α-PSP May Prevent Neurovulnerability in Alzheimer Model in Vitro (Preliminary, Unpublished Data)

Col. Sayan Sawatsri, M.D.
Clinical Associate Professor of Gynecology and Obstetrics, Emory University School of Medicine, Atlanta, GA, USA
Director, Div. of Family Planning, OB-Gyn Dept. Royal Thai Army Hospital and College of Medicine

Advisors:
Col. Ass. Prof. Ph.D. Chainarong Cherdchoo
Royal Thai Army College of Medicine

Col. Warayut Satisatean, M.D.
Royal Thai Army Hospital
Abstract (Preliminary data)

**Objective:** The current study investigated the neurotrophic and neuroprotective action of a unique formulation of $\alpha$-PSP which consists of carbohydrate, crude protein, and essential minerals by using pressure and mechanical hydrolysis to make a complex formulation designated alpha polysaccharidepeptides ($\alpha$-PSP).

**Methods:** Using neuronal cell lines prepared from the LA-N-5 (Los Angeles Neuroblastoma) for Alzheimer’s disease (AD) in complete media and treated with indicated manner, inverted microscopic evaluated morphological and biochemical analysis were conducted to determine the neurotrophic and neuroprotective properties of $\alpha$-PSP.

**Results:** Results of these analysis demonstrated that $\alpha$-PSP significantly decreased neuronal cell death, a cellular marker of memory formation. Dose response analysis (experiment going on) indicated that the lowest effective concentration of $\alpha$-PSP exerted the minimal neurotrophic effect. Results of neuroprotection studies demonstrated that $\alpha$-PSP induced highly significant neuroprotection against beta-amyloid, hydrogen peroxide, and glutamate-induced toxicity.

**Discussion:** Abnormality of glucose/energy metabolism shows relation to AD (1, 2, 3, 4). $\alpha$-PSP may prevent impairment of glucose/energy metabolism and may improve the ability of neurons to reduce the levels of (“scavenger”) free radicals and thereby affecting ATP levels (11, 12).

**Conclusion:** $\alpha$-PSP induced cellular markers of memory function in neurons critical to memory and vulnerable to negative effects of aging, cellular degeneration and Alzheimer’s disease. Results of the current study could demonstrate the cellular mechanism of $\alpha$-PSP on cognitive function and a possible intervention in Alzheimer’s disease.

**Key Words:** Alpha Polysaccharidepeptides ($\alpha$-PSP), Alzheimer’s disease (AD), Cell Death, Neuroprotective.
Introduction

1.1 Alzheimer's disease: A scientific mystery and major impact. Abnormality of glucose/energy metabolism shows relation to Alzheimer’s disease (1, 2, 3, 4). Degenerative and cell death are major causes in AD.

1.2 α-PSP is a complex formulation that consists of carbohydrate, crude protein and essential minerals by using pressure and mechanical hydrolysis to make a complex formulation designated alpha polysaccharidepeptides (α-PSP) (5).

1.3 α-PSP is very safe because it contains phytochemicals that have components of carbohydrates, crude protein and essential minerals by using pressure and mechanical hydrolysis to make a complex formulation designated α-PSP. Evidence by observation from animal (pig) data showed that α-PSP can decrease morbidity from Ataxia (α-PSP may improve cerebral blood flow). In clinical use we found that α-PSP improves short and long term memory (6).

1.4 LA-N-5 (Neuroblastoma cell lines) have been used for model of
1. To determine if $\alpha$-PSP may promote neurotrophic and neuroprotective actions that show decreased cell death (Apoptosis) in the Alzheimer model \textit{in vitro}.

2. To determine if $\alpha$-PSP shows neurotrophic and neuroprotective action in AD model and to determine what the mechanism of $\alpha$-PSP is.

For proposed mechanism of $\alpha$-PSP induced neuroprotection in AD model \textit{in vitro}.
Materials and Methods (1)

1. Neuronal culture
   Neuronal cell lines were prepared from the LA-N-5; Los Angeles Neuroblastoma derived from bone marrow metastasis of 4-month-old male patient. Cells were propagated in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 50 IU/ml penicillin, 50 µg/ml streptomycin, and 1 µg/ml fungizone (complete medium).

2. Morphological analysis
   By using inverted microscopic evaluated morphological analysis of LA-N-5 in indicated conditions.

3. Neuronal survival
   Neuronal viability was determined by inclusion criteria of trypan blue.

4. The neurotrophic and neuroprotective action of α-PSP was determined by inducing other neurotoxic substrates as indicated conditions.
   4.1 Estrogen deprivation exposure: Neuronal viability was determined by estrogen deprivation exposure.
   4.2 Hydrogen peroxide exposure: 1 µM H₂O₂ in HBS for 5 minutes at 37°C. During exposure, E₂ or α-PSP were added concurrently with H₂O₂. After 5 min. the culture was rinsed two times with HBS, and fresh medium with E₂ or α-PSP was replaced.  
   4.3 Glutamate exposure: 0.2 µM Glutamate 20 min. at room temperature
Materials and Methods (2)

AD Model (LA-N-5)

Regular CM.

Control

Pretreated 2 days

α-PSP

Induced neuronal toxicity by indicated conditions

Overnight

morphologic cell count, biochemistry

Determination: morphologic, cell count, biochemistry
Fig. 1 Dose dependent show neuroprotective effect of α-PSP against H₂O₂-induced toxicity in neuroblastoma (LA-N-5). After pretreated with 6.66 mg/ml α-PSP for 2 days and treated with 1 μM H₂O₂ for 5 min exhibit after 1 day neuronal cells death, A. Control LA-N-5 exposed to 1 μM H₂O₂ after 24 h display numerous cell death, degeneration of neuronal process. B. LA-N-5 grown in the presence of 0.26 mg/ml α-PSP for 2 days prior to exposure to 1 μM H₂O₂. C. similar B but when dosed with α-PSP at 1.33 mg/ml, showed decrease in neuronal cell death compared with control. D. similar C but if increased dose α-PSP to 6.66 mg/ml, showed decrease in neuronal cell death compared with control. (X 400)

Fig. 2 Dose dependent shows neuroprotective effect of α-PSP against H₂O₂-induced toxicity in neuroblastoma (LA-N-5). If increased dose α-PSP to 6.66 mg/ml, showed decrease in neuronal cell death compared with control.
Fig. 3 Dose dependent of α-PSP against glutamate-induced toxicity to cells and dendrites. A. LA-N-5 under control conditions appear healthy (cytoplasm and neuronal processes). B. LA-N-5 exposed to 0.2 μM Glutamate after 24 h display shrunken cell bodies and degeneration of neuronal process. C. LA-N-5 grow in the presence of 0.066 mg/ml α-PSP for 2 days prior to exposure to 0.2 μM Glutamate after 24 h display exhibit clear features of neuronal viability for cell bodies and clearly defined neuronal process similar to those of control neurons not treated with 0.2 μM Glutamate. D. similar to C but if increased dose α-PSP to 6.66 mg/ml, showed neuronal viability and neuronal process obviously similar with control (C compare B, D compare B). X 400

Fig. 4 Dose dependent of α-PSP against glutamate-induced toxicity to cells and dendrites. If increased dose α-PSP to 6.66 mg/ml, showed neuronal viability and neuronal process obviously similar with control.
Fig. 5 Dose dependent show neuroprotective effect of \( \alpha \text{-PSP} \) against \( \text{A}\beta_{25-35} \)-induced toxicity in neuroblastoma (LA-N 5). After pretreated with 6.66 mg/ml \( \alpha \text{-PSP} \) for 2 days and treated with \( \text{A}\beta_{25-35} \) for 24 h exhibit after 1 day neuronal cell death, A. Control LA-N-5 exposed to \( \text{A}\beta_{25-35} \) after 24 h display numerous cells death, degeneration of neuronal process. B. LA-N-5 grow in the presence of 0.26 mg/ml \( \alpha \text{-PSP} \) for 2 days prior to exposure to \( \text{A}\beta_{25-35} \). C. similar B but when dosed with \( \alpha \text{-PSP} \) at 1.33 mg/ml, showed decrease in neuronal cell death compared with control D. similar to C but if increased dose \( \alpha \text{-PSP} \) to 6.66 mg/ml, showed decrease in neuronal cell death compared

Fig. 6 Dose dependent show neuroprotective effect of \( \alpha \text{-PSP} \) against \( \text{A}\beta_{25-35} \)-induced toxicity in neuroblastoma (LA-N 5). If increased dose \( \alpha \text{-PSP} \) to 6.66 mg/ml, showed decrease in neuronal cell death compared with control.
Results

• Neuroprotection by $\alpha$-PSP against other neurotoxic results
  * Estrogen deprivation induced neurotoxicity
  * Hydrogen peroxide induced neurotoxicity
  * Glutamate induced neurotoxicity
  * Beta amyloid$_{25-35}$ induced neurotoxicity

• Dose dependent of $\alpha$-PSP shows neuroprotection against other neurotoxins (experiment going on)

Discussion

• Abnormality of glucose/energy metabolism shows relation to AD (1, 2, 3, 4). $\alpha$-PSP may prevent impairment of glucose/energy metabolism and may improve the ability of neurons to reduce the levels of (“scavenger”) free radicals and thereby affecting ATP levels (11, 12).
Conclusions

• $\alpha$-PSP contains a unique formulation consisting of a complex form of carbohydrate, crude protein and essential minerals by using high pressure and mechanical hydrolysis to make a complex formulation designated alpha polysaccharidepeptides ($\alpha$-PSP). $\alpha$-PSP, in vitro, has consistently demonstrated that it prevents cell death from other neurotoxicity agents.

• $\alpha$-PSP induced cellular markers of memory function in neurons critical to memory and vulnerable to negative effects of aging, cellular degeneration and Alzheimer’s disease. Results of the current study could demonstrate the cellular mechanism of $\alpha$-PSP on cognitive function and a possible intervention in Alzheimer’s disease.

• In clinical application, $\alpha$-PSP may promote cellular mechanism in memory and neuronal survival and may be used as a nutritional supplement in aging, cellular
References

6. Interview with Medical Doctors Testimonial No.007, 018, 058 and 068.