later using the 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1 reagent from Roche Diagnostics, Mannheim, Germany) assay, as described previously.[35] The measurement is based on the ability of viable cells to cleave tetrazolium salts by mitochondrial dehydrogenases. The optical density (OD) was determined at 450 nm using a microplate reader (Multiskan EX Thermo-Fisher scientific Waltham, MA, USA).

2.7. Statistical analyses

The results shown represent the mean ± standard deviation from three experiments (n = 3). Mann-Whitney's U-test was used to compare $A\beta_{25-35}$ alone with citrate-treated $A\beta_{25-35}$ groups or control (cell). *P*-values < 0.05 were considered statistically significant.

III. RESULTS

3.1. Fluorometric studies of effects of citrate on the formation of $A\beta_{25-35}$ fibril

The concentration 100 μM was chosen as working concentration of $A\beta_{25-35}$ as described previously.[36] At 7 days, the presence of citrate led to a significant decrease in the Aβ fibril formation as compared to $A\beta_{25-35}$ peptide alone (Fig. 1). The Th-T fluorescent assay, a valuable method for a real-time evaluation of formation of Aβ aggregation in vitro, was performed to verify the inhibition of Aβ fibril formation by citrate and revealed that citrate remarkably suppressed Aβ aggregation. Figure 1 shows that following 7-day incubation, 1, 2.5, and 5 mM citrate attenuates significantly $A\beta_{25-35}$ fibril formation. The percentages of Aβ fibril formation in the presence of citrate (1, 2.5, and 5 mM) were 69 %, 40 %, and 32 % of $A\beta_{25-35}$ alone, respectively ($P<0.05$).

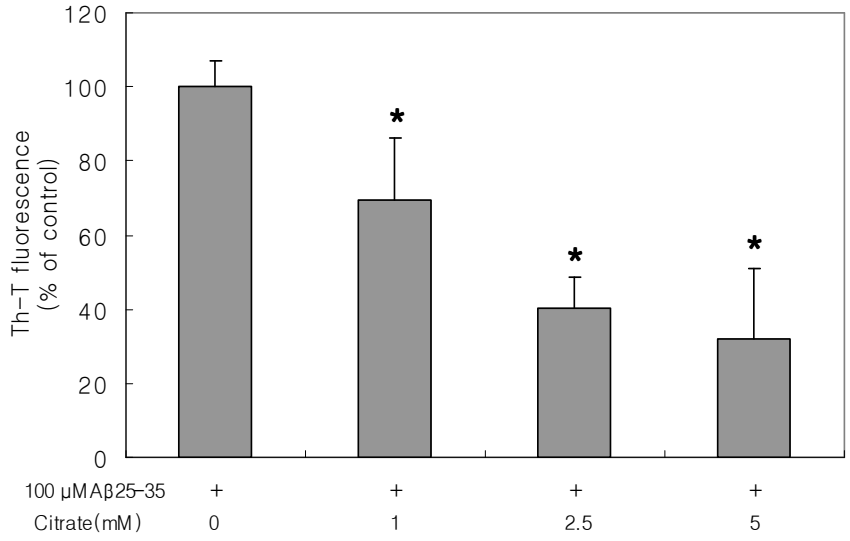

Fig. 1. The effect of citrate on fibril formation of $A\beta_{25-35}$ by Th-T fluorometric assay.

$A\beta_{25-35}$ at 100 μM was incubated with (1, 2.5, and 5 mM final concentration) citrate at 37°C for 7 days. The Th-T was mixed with each sample and fluorescence intensity was measured using a spectrofluorometer. The data are summarized as the mean ±S.E.M. of three experiments. Asterisk (*) indicates a significant ($P<0.05$) difference between Aβ alone and citrate-treated Aβ group.

3.2. Changes in cell morphology after $A\beta_{25–35}$ and citrate application

Cells without any treatment (control) are shown in Fig. 2A. Most of cells were well attached to the substrate and developed neurites. Cell morphology changed dramatically after exposure to 50 μM $A\beta_{25–35,}$ characterized by swelling, degeneration of neuritis, and detachment from the substrate (Fig. 2B). In contrast to Fig. 2B, destruction of cells has been avoided by the co-treatment with 5 mM citrate (Fig. 2C).

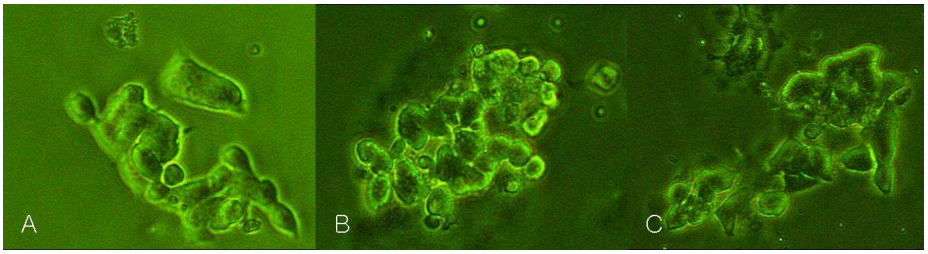

Fig. 2. Changes in PC12 cell morphology.

(A) : Control PC12 cells. Cells are well attached to the substrate and have neurites. (B) : PC12 cells were treated with 50 μM $A\beta_{25-35}$. Cells, which were detached from the substrate, are swollen, and have lost their neurites. (C) : PC12 cells with both 50 μM $A\beta_{25-35}$ and 5 mM citrate. Cells are relatively normal in appearance.

3.3. Protective effect of citrate in $A\beta_{25-35}$-treated cells: WST-1 assay

The $A\beta_{25-35}$-induced cytotoxicity was determined by the percentage of WST-1 reduction assay after culture for up to 24 h with increasing concentrations (1.0, 2.5 and 5.0 mM) of citrate (Fig. 3). 50 μM $A\beta_{25-35}$ alone decreased about 20 % cell viability in WST-1 values compared with the control (cell). The cytotoxic effects were attenuated by the co-treatment with citrate dose dependently ($P<0.05$). The percentages of neuroprotection by citrate (1, 2.5, and 5 mM) were 19 %, 31 %, and 34 %, respectively.

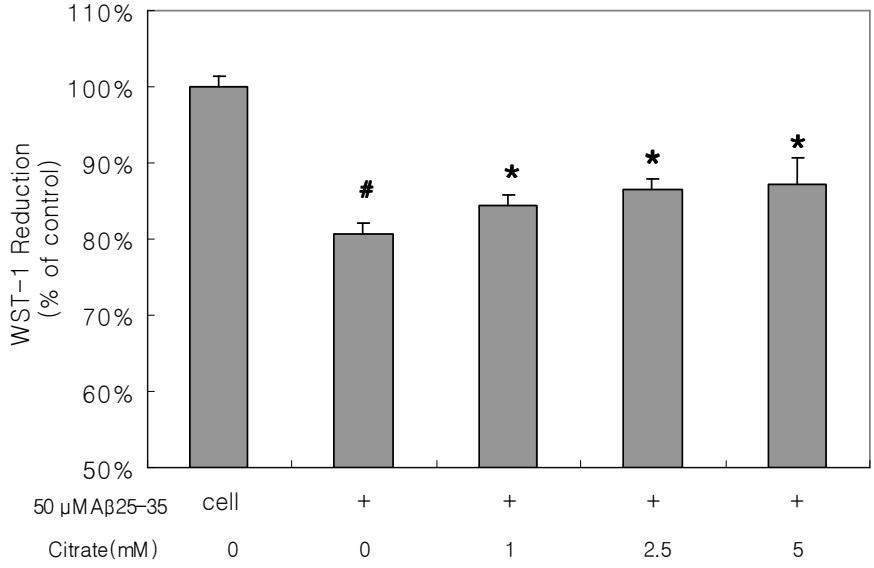

Fig. 3. The effect of citrate on the viability of PC12 cell line.

The cells were incubated for 24 h with 50 μM $A\beta_{25-35}$ preincubated in the absence or presence of citrate at 37°C for 7 days. The data are summarized as the mean ±S.E.M. of three experiments. Asterisk (*) indicates a significant ($P<0.05$) difference between Aβ alone and citrate-treated Aβ group and $^{\#}$ $P<0.05$ compared Aβ alone with control (cell).

IV. DISCUSSION

The formation of insoluble Aβ deposits in the brain is a central event in the pathogenesis and a pathological hallmark of AD. Aβ has been shown to be toxic to neuronal cells in culture after an aging process of several days, which corresponds to the kinetics of *in vitro* amyloid fibril formation.[9] A number of therapeutic approaches to reducing the load of amyloid plaque seem possible, focusing on βAPP production, βAPP processing, fibril formation by Aβ, or removal of Aβ from critical areas in the brain. A fruitful approach in the study of the relationship between the structure and fibrillogenicity of Aß has been to alter the sequence of the peptide and to analyze the effect on amyloid formation. Replacing hydrophobic for hydrophilic residues in the internal Aß hydrophobic region impairs fibril formation, suggesting that Aß assembly is driven partially by hydrophobic interactions.[37] On the other hand, several reports indicate that the conformation adopted by Aß peptides is crucial in amyloid formation. Barrow and Zagorski demonstrated that Aß incubated at different pHs or in distinct solvents shows either mainly random coil/α-helical or β-sheet secondary structure. Under the conditions in which the peptide adopts the random coil/α-helical structure, it aggregates slowly, but when Aß adopts the β-sheet conformation, it aggregates rapidly.[38-40] Therefore, it seems likely that a key event in amyloidogenesis is the conversion of the normal

soluble Aß conformer into the β-sheet-rich amyloidogenic intermediate. The emerging picture from studies with synthetic peptides is that Aß amyloid formation is dependent on hydrophobic interactions among altered Aß peptides that adopt an antiparallel ß-sheet conformation.

Many of studies for inhibition of Aß fibril formation have been done. Based on the fact that the major force driving Aß aggregation is hydrophobicity, Murphy and colleagues have added charged residues to the ends of the recognition motif as a disrupting element.[41] Having shown that at least three lysines are required as an appropriate disrupting element, the compound (KLVFFKKKK) showed activity in altering fibril morphology and reducing cellular toxicity in vitro suggesting that the charged nature of the disrupting element is critical.[42] Another approach to produce peptide inhibitors have been reported by Findeis et al.[43,44] Their strategy was to retain a peptide sequence that could bind to Aß and add a bulky group, such as a steroid, at its terminus to hinder Aß polymerization. The all-D-amino acid peptide cholyl-LVFFA-OH was shown to be a potent inhibitor of Aß polymerisation, but was cleared up almost completely upon hepatic first pass, possibly because the cholyl group was recognised as an endogenous bile component. Several other molecules with different chemical groups for improved pharmacological properties of inhibition of Aß fibril formation are under development.

A few reports have been focused on the interactions between Aβ and

surfactant molecules. Three micelle systems, the negatively charged utilized sodium dodecyl sulfate, the neutral zwitterionic dodecylphosphochline, and the positively charged dodecyltrimethylammonium chloride, in heterogeneous amphiphilic environment have been utilized to interact with Aβ peptide fragments.[31] Their results showed that the promotion and the stabilization of α-helix secondary structure were highly correlated with the availability of the surfactant's charged surface, indicating the significant role of electrostatic forces in the interaction between Aβ and surfactants. Citrate is the low molecular weight organic acid carrying three valent anionic carboxyl groups (Fig. 4). Therefore, in aqueous phase, citrate shows the high charge characteristics. It has been used as non-soap, oganic anionic surfactant. Furthermore, this is widely available and economical, and generally recognized as safe material.[45,46] Moreover, citrate, as the first Krebs cycle intermediate, has antioxidant properties due to its ability to chelate iron by forming inactive complexes at physiological range of concentrations.[47]

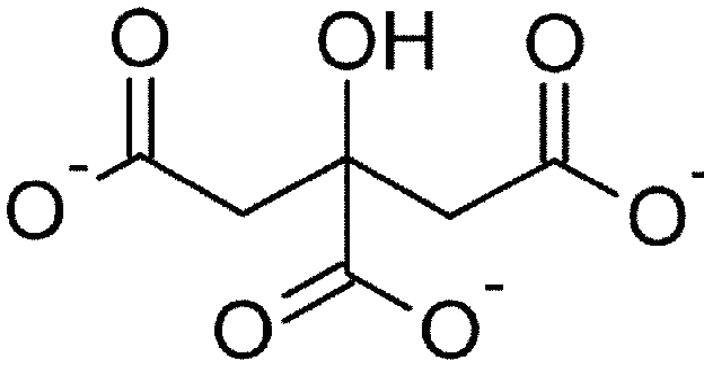

Fig. 4. Molecular structure of three-valent citrate anion in physiological buffer solution (pH 7.4).

Considering plasma citrate concentration ranges from 70 to 700 μM,[48] our results demonstrated that citrate could have the anti-amyloidogenic and neuroprotective effects at supra-physiological range of concentrations while antioxidant effect of citrate was at physiological concentrations. This finding supports that the future therapeutic use of citrate might be considered.

The mechanisms that underlie the anti-amyloidogenic effect of citrate are still unclear, but as for the possible inhibitory mechanisms of citrate, we speculate that citrate might have two effects, salting-in and surfactant effects. The surfactant effect of citrate could provide a possible explanation for inhibition of fibril formation, based on the fact that the major force driving Aß aggregation is hydrophobicity. Putative binding of citrate through hydrophobic backbone to a hydrophobic region of Aß seems to block associations between Aβ molecules by encapsulating hydrophobic portions of Aβ and forming micelle, thereby increasing solubility and inhibiting fibril formation of Aß. Wang et al. have indicated the anti-amyloidogenic activity of the amphiphilic surfactants, 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (3-di-C6-PC) and 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine (di-C7-PC), in physiological condition.[32] Our experiments showed that citrate inhibited Aβ fibril formation at physiological pH. Inhibitory action of citrate on fibrillization of $A\beta_{25-35}$ in citrate-treatment conditions, was significant, being compared with $A\beta_{25-35}$ alone (Fig. 1). Further verification of the exact

mechanism of Aβ-surfactant interaction needs to be investigated. Another possible mechanism is salting-in (solubilizing) effect of citrate. The salt ions change the chemical potential of the macromolecule. According to the Hofmeister series, there are two species of anions, kosmotropes and chaotropes. The kosmotropes are strongly hydrated and have stabilizing and salting-out effects on proteins and macromolecules. On the other hand, chaotropes are known to destabilize folded proteins and give rise to salting-in or solubilizing behavior.[49] The effect of the anions could be explained on the basis of three direct interactions of the anions with the macromolecules. First, kosmotropic anions were found to polarize water molecules that were directly hydrogen bonded with the amide moieties of macromolecule. Second, both chaotropes and kosmotropes could increase the cost of hydrophobic hydration (i.e. raise the surface tension of the polymer/water interface). Third, chaotropic anions could bind directly to the side-chain amide moieties. The first and second of these interactions led to the salting-out (aggregation) of the polymer, whereas the third effect causes the polymer to salt-in.[50] It is possible that citrate, as a chaotropic anion, have exerted the solubilizing effect through the third effect.While citrate would be an important molecule for therapeutic strategy, the mechanism of anti-amyloidogenic activity is unknown so a molecular approach to these interactions is required. We suggest surfactant and salting-in effects as the possible mechanisms that underlie the anti-

amyloidogenic effect of citrate. The neuroprotective effect of citrate against Aβ-induced toxicity *in vivo* needs to be investigated and the ability of Aβ fibril formation inhibitors to suppress the progression of Alzheimer's disease in humans needs to be evaluated.

Our findings have demonstrated that citrate inhibited Aβ fibril formation and reduced Aβ-induced toxicity. The concept of Aβ fibril formation inhibitors provides a potentially general strategy to treat amylodogenic diseases. A decrease in the occurrence of AD is a major treatment goal for patients, families, and society. We hope that citrate, as one of the inhibitors of Aβ fibril formation with antioxidant and neuroprotective effects, will prove to be useful therapeutic agents to prevent or retard amyloidogenesis in AD.

V. CONCLUSION

Citrate inhibits the formation of Aβ fibrils *in vitro* and has neuroprotective effect against Aβ-induced toxicity in PC12 cells.

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ABSTRACT IN KOREAN

시험관내 환경에서 알츠하이머병 베타아밀로이드 원섬유의 응집에 대한 구연산의 억제효과

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양 현 덕

베타아밀로이드 원섬유 형성은 알츠하이머병의 주된 특성 중에 하나로 확인되고 있다. 베타아밀로이드 원섬유 형성 억제는 이 질병 치료에서 주된 치료목표로 인식되고 있다. 시험관내 환경에서 아밀로이드의 형성이나 신경독성을 억제할 수 있는 몇 가지 작은 크기의 분자들이 알려져 있다. 구연산은 잘 알려진 항산화와 항세균 특성 이외에 고음이온 전하밀도를 가지는 분자구조의 특성 때문에 계면제의 기능도 가지고 있다. 따라서, 구연산은 베타아밀로이드 원섬유의 형성을 억제하고 PC12 세포에서 베타아밀로이드 유발 신경독성에 대한 보호효과를 나타낼 수 있음을 가정하였다. $A\beta_{25-35}$ 와 $A\beta_{25-35}$ 에 구연산을 처리한 그룹간에서 구연산에 의한 베타아밀로이드 원섬유 형성에 미치는 효과를 조사하기 위해서 thioflavin-T (Th-T) 분석과정에서 형광을 측정하였다. 또한 PC12 세포에서 베타아밀로이드 유발 독성에 대한 구연산의 신경보호 효과를

알아보기 위해 세포생존율 분석법인 WST-1 분석법을 실시하였다. 결과로서는, 구연산은 베타아밀로이드 원섬유 형성을 억제하였다. 반응 시작 7일 경과 후 thioflavin-T 분석시, 구연산(1, 2.5, and 5 mM)에 의해 베타아밀로이드 원섬유 형성 억제 백분율은 각각 31, 60 그리고 68 %를 나타냈다. 세포형태분석에서 구연산은 세포 파괴를 방지하는 효과를 보였으며 세포생존율 분석법인 WST-1 에서 구연산에 농도 의존적으로 $A\beta_{25-35}$의 독성이 감소하였다. 신경보호효과에 대한 백분율은 19, 31 그리고 34%를 각각 나타내었다. 결론적으로, 구연산은 시험관내 환경에서 베타아밀로이드 원섬유 형성을 억제하고 PC12 세포에서 베타아밀로이드 유발 독성을 억제하는 효과를 보였다. 구연산이 베타아밀로이드에 대항해서 신경보호효과를 보일 수 있는 것은 베타아밀로이드 원섬유 형성의 억제로 생각할 수 있다. 비록 항아밀로이드 특성 기전에 대해서는 명확하지는 않지만, 구연산의 가능성 있는 기전으로는 염용효과와 계면제효과 때문일 것으로 추정된다. 이와 같은 결과를 바탕으로 구연산은 알츠하이머병의 치료 효과를 기대할 수 있으며 이를 동물 실험으로 입증할 필요가 있다.

핵심 되는 말 : 구연산, 알츠하이머병, 베타아밀로이드, 원섬유