The protective and antioxidant effects of Colostrinin™ (CLN) on hippocampal cells in culture

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Background

- Colostrinin™ (CLN) is a uniform mixture of low molecular weight proline rich polypeptides derived from colostrums. It has been found to have beneficial effects on patients with Alzheimer's disease (AD). The aim of this project is to gain more understanding of the mechanism of action of CLN.
- Beta-amyloid (Aβ) aggregates into extracellular plaques in AD and this process is thought to be the major initiator of neurodegeneration [1]. The longer 1-42 form of Aβ has been found to be more toxic *in vitro* than the shorter forms.
- Previous work has demonstrated that CLN has antioxidant effects [2] and the protein levels of the antioxidant enzyme Cu/Zn superoxide dismutase (SOD) have been demonstrated to be increased in AD patients [3].
- Furthermore CLN has previously been shown to be a modest inducer of the cytokine tumour necrosis factor (TNF)α [4]. TNFα has been found to protect against Aβ-induced toxicity by decreasing the protein and activity levels of the cyclin dependent kinase (Cdk)5 [5] which can phosphorylate Tau and therefore contribute to further neurodegeneration [6].
- Here we investigated the effect of CLN on Cu/Zn SOD protein levels and the ability of CLN to reduce protein levels of Cdk5 was analysed in order to elucidate whether CLN may be protective against Aβ-induced toxicity via the release of TNFα and reduction of Cdk5.

Methods

The model system used for these experiments was primary hippocampal cultures from embryonic day 18 (E18) rats.

Immunocytochemistry

Cultures were treated with 5μg/ml CLN and/or 25μM $A\beta_{1-42}$ for 48 hours. Immunocytochemisty for the neuronal marker MAP-2 and the astrocytic marker GFAP was then carried out.

Western blotting

Cultures were treated for 24hr with 10μM $A\beta_{1-42}$ with or without 5ng/ml or 5μg/ml CLN and were then prepared for western blot analysis. 20μg of protein was run on SDS PAGE gels and western blots were carried out for Cu/Zn SOD and Cdk5.

CLN is protective against $A\beta_{1-42}$ induced toxicity

$A\beta_{1-42}$ caused a decrease in neurons of 44±13% and reduced the number of astrocytes by 54±16% in hippocampal cultures. When CLN was pre-incubated and co-administered with $A\beta_{1-42}$ there was protection against this $A\beta_{1-42}$ induced toxicity for neurons and to a lesser extent for astrocytes (see figure 1).

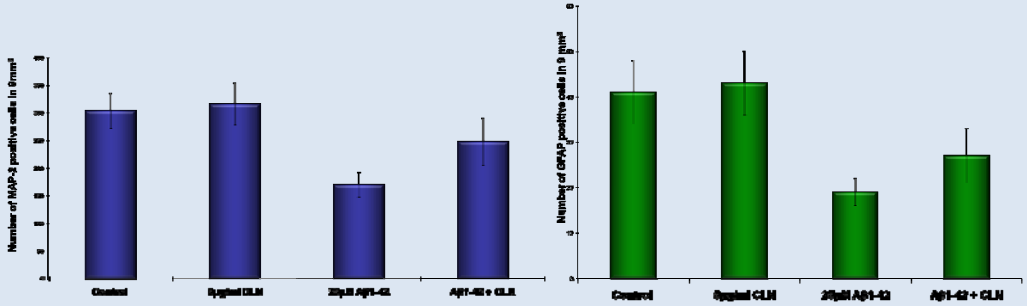

figure 1: Quantification of immunolabelling for MAP-2 positive and GFAP positive cells showing a decrease in the numbers of both cell types upon treatment with $A\beta_{1-42}$ which is partially reversed when CLN is pre-incubated and co-administered with $A\beta_{1-42}$

CLN provides antioxidant protection

There was no significant change in Cu/Zn SOD protein levels with CLN treatment alone. However an increase of 29±8% in Cu/Zn SOD protein levels was observed with $A\beta_{1-42}$ treatment and this was reduced to control levels when CLN was pre-incubated and co-administered with $A\beta_{1-42}$ (see figure 2).

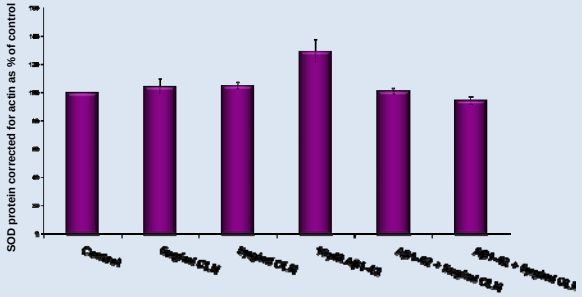

figure 2: Quantification of western blot data showing an increase in the protein levels of Cu/Zn SOD upon treatment with A β_{1-42} which is reversed when CLN is pre-incubated and co-administered with $A\beta_{1-42}$

CLN does not appear to reduce Cdk5 protein levels

Both $A\beta_{1-42}$ and CLN treatment led to a small increase in the levels of Cdk5 in the cultures (see figure 3).

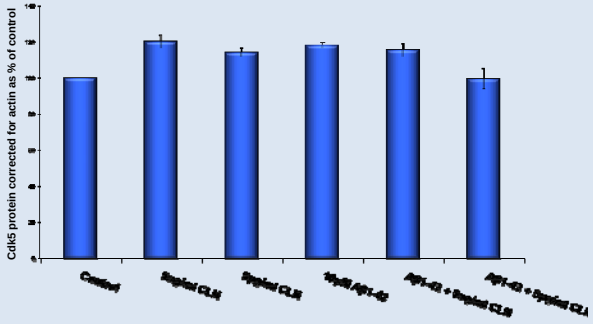

figure 3: Quantification of western blot data showing a small increase in the protein levels of Cdk5 upon treatment with CLN or A β_{1-42}.

Conclusions

CLN provides protection against $A\beta_{1-42}$-induced toxicity in primary hippocampal cultures and the mechanism of this protection appears to involve reducing oxidative stress but not decreasing Cdk5 protein levels.

Acknowledgments

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Animal house staff
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References

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