Colostrinin inhibits the aggregation of β-amyloid peptide in SHSY-5Y neuroblastoma cells.

Anitha Rajendran, Sek Wen Hui, Marian L. Kruzel††, Thomas Nicotera† & Thamarapu Srikrishnan, Departments of Cancer Biology and Cancer Prevention†, Roswell Park Cancer Institute, Buffalo, New York 14263, USA & ††ReGen Therapeutics Plc., London, England

Abstract

Colostrinin (CLN), a proline-rich polypeptide complex, have shown a stabilizing effect on cognitive function in Alzheimer's Disease Assessment Scale-cognitive (ADAS-cog) and on daily functions in Instrumental Activities of Daily Living (ILDL) in recently conducted Phase II clinical trials. The aim of this study was to elucidate a possible mode of action for CLN in the treatment of Alzheimer's Disease. Here we report the ability of CLN to prevent the aggregation of beta-amyloid peptides (Aβ 1-40 and Aβ 1-42) *in vitro*. The effects of CLN on the fibril formation were monitored using a Nikon Eclipse E400 optical microscope and a Hitachi H-600 transmission electron microscope (EM). The EM pictures illustrate that Aβ 1-40 and Aβ1-42 show dense fibers which grow as the incubation time increases. We have also demonstrated that both Aβ 1-40 and Aβ 1-42 fibrils grow to much lesser density in the presence of CLN. Also, CLN added to the twenty day old incubation mixture reduced the length of Aβ fibrils when analyzed a day later. These optical observations were extended to a cell culture model (SHSY-5Y), in which we studied the effect of CLN on the neurotoxic activity of beta-amyloid peptides. The beta-amyloid peptides were pre-incubated with CLN at various times and used to treat SHSY-5Y neuroblastoma cells for up to 4 days. The cytotoxic effect was monitored for viability by trypan blue and for protein content (sulforhodamine assay) of SHSY-5Y cells. We demonstrated that 48 h pre-incubation of CLN with beta-amyloid peptides leads to near-quantitative cytotoxic protection, which was observed immediately post-treatment at the highest dose concentrations (50μg/ml). Our data also demonstrate that at longer incubation times, low doses of CLN can attain cytotoxic protection levels similar to that of the highest doses. Thus, the prevention of aggregation of beta-amyloid peptides by CLN is consistent with the reduction of the cytotoxic effects of beta-amyloid on SHSY-5Y neuroblastoma cells.

Introduction

AD is a progressive neurological disease caused by the deposition of amyloid aggregates on neuronal cells. Due to the obvious relation of amyloid to AD pathology, many efforts are underway to develop various clinical protocols which may interfere with the process of amyloid formation. CLN is a proline-rich polypeptide that has been used successfully in phase I and II clinical trials in the treatment of AD. We hypothesize that CLN can prevent the formation of amyloid beta aggregates, as well as dissolve previously formed fibrils. This investigation centers on the mode of action of CLN on neuroblastoma SHSY-5Y cells.

Methods

Cell Culture: The SHSY-5Y cells were cultured in Dulbecco's minimal essential medium (MEM)/Nutrient mixture F-12 (1:1, v/v; Gibco BRL) containing 10 I.U penicillin/ml and 100 μg/ml streptomycin,15% fetal calf serum, 1% non essential MEM amino acid supplement and 2 mM freshly prepared glutamine at 37°C in a humidified incubator with 5% CO_2 /95% air (Sian et al, 2000).

Cell Viability: Viability was scored by light microscopy using trypan blue exclusion (0.4% in PBS).

SRB Cell growth assay: The sulforhodamine assay (SRB) is a measure of total protein and used as a measure cell viability. Cells were seeded at 5000 cells/well in 96 well plates and grown for 48 hrs at 37°C. Aβ (1-40) peptide was incubated at 0.5 mg/ml in 0.1M tris pH 7.4 at 37°C for 0, 1, 5 and 48 hrs. The medium was removed and replaced with Opti MEM containing amyloid at concentration from 200-0.1 μg/ml incubated sample and cells were again incubated for 48 hrs. The media was removed and the cells fixed for 1 hr in 10% TCA, washed in PBS and stained with 0.4% SRB for 15 min. Unbound dye was removed with 1% acetic acid and (protein) bound dye was extracted in 10 mM tris. The absorbance was measured in multiwell plate reader at 570nm.

Results

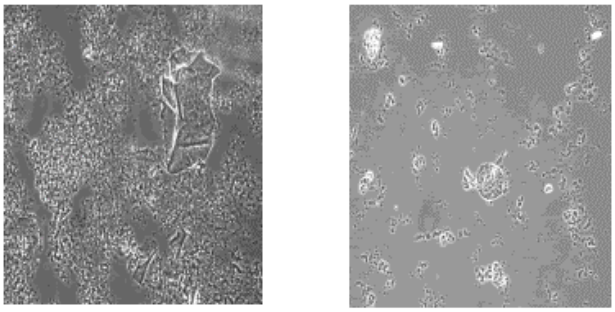


Fig. 1. Optical microscopic picture of $A\beta_{(1-40)}$ (0.346mM) in Tris. HCl buffer at pH 7.4 (a) control; (b) $A\beta_{(1-40)}$ (0.346mM) + CLN (100μg) at 7th day of incubation.

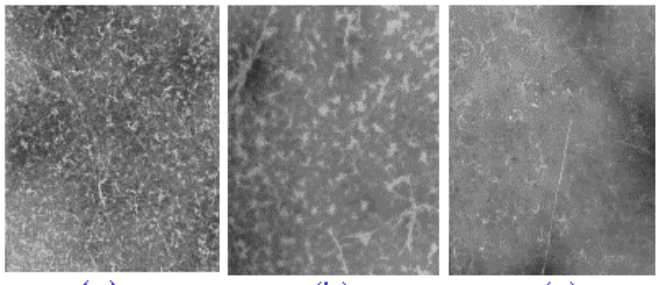
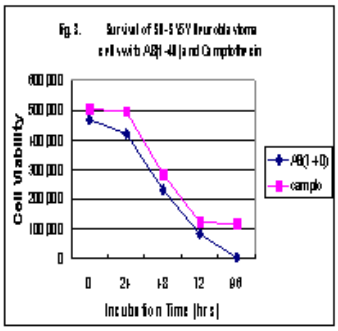
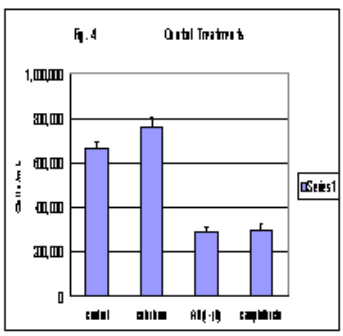
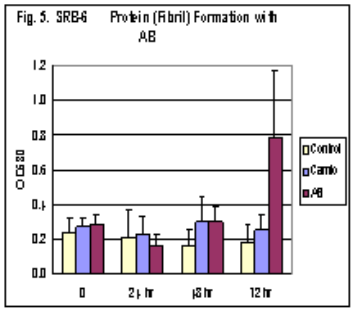


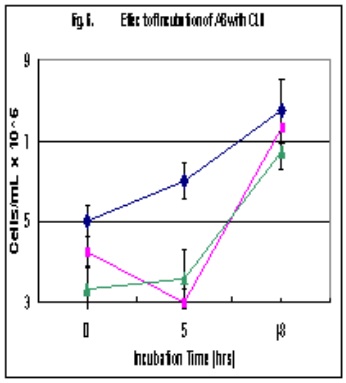
Fig. 2. Negatively stained electron micrograph picture of (a) fresh $A\beta_{(1-40)}$ (0.346 mM); (b) $A\beta_{(1-40)}$ (0.346 mM) + CLN (100μg) at 0 hr incubation; (c) $A\beta_{(1-40)}$ (0.346 mM) + CLN (100μg) at 24 hr incubation. Treatment of $A\beta_{(1-40)}$ with CLN for 24 hr shows a dramatic reduction in Aβ fibril content.





Figs 3-6. Incubation of SHSY-5Y cells with Aβ resulted in a dose dependent reduction in viability (Figs 3 & 4). The SRB method did not provide adequate viability information but showed a substantial increase in protein content, likely fibril formation, at 12 hr (Fig. 5). Treatment with CLN indicated dose and time dependent protection against Aβ fibril formation (Fig.6).





Conclusions

1. **We have used both optical microscopy [Fig. 1] and transmission electron microscopy [Fig. 2] to demonstrate that CLN reduces the level of Aβ-amyloid aggregates.**
2. **The cytotoxic effects of Aβ-amyloid fibers were monitored by cell viability using tryphan blue [Figs. 3 & 4] and total protein content using SRB [Figs. 5] in SHSY-5Y neuroblastoma cell. We demonstrated that the cytotoxic effects of Aβamyloid were reduced in the presence of CLN [Fig 6]. CLN was found to be very effective at much lower concentrations and shorter time frame (48 hours) than previously reported.**
3. **The enhanced survival of SHSY-5Y in the presence of CLN correlates with the ability of CLN to interfere with the process of Aβ-amyloid formation.**