Review Article

Colostrinin™: An Oxidative Stress Modulator for Prevention and Treatment of Age-related Disorders

Istvan Boldogh and Marian L. Kruzel

Department of Microbiology and Immunology, University of Texas Medical Branch at Galveston, TX 77555, USA
PharmaReview Corporation, Pearland, TX 77584, USA

Communicated by Thamarapu Srikrishnan

Abstract. Colostrum-derived proline-rich polypeptide, also known as Colostrinin™ (CLN), has been shown to have a stabilizing effect on cognitive function in Alzheimer’s disease patients. This complex action of CLN could be related to prevention of amyloid-β peptide aggregation, as shown in in vitro studies, and its impact on delicate cassettes of signaling pathways common to cellular redox regulation, proliferation and differentiation. Studies on cultured cells showed that CLN modulates intracellular levels of reactive oxygen species (ROS), via regulation of glutathione metabolism, activity of antioxidant enzymes and mitochondria function. Due to an improvement in senescence-associated mitochondrial dysfunction and a decrease in ROS generation, CLN decelerates the aging processes of both cultured cells and experimental animals. When given orally to mice, CLN increased the lifespan and improved various motor and sensory activities. Although the molecular basis by which CLN exerts its diverse effects are still under investigation, the regulatory effect on the cellular redox state via maintenance of mitochondrial function and modification of ROS-induced cell signaling seem to be of great importance. In this article, we examine experimental data pertinent to the mechanism of action, including a review of CLN’s utility in the maintenance of physiological processes in which oxidative stress has an etiological role.

Keywords: Age-related disorders, Alzheimer’s disease, amyloid-β, immunomodulation, oxidative stress, proline-rich polypeptide

INTRODUCTION

Proline-rich regions of proteins are widely occurring in both prokaryotes and eukaryotes. They are frequently found as multiple tandem repeats in proteins of structural and functional importance. Despite significant progress in understanding the structure–function relationship in proteins, the biological relevance of proline rich regions is still unclear. Proline is a very unusual amino acid in which the side-chain is cyclized back on to the backbone amide position. This conformation produces a so-called “structural disturbance” and results in proline-rich proteins and peptides (PRPs) as helix and β-sheet breakers [130]. In many ways, it is not surprising that proline-rich peptides are highly bioactive molecules. The unique chemical characteristics of proline make these peptides responsible for a variety of biological effects. PRPs have been reported to possess activities ranging from antimicrobial action [83] to prevention of neurodegenerative disorders [48, 87]. Numerous PRPs have been isolated from mammalian salivary glands and characterized as a “first line defense” against the detrimental effects of tannins and
polyphenols in diet [26]. Also, a family of PRP-related immunomodulators was discovered in the neurosecretory granules of the neurohypophysis [46]. However, the most extensive studies involving human clinical trials have used colostrum-derived PRP, also known as Colostrinin®. The aim of this article is to review data generated on cultured cells, experimental animals and human trials with regard to the possible mechanism(s) of action of colostrum-derived PRP.

**COLOSTRININ®: STRUCTURE AND FUNCTION**

Colostrinin® (CLN) is found at particularly high concentrations in colostrum, or “first milking” of all mammals. It has long been recognized that breast-feeding offers a pronounced enhancement of passive immunity and promotes infantile gut immunity [25, 50,68]. Thus, the constituents of colostrum, and, to some extent also of mature milk, not only ensure adequate resistance to pathogens by delivery of maternal immunoglobulins and other protective factors, but also play a crucial role in promoting maturation of the immune and central nervous system. Milk proteins are often precursors of different biologically active peptides, which are inactive within the sequence of the precursor protein and become active upon physiologically controlled proteolysis. Many colostrum-derived proteins and peptides, such as PRPs and caseinophosphopeptides, reveal multiple bioactivities [29]. Immunotropic properties of these peptides have been extensively studied, predominantly in animal experimental models [70, 140].

CLN was first isolated from ovine colostrum and characterized as a complex of low-molecular-weight proline-rich polypeptides [61]. CLN is comprised ~22% of proline, non-polar amino acids, and low percentage of glycine, alanine, arginine and histidine. There are no tryptophan, or cysteine residues [62]. CLN is not phosphorylated or glycosylated and the hydrophobic amino acids constitute ~50% of the total mass. The amino acid composition of CLN is very similar in all mammalian species [71,116]. However, due to individual variations in colostrums, it is very difficult to obtain a consistently identical end-product. A new purification method, consisting of alcohol extraction and membrane filtration, seems to improve the reproducibility of CLN [72]. More importantly, avoidance of excessively harsh conditions during purification preserves the peptides’ structure and biological activity. CLN obtained by this method appears to be consistent in: 1) molecular size of peptides by SDS PAGE and high performance liquid chromatography (HPLC); 2) similar amino acid composition, characterized by high content of proline (~20%; Table 1); and 3) ability to induce cytokines.

Further studies utilizing HPLC and mass spectroscopy revealed that CLN is a complex of peptides with homology to three protein precursors: annexin, β-casein and a hypothetical β-casein homolog [71]. Remarkably, the sequence of these peptides shows no significant homology to any specific protein in the current GenBank database. The synthetic peptides of various lengths representing the N-terminal sequence of the CLN peptides are now available for the peptidomimetic studies.

The immunotropic activity of CLN was recognized by Janusz and colleagues in the early seventies [61,63,139]. Initially, the immune regulatory effect of CLN was demonstrated on the humoral immune response in mice against sheep red blood cells [129]. CLN has been shown to induce maturation and differentiation of murine thymocytes, formation of helper cells from PNAhigh thymocytes, and cytotoxic T cells from PNAlow thymocytes [60,141,142]. In addition, it was demonstrated that CLN is a potent inducer of cytokines both in vitro and in vivo [58]. Furthermore, the ability of CLN to induce IFN-γ was correlated with cell proliferation shown by 3H-thymidine incorporation into the DNA [117]. CLN has also been shown as a B cell-trophic mitogen. It activated resting mouse B cells to enter the cell cycle at frequencies comparable with those seen for LPS [64]. CLN, when administered intraperitoneally, significantly lowered the incidence of positive Comb’s reaction in New Zealand black mice. From a precursor pool of cells, CLN also induced suppressor cells that control the development of hemolytic anemia [138].

**COLOSTRININ®: DIVERSE BIOLOGICAL ACTIVITY AND MECHANISM OF ACTION**

Because oxidative stress has been proposed as an etiologic factor in many age-related disorders, it has become increasingly important to understand the mechanism of action for therapeutic compounds that may influence/affect cellular redox homeostasis. Although a substantial body of evidence showed that CLN has diverse immune regulatory functions and cell/neuroprotective ability, it has been only recent-
Table 1
Amino acid analysis of CLN

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Colostrinin</th>
<th>Ovine Original</th>
<th>Ovine MeOH</th>
<th>Bovine MeOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp/Asn</td>
<td>3.42</td>
<td>2.80</td>
<td>5.13</td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>5.66</td>
<td>5.05</td>
<td>6.94</td>
<td></td>
</tr>
<tr>
<td>Glu/Gln</td>
<td>15.48</td>
<td>15.77</td>
<td>17.99</td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>3.15</td>
<td>3.03</td>
<td>2.95</td>
<td></td>
</tr>
<tr>
<td>His</td>
<td>2.54</td>
<td>2.14</td>
<td>3.28</td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>2.32</td>
<td>3.34</td>
<td>1.62</td>
<td></td>
</tr>
<tr>
<td>Thr</td>
<td>5.73</td>
<td>5.30</td>
<td>4.32</td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>2.78</td>
<td>2.13</td>
<td>3.06</td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>21.07</td>
<td>22.50</td>
<td>20.79</td>
<td></td>
</tr>
<tr>
<td>Tyr</td>
<td>1.36</td>
<td>1.54</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>9.27</td>
<td>11.10</td>
<td>8.15</td>
<td></td>
</tr>
<tr>
<td>Met</td>
<td>3.33</td>
<td>1.70</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>5.30</td>
<td>4.93</td>
<td>8.15</td>
<td></td>
</tr>
<tr>
<td>Ile</td>
<td>3.17</td>
<td>3.42</td>
<td>3.21</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>11.04</td>
<td>10.47</td>
<td>10.33</td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>4.38</td>
<td>4.77</td>
<td>5.41</td>
<td></td>
</tr>
</tbody>
</table>

* Janusz et al. [61].
** Kruzel et al. [71,72].

ly demonstrated that it helps to maintain physiological levels of oxygen- and nitrogen-centered reactive species [20,135]. As a pleiotropic factor, CLN induces not only cellular physiological responses but also preserves redox homeostasis in stressed cells, tissues and entire organisms (Fig. 1).

The role of CLN in homeostasis of cellular redox and signal transduction

Reactive oxygen species (ROS), along with reactive nitrogen species (RNS), are essential secondary messengers in inter- and intracellular signaling cascades. However, ROS/RNS can also induce cellular pathological responses that may lead to diseases conditions or senescence processes and apoptosis. The cumulative production of ROS/RNS through either endogenous cellular sources or environmental insults causes oxidative stress, which is common to carcinogenesis, inflammation, aging-associated diseases and aging processes (Fig. 2), in which levels of oxidative damage to proteins, lipids and DNA are increased. Oxidatively damaged proteins are degraded and DNA is repaired; however, oxidation of fatty acids leads to the generation of even more potent reactive molecules, such as 4-hydroxynonenal (4-HNE) and malondialdehyde(s) [39]. 4-HNE can react with the nucleophilic sites in DNA, mitochondrial proteins and a variety of other nucleophiles, including GSH, resulting in cellular stress responses and oxidative stress [36]. While micromolar concentrations of 4-HNE are cytotoxic, at nanomolar levels it can be involved in activation of signal transduction pathways [28,36,77]. Most importantly, 4-HNE has been shown to be associated with the etiology of degenerative disorders such as atherosclerosis, Parkinson’s disease, and Alzheimer’s disease (AD) [40,114].

Recent studies have investigated the effects of CLN on 4-HNE-protein adduct formation, generation of ROS, glutathione (GSH) metabolism, and on the modification of the signal transduction cascade [17] in cultured PC12 cells. Results show that treatment of PC12 cells with 4-HNE causes c-Jun N-terminal kinase (JNK) activation (15–30 min post-treatment). However, when CLN was added prior to 4-HNE treatment, JNK activation was not only delayed or reduced, but was abolished. Additional results show that CLN: 1) reduced the levels of 4-HNE-protein adducts, as shown by fluorescent microscopy and Western blot analysis; 2) decreased 4-HNE-induced intracellular levels of ROS, as shown by 2’7’-dichlorodihydrofluorescein-mediated fluorescence; and 3) inhibited 4-HNE-mediated extrusion of GSH via the cytoplasmic membrane into the extracellular milieu as determined fluorimetrically.

It is well-documented that JNK regulates major cellular signaling cascades, along with mitogen-activated protein (MAP) kinases, the extracellular signal-regulated kinase, and the p38 MAP kinase [94]. JNKs are activated by a wide variety of stimuli, including ROS, DNA-damaging agents and inhibitors of protein synthesis, heat or osmotic shock [94]. These
stimuli appear to operate through small GTP-binding proteins such as Ras resulting in sequential activation of various protein kinases. Targets of the JNK signal transduction pathway include the transcription factors ATF2 and c-Jun (binds to the N-terminal region of ATF2 and c-Jun and phosphorylates two sites within the activation domain). These factors are members of the basic leucine zipper group that bind as homo- and heterodimeric complexes to AP-1 and AP-1-like sites in the promoters of several genes, resulting in increased transcriptional activity. Although the biological significance of a CLN-mediated decrease in JNK activation needs to be established further, we can speculate that it may be important, e.g., in the prevention of amyloid-β (Aβ)-induced apoptosis, and extension of lifespan in cell cultures or animal models, as well as neurite outgrowth of neuronal precursor cells [5,8,9,109].

There is evidence that p53 lies at the center of a network of complex redox interactions [81]. In this network, p53 can control the timely production of ROS, but this activity itself is under the control of changes in cellular redox status [81]. Activation of p53 can occur in multiple ways: examples include an increase in ROS levels, DNA damage, activations of cell cycle-check kinases or to 4-HNE exposure [81]. Most importantly, CLN showed a potent inhibitory effect on 4-HNE-induced activation (Fig. 3). Based on these results, CLN modulates the balance between oxidants and antioxidants, thereby also regulates redox-sensitive cel-

Fig. 1. CLN’s potential mode of action.

Fig. 2. CLN’s hypothetical mode of action via cellular redox mechanisms.

Fig. 3. Inhibition of 4-HNE-induced p53 activation by CLN. Cells were incubated for 24 h with CLN concentrations as indicated and 4-HNE (50 nM) was added. Inset: Cells were lysed and Western blot analysis was undertaken to determine levels of p53. 4-HNE, 4-hydroxynonenal.
A B

Fig. 4. CLN decreases intracellular ROS levels and oxidative damage to DNA. A, CLN pre-treatment of cells prevents oxidant-induced increases in ROS levels. Cells were grown for five population doublings in media containing 100 ng per ml CLN. Cells were loaded with H$_2$DCF-DA (final concentration: 5 µM) and challenged with H$_2$O$_2$ (100 µM), glucose oxidase (GO, 0.2 unit), or Aβ (10 µg per ml). Changes in DCF fluorescence were determined by flow cytometry (FACSAria, Beckton Dickinson). B, CLN decreases the augmentation of 8-oxo-7,8-dihydro-2′-deoxyguanosine levels induced by Aβ (left upper panel). SH-SY5Y cells were grown for five population doublings in media containing 100 ng per ml CLN. Then cell monolayers were treated with polymerized Aβ (10 µg per ml) for 3 hrs, fixed in formalin, and permeabilized by acetone:methanol (1:1). Levels of 8-oxodG were determined by fluorescent confocal imaging, as we previously described [59]. Lower panels show 4′,6-diamidino-2-phenylindole (DAPI)-stained images of nuclei. H$_2$DCF-DA, 2′,7′-Dichlorodihydrofluorescein diacetate; DCF, Dichlorofluorescein; Aβ, amyloid-β.

CLN has no direct antioxidant activity, so the basic question is how is the cellular redox state modulated by CLN? As it was demonstrated, CLN inhibited the 4-HNE-mediated extrusion of GSH into the extracellular milieu, which suggests that CLN might regulate levels of the GSH pool by modulating the activity of cell membrane-associated thiol transporters [20]. Cellular thiol levels and the cellular redox state of thiols are critically important molecules in determining the redox status of the intracellular milieu, and therefore effects of CLN on multiple cellular processes, such as cell proliferation, differentiation, and apoptosis are not surprising. Results from our microarray analyses show altered gene expression profiles of several genes that are directly or indirectly involved in the maintenance of the cellular redox state. Examples include mitochondrial respiratory complex proteins, cytoplasmic and mitochondrial superoxide dismutases, heat shock protein 32 (hemeoxygenase-1), and glutathione reductase that catalyzes the reduction of glutathione disulfide (GSSG) to GSH [13].

Furthermore, our data show that pre-treatment of cells with CLN protects against exogenous oxidants such as H$_2$O$_2$, and Aβ, or against ROS generated enzymatically. CLN did not change redox status of the redox-sensitive 2′, 7′-dihydro-dichlorofluorescein (H$_2$DCF) but resulted in decreased H$_2$DCF oxidation, when CLN pre-treated human cells (SH-SY5Y: human neuroblastoma cell) were exposed to H$_2$O$_2$, glucose oxidase (GO), or Aβ (Fig. 4A). In line with this observation, CLN decreased Aβ-mediated guanine-base oxidation in the DNA, as shown by the immunochemical detection of 8-oxo-7,8-dihydro-2′-deoxyguanosine (Fig. 4B).

An increase in the intracellular levels of ROS, as shown by augmentation of lipid peroxidation, protein oxidation, and DNA oxidation, is a hallmark of various disease conditions, including cognitive decline and aging. Intracellularly, ROS may be generated by cytoplasmic and membrane-associated oxidases (e.g., NADPH oxidase, diamine oxidase, and xanthine oxidoreductase), but the major source of ROS is the mitochondria. Mitochondria are discrete organelles that form a highly dynamic and interconnected cellular network, and their numbers may exceed thousand per cell. The roles of mitochondria are well-established in energy generation and metabolism, and they are considered to be major intracellular sources of ROS. A growing number of neurological diseases are being linked to mitochondrial dysfunctions described in sarcopenia, AD, Huntington’s, and other degenerative diseases [95]. Mitochondria-generated ROS play an important role in the release of cytochrome c and other pro-apoptotic proteins that can trigger caspase activation and apoptosis. Importantly, CLN stabilizes mitochondrial function in Aβ-treated human SH-SY5Y and rat PC12 cells (Fig. 5) and during cellular aging/senescence processes [9].
Fig. 5. Amyloid-β-induced mitochondrial H$_2$O$_2$ production is decreased by CLN. SH-SYSY and PC12 cells were maintained in media containing 100 ng CLN per ml. Cells were treated with Aβ, and 24 h later mitochondria were isolated. Levels of H$_2$O$_2$ released from intact mitochondria were determined by Amplex red assays, as we described earlier [10]. Rotenone, 3-NPA (3-nitropropionic acid), and Antimycin A are inhibitors of respiratory complexes I, II and III, respectively. Catalase was used as a control to degrade H$_2$O$_2$. FC-CP (carbonylcyanide-p-trifluoromethoxyphenylhydrazone) is a decoupler of mitochondrial inner membrane potential. 3-NPA, 3-nitropropionic acid. *p = 0.001, **p = 0.0001.

CLN protects against the spontaneous and induced mutations

Oxidatively damaged proteins are usually eliminated by degradation, while damaged DNA is repaired [91]. ROS-, chemical- and physical-agents-mediated DNA damage and their incorrect repair lead to DNA sequence alterations, which are implicated in carcinogenesis [49]. Modifications in DNA sequence order also result in altered protein structures and/or activities (e.g., p53), leading to changes in cell cycle progression, and dysregulation of the cellular redox balance, both of which culminate in the manifestation of pathobiological processes and diseases [91].

Mutational analysis has been an important approach for testing the genotoxicity of biologically active, natural, industrial and environmental compounds. A recently published study showed that CLN decreased ROS-induced mutagenicity [5]. In these studies, the protective effect of CLN against spontaneous and induced mutations was tested in Chinese hamster V79 cells at the hypoxanthine phosphoribosyl-transferase gene locus. As described, CLN had no effect on cell proliferation, and plating efficiency, and did not induce the expression of phosphatidyl serine on the outer cytoplasmic membrane, considered to be an early sign of cellular toxicity [5]. Moreover, it has been demonstrated that CLN significantly decreases the frequency of mutations developing spontaneously or induced by ROS, chemical agents, and UV irradiation [5].

Since oxidative damage to DNA is the most common, studies were undertaken to investigate the antimutagenic effect of CLN in oxidatively stressed cells. Cellular oxidative stress was produced by glucose oxidase, a known ROS generator [33] or by addition of H$_2$O$_2$. Data show that when CLN was present during oxidative insult of the cells, the mutation frequency decreased significantly (≥ p = 0.001) (Fig. 6). At present, the mechanism by which CLN exerts its antimutagenic activity in oxidatively stressed cells is unclear. It is possible that CLN increases the activity of antioxidant enzymes and/or levels of cellular low-molecular-weight antioxidants, such as glutathione. Another possibility is that CLN increases the efficacy of DNA base excision repair, the major pathways repairing oxidatively damaged DNA [91]. For example, there is evidence showing the importance of redox-dependent, posttranslational modifications of apurinic/apuriminidic endonuclease-1 (APE1/ref-1), a rate-limiting protein in base excision repair processes. These modifications of APE1 are critical for its interaction with members of the repair machinery and its endonuclease activity [67,101].

To test further whether CLN was only effective in suppressing ROS-induced mutagenesis, cells were irradiated with ultraviolet A (UVA). UVA radiation-induced genomic instability has been linked to the production of ROS [53,128]. At concentrations of 100
ng per ml or higher, CLN significantly inhibited UVA-induced mutagenesis. Regardless of the mechanism, these findings have great significance, since UV irradiation is the primary cause of squamous cell carcinomas [35] and malignant melanomas [82,111]. The UV doses that were chosen in this study are physiologically relevant because they were equivalent to midday sun exposure for 30 and 100 min [69,90]. These data imply that CLN is likely to be suitable for application in humans to decrease UV-induced cellular damage and its well-established carcinogenic effects [82,111].

CLN significantly decreased the mutation frequency induced by the DNA-damaging chemical agents methyl methanesulfonate (MMS) and mitomycin C (MMC). MMS primarily alkylates guanine at the O\textsuperscript{6}-position, which is repaired by O\textsuperscript{6}-methylguanine DNA methyltransferase [89]. CLN substantially decreased MMS-induced mutation frequency (Fig. 6). The explanation may be that MMS-induced genomic damages are primarily due to guanine alkylation and are repaired by O\textsuperscript{6}-methylguanine DNA methyltransferase’s action, which is not redox regulated [89]. These results suggest that the substantial CLN-mediated decrease in mutation frequency is independent from CLN’s impact on cellular redox in MMS-treated cells. MMC is a DNA cross-linking alkylating agent that, after getting through the cell membrane, is activated by NAD(P)H quinone oxidoreductase, xanthine dehydrogenase, xanthine oxidase, cytochrome P450 reductase, and/or cytochrome b\textsubscript{5} reductase [30]. By directly interacting with DNA, MMC induces an array of damage, including DNA inter- and intra-crosslinks, single-strand breaks and double-strand breaks. Taking into consideration that CLN decreases mutation frequency in GO, H\textsubscript{2}O\textsubscript{2} and UV-irradiated cells, the significant decrease in MMC-induced mutation frequency by CLN is not surprising.

In conclusion, these results suggest that the anti-mutagenic properties of CLN are mediated by multiple but overlapping mechanisms: 1) a decrease in the cellular levels of ROS and oxidative damage to DNA; and/or 2) CLN may increase the efficacy of DNA repair via the generation of favorable cell activation signals and/or cellular responses to DNA damage.

**CLN decreases allergic inflammation**

Allergenic molecules are processed via dendritic cells and presented as peptides by class II major histocompatibility molecules to allergen-reactive T helper type 2 (Th2) cells [124]. These interactions orchestrate a cascade of events that lead to IgE synthesis and recruitment of inflammatory cells [74,124]. A study, which investigated potential allergic responses in a mouse model to CLN and its impact on allergic sensitization and reactions caused by common allergens, showed that CLN did not increase IgE/IgG1 levels, or induce cutaneous hypersensitivity reaction, airway inflammation and mucin production [19]. Importantly, CLN significantly \((p < 0.01)\) decreased IgE/IgG1 production, eosinophilia, mucin production and airway hypersensitivity induced by allergenic extracts from ragweed pollen grains and house dust mites [19]. Figure 7 shows decreases in eosinophil accumulation and mucin production in lungs induced by the potent allergen ragweed extract. The pollen of short ragweed (A. artemisiifolia) is one of the most abundant aeroallergens causing severe seasonal allergic rhinitis, conjunctivitis and airway inflammation in the United States and
Europe [122,132]. The house dust mite extract was isolated from *D. pteronyssinus* and *D. farinae*, the most common sources of indoor allergens worldwide [123]. More than 50% of patients with allergies and up to 80% of asthmatic children are sensitized to mite allergens [22,34].

It has been shown previously that allergen-bearing pollens induce airway inflammation in sensitized individuals, and the recruited inflammatory cells produce oxidative stress in the airways [37,86]. Furthermore, in addition to their allergenic proteins, pollen grains or their extracts contain NAD(P)H oxidases, which increase oxidative stress levels in mucosal epithelial cells and the airway epithelium within minutes of exposure [7,16]. This oxidative stress is required for antigen-mediated robust inflammation in the lower airways and conjunctiva [7,16]. CLN-mediated decreases in inflammatory responses to ragweed pollen extract may be explained by the fact that CLN-treated cells have an increased ability to cope with oxidative stress [5,20,109]. CLN conditioning also decreased house dust mite extract-mediated inflammatory responses, although it contains proteases to invade the site of exposure and potentiate allergic immune responses [66,123].

Although these studies convincingly show CLN’s antiallergenic capacity, the mechanism implicated in these processes is not known. Whether CLN-induced immunomodulators (e.g., IFN-γ, IL-6, IL-10) have an impact on the allergic sensitization processes or the manifestations of allergic reactions is the subject of speculation. For example, it may be proposed that CLN alters Th2 responses and switches cellular chemokine and cytokine production towards Th1. Our speculation relies on previously published data showing that CLN alters the production of IFN-γ, IFN-α, IL-6 and IL-10 [134,136]. Th1 cells have been characterized by the production of IL-2 and IFN-γ, whereas Th2 cells secreted IL-4, stimulating IgE production [93]. At this time, it is not known if CLN mediated any change in IFN-γ levels in our model system; however, it has been shown that IFN-γ counteracts IgE production in cell culture systems of both human and mouse origin [43,102]. Indeed, CLN significantly (*p = 0.001*) decreased serum levels of IgE/IgG1, cutaneous reactions in RWE-challenged animals and those exposed to house dust mite extracts [9]. It has also been shown that CLN mediates maturation and differentiation of murine thymocytes, promotes proliferation of peripheral blood leukocytes, and induces immunomodulators, including various cytokines [60,62,117,141]. These results further indicate the existence of potential mechanisms by which CLN could modulate a cellular network resulting in cytokine and chemokine production that decreases/prevents inflammatory processes induced by allergens.

**CLN induces neurite outgrowth in cultured cells**

Neurites are essential for intercellular communication in the nervous system. Recent studies have shown that CLN induces neuronal differentiation via two interrelated cellular processes: 1) inhibition of cell proliferation and 2) progression through the stages of neurite outgrowth [8]. The induction of neuronal differentiation by CLN can be demonstrated in cultures of normal human epithelial, human neuroblastoma (SH-SY-5Y) and rat medullary pheochromocytoma (PC12) cells (Fig. 8). Detailed mechanistic studies on CLN-mediated neurite outgrowth were carried out using PC12 cells, developed from a transplantable chromaffin tumor. PC12 cells respond to nerve growth factor (NGF) and other factors with a change in gene expression and/or morphology [84] and are suitable for testing biological activity of materials such as CLN.

Neurite outgrowth is a tightly regulated cellular process induced by hormones and neurotrophins such as NGF both *in vivo* and in cultured cells. For example, neurite growth induced by NGF requires the tyrosine kinase (TrkA) receptor and a cascade of events through the Ras/MAPK pathway [32,131]. PC12 cells contain both the TrkA and low-affinity NGF receptors [32,54]. Studies on CLN-mediated PC12 differentiation show an anti-proliferative effect, followed by neurite outgrowth. The percentage of cells showing CLN-induced neurite outgrowth was concentration-dependent in a manner similar to that of NGF, the best characterized agonist [80,126].

Studies show that CLN inhibited cell proliferation via the p53-p21WAF1 pathway, resulting in an accumulation of cells in the G1 phase of the cell cycle. Indeed, the major step in neurite outgrowth is cell cycle arrest via p53 stabilization. p53 exists in latent and antiproliferative forms that differ in their degree of post-translational modification, e.g., phosphorylation, acetylation [52,81] and their subcellular distribution [112]. Activated p53 alters the transcription of genes, many of which regulate cell proliferation [52,81,84] and are required for differentiation [4,98]. The observed stabilization of p53 was due to phosphorylation of p53 at serine 15 that accumulated in the nuclei of CLN-treated PC12 cells. These events oc-
CLN induces cell differentiation. Human primary epithelial cells, human neuroblastoma (SH-SY-5Y) and pheochromocytoma (PC12) cells were cultured in their well-defined culture media [6,8] and then treated with 100 ng per ml CLN. Neurite outgrowths were visualized microscopically and photographed with a Photometrix CoolSNAP Fx camera mounted on a NIKON Eclipse TE 200 UV microscope.

curred in parallel with an increase in p21WAF1 level and cell cycle arrest. PC12 cells transfected with inhibitory oligonucleotides specific for p21WAF1 knocked out CLN-induced p21WAF1 expression, while p53 activation was not altered. These inhibitory oligonucleotides significantly decreased p53-p21WAF1 activation and the percentage of cells showing neurite outgrowth in both CLN and NGF-treated cultures. These results strongly suggest that CLN induced a cascade of events in PC12 cells similar to that of neurotrophins.

CLN, like NGF, induces GAP-43 expression in PC12 cells during differentiation processes [8]. GAP-43 is a marker of neuronal differentiation whose expression is restricted to the nervous system during development and regeneration [12]. GAP-43 expression requires Ras-dependent integrated signaling through extracellular signal-regulated kinase, JNK, and MAP [88,103,133]. Neurite outgrowth of PC12 cells induced by CLN showed kinetics similar to that caused by NGF. This raised the possibility that CLN may interact with the NGF receptor, TrkA and utilize a cell activation pathway similar to that of NGF. The neurite outgrowth induced by combination of NGF and CLN was only slightly higher than that caused by NGF or CLN alone, suggesting that the cell surface receptor(s) may not be different [8]. Taken together, it may be proposed that CLN via cell surface receptor(s) mediates a wide spectrum of activities similar to those induced by hormones and neurotrophins leading to neurite outgrowth.

CLN increases the lifespan of cultured cells by preventing mitochondrial dysfunction

Increased ROS generation and mitochondrial dysfunction contribute to aging and neurodegenerative diseases, as shown in human studies, in transgenic rodent models and cell culture studies [11,23,24,38]. To study whether CLN delays the onset of replicative senescence, murine diploid fibroblast cells (MDF) were used from senescence-resistant (MDFR) and senescence-prone (MDFS) mice. In studies by Bacci and colleagues [9], MDF cultures were continuously passaged at ambient oxygen concentration until they reached a crisis in growth, and showed morphological characteristics of senescence. Cells in the state of replicative senescence exhibited a low saturation density, and an increased population-doubling time, along with increasing number of enlarged and polyploid cells. These changes occurred in cultures of MDFS at 10–12 population doubling levels (PDL), much earlier than that of MDFR cells (16–19 PDL). When CLN was added (250 ng per ml) into the growth medium, the lifespans were increased in both cell cultures. The MDFS cell’s lifespan increased significantly ($p = 0.001$) from 11–12 PDLs to 15–17 PDLs. CLN has increased the lifespan of MDFR cultures from 15–16 to 20–21 PDLs. Such an effect of CLN on PDL of MDFS cells was concentration dependent [9]. The most remarkable observation was that CLN significantly ($p = 0.001$) decreased spontaneous immortalization frequency.

As described by Bacci and his colleagues [9], at PDL4 and PDL7 there was no significant difference in ROS levels between MDFS and MDFR cells. Compared to cells at PDL4, MDFS cells at PDL9 showed a two-fold increase in ROS levels while, at the stage of replicative senescence (PDL 12), MDFS cells there was a greater than three-fold increase in ROS levels. ROS levels in MDFR cells gradually increased from PDL12 and reached a greater then two-fold increase at PDL16. Most importantly, in MDFS and MDFR cultures that were maintained in a medium containing CLN, ROS levels were significantly lower, even at a stage of replicative senescence, when compared to cultures that contained no CLN in their media.

Oxidative damage to mitochondrial respiratory complexes and other molecular components leads to a vicious cycle of sustained ROS generation, and increasing mitochondrial damage, that adversely affects
functions that cause a loss of ATP-generating capacity [76,113]. These observations may be valid for MDF\textsuperscript{SP} and MDF\textsuperscript{R} cells, because the higher oxidative stress observed in the accelerated senescence-prone strains of mice are associated with mitochondrial dysfunctions [55]. Indeed, in-depth investigations using microscopic imaging (Fig. 9A) and biochemical assays showed that mitochondria are the source of ROS in these cells [9]. Further, mitochondria from cells reaching the senescence stage generated significantly higher amounts of ROS than did those actively replicating MDF cells (Fig. 9B). Remarkably, mitochondria from cells maintained in the presence of CLN generated significantly less ROS (Fig. 9A). The CLN-mediated decrease in mitochondrial ROS levels (Fig. 9B) is in agreement with its ability to lower levels of oxidative stress [17,18] and extend lifespan [9]. An increase in the lifespan of diploid cultured cells over-expressing mitochondrial antioxidant enzymes was previously documented [137]. Based on observations described above and shown in Fig. 9, we speculate that CLN increases mitochondrial GSH levels and/or activity of antioxidant enzymes thus improving mitochondrial function and delaying the development of an increased oxidative state and cellular senescence processes.

Those approaches that aimed to improve mitochondrial dysfunction and/or lower oxidative stress levels in age-associated diseases have attributed a great importance to CLN. Oxidative damage to mitochondrial respiratory complexes and other inner membrane proteins lead to a vicious cycle of increasing mitochondrial damage and sustained ROS generation, which adversely affects tissue function in vital organs and the central nervous system [11,41,56,76,96,113]. The precise mechanism by which CLN delays senescent processes is unknown; however, these data suggest that CLN may be used in preventive and therapeutic approaches to delay the onset of symptoms of age-associated diseases that have been shown to be associated with mitochondrial dysfunction.

**CLN protects against amyloid-β-induced apoptosis in neuroblastoma cells**

Amyloidosis is a neuronal disease caused by aberrant protein folding in which insoluble globular proteins are deposited in the brain tissues, leading to tissue damage and disease [51]. Epidemiological studies have shown that the alterations in the metabolism of the amyloid-β protein precursor (A\textbeta PP) and the formation of A\textbeta plaques are the main cause of neuronal death in AD [47]. These plaques promote oxidative stress-
induced injury in the brains of AD patients. Moreover, Aβ plaques have been shown to instigate cells to produce excessive quantities of free radicals, which cause further damage to brain cells [75,104,125]. The successful development of clinical protocols using antioxidants for AD solely depends on the availability of products that are safe and have a proven mechanism of action.

Schuster and his colleagues reported that CLN prevented the aggregation of Aβ peptide (1–40) in vitro [109]. The impact of CLN on fibril formation was monitored by optical and electron microscopy. The microscopic investigations showed that Aβ (1–40) peptides-formed fibrils (25 µm in length) after 24–48 hours of incubation. The presence of 250 nM CLN completely abolished the fibril formation. Aβ (1–42) peptides grow into dense fibers when examined at the 20th day. In the presence of CLN, however, the fibrils are much shorter and less dense. More importantly, the addition of CLN as late as the 17th day can dissolve the preformed fibrils. These observations were compared to the effects of CLN on the neurotoxic activity of Aβ peptides in cell culture model (SHSY-5Y). The Aβ peptides were pre-incubated with CLN at various length of times and SHSY-5Y cells were treated for up to 4 days [109]. The cytotoxic effect was monitored by trypan blue exclusion. The results showed that 24–48 hrs treatment resulted in toxicity of 10–50 µM of Aβ peptides. Pre-incubation of 0.0025–0.25 µM of CLN with 25 µM of Aβ peptides led to near-complete abolition of cytotoxicity. Importantly, low doses of CLN (e.g., 2.5 nM) attained cytotoxic protection similar to those seen at the highest doses (e.g., 0.25 µM). The time course for the appearance of Aβ fibrils coincides with that for cytotoxicity, and the reduction of fibrils of Aβ peptides by CLN is concomitant with the reduction of the cytotoxic effects of Aβ on SHSY-5Y neuroblastoma cells. These studies suggest that the neuroprotective effects exerted by CLN are related to the decrease in Aβ fibrils [109].

The impact of CLN on fibril formation was measured by the thioflavin T (ThT) method [21]. The assay measures changes in fluorescence intensity of ThT upon binding to amyloid fibrils. Unbound ThT does not fluoresce. Consequently, it can be used to monitor the effect of drugs in their capacity to inhibit Aβ aggregation. The enhanced fluorescence is easily observed using this spectroscopic assay, and is commonly used to monitor fibril formation over time. It has been demonstrated that CLN effectively decreases the aggregation of Aβ and fibril formation [109] in vitro.

Whether CLN inhibits fibril formation by Aβ and also decreases the toxicity of soluble Aβ monomers and oligomers in vivo are yet to be assessed. Inhibition of fibril formation may lead to increased levels of soluble Aβ monomers/oligomers that are thought to be important effectors of synapse loss and neuronal injury [127]. CLN has been shown to have a stabilizing effect on cognitive functions, including long-term memory enhancement in AD patients [78], and therefore it may be proposed that CLN not only inhibits fibril formation, but decreases the toxicity of Aβ monomers/oligomers in vivo.

COLOSTRININ™: EFFECTS ON LEARNING, MEMORY AND LIFESPAN IN ANIMAL MODELS

In the late nineties, the in vivo and in vitro immunomodulatory properties of CLN were linked to neuroprotective activity in humans and animals. In particular, the effects of CLN on spatial learning and incidental memory in rats of two age groups, 13-month-old (aged) and 3-month-old (young), were spectacular [99]. CLN at low doses (4 µg per rat) facilitates the acquisition of spatial learning of aged (13 months old), but not young (3-month-old) rats. The statistically significant difference between placebo-treated and CLN-treated animals suggests that CLN improves the ability of aged animals subjected to the Morris Water Maze test to find safety on a platform in the tank. Detailed analysis of swimming behavior indicated that the aged placebo-treated rats were impaired in the latter stages of spatial learning. Treatment with CLN resulted in a significantly (p = 0.05) higher precision in the animals ability to find the platform as revealed by the higher number of direct hits. The fact that aged CLN-treated animals were not impaired suggests that CLN improved the ability of aged subjects to find the platform more effectively [99]. In similar studies, treatment with one of the constituent peptides of CLN (called nonapeptide: Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro) substantially, but not significantly affected the acquisition of spatial learning and memory retrieval in the Morris Water Maze test [100]. The difference in cognitive effects between CLN and the CLN-derived nonapeptide may be explained by a possible modification of its chemical structure when it is isolated or by the absence of other peptides also required for mediation of the highly complex cognitive activities.
Untreated mice were compared to the solvent-fed group: male mice (n = 41); female mice (n = 40), and female mice (n = 37), female mice (n = 34). A control group of animals (female: n = 27; male n = 31) were left untreated.

To have a more comprehensive understanding of the effect of CLN on experimental animals, the lifespan of New Zealand black mice was determined [138]. In these studies parallel groups of mice were treated with 10 ng, 100 ng, and 1000 ng of CLN twice a week beginning on day 165 of their life. Importantly, the lifespans of these mice were increased significantly (p = 0.01) when treated with 100 ng of CLN, compared to mock (saline)-treated animals. Administration of 10 ng and 1000 ng of CLN only substantially increased their lifespan. The senescence-accelerated mouse is a useful animal model to study aging or age-associated disorders due to its inherited aging phenotype [121]. In a recently conducted study, the effects of the oral administration of CLN on the lifespan and on various behavior characteristics in senescence-accelerated mice were investigated (Fig. 10). Results show that CLN administration via drinking water significantly increased the lifespan of these mice [15].

Groups of CLN-fed mice were also tested for their ability to perform an age-sensitive battery of tests for cognitive and motor function. A swim maze task was employed to measure the ability of the mice to learn and remember the location of a hidden platform. This task is dependent on cortical and hippocampal functions. In addition, a set of psychomotor tests was used to evaluate different dimensions of age-associated loss, including spontaneous locomotion, coordinated running, balance, muscle strength, sensory reactivity, and reaction time. Data in Fig. 11 show that CLN administration to mice improves age-associated locomotion, and learning/memory capacities [15]. Improved neurological performance correlated well with the decreased levels of oxidative stress markers measured in various organs. In particular, it has been shown that CLN decreased levels of 8-oxo-7,8-dihydro-2’-deoxyguanosine in DNA and significantly lowered oxidative damage to proteins in brain and liver [15]. The results of these comprehensive studies are in agreement with investigations that describe extended lifespan in cultured cells [9]. We propose that at a cellular level CLN binds to not-yet-identified membrane receptor(s) and modulate cellular signaling and the cellular redox state via mitochondrial metabolic processes, resulting in improved motor and sensory activities and prolonged lifespans.

Stewart and Banks have shown that CLN derived from ovine or bovine sources exhibits potency as a cognitive enhancer of avoidance training on the weak aversive stimulus of 10% methyl antranilate in newly hatched chicks [119]. CLN’s efficacy on improving memory for the weak aversant showed a dose–response curve with potency over a 100-fold range. It brings the retention of memory up to the level normally seen with 100% methyl antranilate [85]. The memory enhancement by CLN for the weak aversant (10% methyl antranilate) is comparable to that shown previously in the chick passive avoidance model [85,106] where memory was improved with compounds which stimulated corticosterone action [85]. The cognitive enhancing effect of CLN was independent of whether it was intracranially or intraperitoneally administered. The likely explanation for this finding is that in the newly hatched chicks, the blood-brain barrier is incomplete, thus allowing rapid access of CLN to the brain. In mammals where there is a notable blood brain barrier, CLN can exert a cognitive effect even when injected intra-peritoneally. These data are supported by a study on a rat model [100]. It has been demonstrated that CLN injected intraperitoneally in older animals (of 13 months of age) at a dose of 4 µg per rat facilitated the acquisition of spatial learning. At this concentration, it also improved incidental learning. CLN also has efficacy in the treatment of humans suffering from mild or moderate AD [14]. Although the precise mechanism of CLN’s action in enhancing memory is unclear, one possibility is that this may involve second step in a two-stage memory process [105]. The first does not require de novo synthetic processes and decays at some 6–8 h post-training, whereas the second stage requires a cascade of synthetic events which ensure that memory is formed as a long-term store. The weak (10% methyl antranilate) avoidance training task fails to stimulate...
the second wave of events and thus memory is lost after some 6–8 h. CLN may exert an effect by acting upon the transmitter/receptor systems which are known to be rapidly up-regulated on weak avoidance learning in the chick, influenced by N-methyl-D-aspartate receptor agonists [118]. If so, then the cascade of processes involved in long-term memory formation including protein synthesis are initiated and, hence, the second phase of memory retention following treatment with 10% methyl antranilate is similar to the test seen with 100% methyl antranilate. These data show a clear enhancing effect of CLN on training for a weak, aversive task, and demonstrate its widespread efficacy as a cognitive enhancer in other animal species and humans.

**COLOSTRININ® CLINICAL UTILITY IN ALZHEIMER’S DISEASE**

Dementia is a brain disorder that seriously affects a person’s ability to carry out daily activities. One of the most common forms of dementia, known as AD, involves the frontal lobe of the brain and affects such cognitive functions as language, thought processes and memory [65,115]. The hallmarks of AD pathology are the neuritic plaques, microscopic foci of extracellular Aβ deposition, and the neurofibrillary tangles, intracellular fibrils composed of hyperphosphorylated Tau protein. There is substantial evidence showing that both Aβ deposits and Tau protein fibrils are the products of misfolded proteins largely involved in increased production of ROS in brain [1,73,110]. Although there is major progress in understanding pathogenesis of AD dementia, the preventive and therapeutic measures still have to be explored.

There are a number of drugs presently employed to alleviate the effects of AD dementia or delay its progression; however, the treatments are symptomatic and there is no cure to reverse AD pathology. A family of cholinesterase inhibitor drugs (tacrine, donepezil, metrifonate, rivastigmine and galantamine), which increase the availability of acetylcholine in central synapses has been approved by the FDA for the treatment of AD dementia since the 1990s. However, these drugs only offer short-term benefits during the early stages of AD. Moreover acetylcholinesterase inhibitors cause significant side effects, including nausea, anorexia, vomiting, and diarrhea [31]. Recently, memantine, a non-competitive antagonist of glutamatergic NMDA receptors has been approved for treatment of advanced stages of AD. Although memantine is less toxic than other potential drugs, its effectiveness in AD is still under continuous evaluation. Inflammation surrounding Aβ plaques is thought to be a key factor in the progression of AD dementia. Therefore nonsteroidal anti-inflammatory drugs have been tested in various preventive protocols [57,120]. In general, nonsteroidal anti-inflammatory drugs show some neuroprotective effect, but the overall benefit for AD patients is not significant [2,107]. Also, evidence is growing that Ginkgo biloba extract improves cognitive functions in AD patients [97]. Although the overall benefit of Ginkgo therapy is accepted, there have been some side effects, including coma, bleeding, and seizures [42,45,92]. In another approach, scientists have been exploring the possibilities of a vaccine-based therapy for AD. Vaccination of transgenic mice against human Aβ, a constituent of senile plaques in AD, led to clearance of this protein from the brain [108]. However, in human trials, some subjects developed autoimmune encephalitis that prompted termination of the trial [27]. Evidence continues to accumulate that protection against increased levels of intracellular ROS may provide the best approach for the prevention of cognitive decline in elderly patients. Oxidative stress is one of the earliest events of AD, and may prove to be an important mediator in the onset, progression and pathogenesis of the disease. The sources of ROS-mediated damage appear to be multi-faceted in AD, with interactions between abnormal mitochondria, NADPH oxidase, transition metals, and other factors.

Recently, significant progress has been made in testing the clinical utility of CLN. In the first observational clinical trials, volunteers were treated with low doses of CLN (100 and 200 µg/day/patient) for three weeks. As a result of the treatment, volunteers developed a transient tolerance to CLN, as measured by the induction of IFN-γ and TNF-α in the whole blood cultures [58]. It is worth mentioning that CLN tolerance has been documented in only two volunteers. No statistical data were obtained, and, more importantly, the two volunteers responded differently. Interestingly, these initial findings were used for the development of a clinical protocol for a study in AD. Hence the protocol for the subsequent interventional study included a 2 weeks hiatus between 3 weeks long sessions of CLN treatment [78,79]. Initially 46 AD patients were recruited into the study. Patients were randomly assigned for treatment with CLN (100 µg per dose, every other day); commercially available bioorganic selenium (100 µg per dose, every other day) or placebo tablets. Each patient received 10 cycles
of treatment during the year of the clinical trial (3 weeks of treatment and a 2 weeks hiatus). The clinical benefits were assessed by psychiatrists who were blinded to the type of treatment. Eight of the 15 AD patients treated with CLN improved and the disease had stabilized in 7 others. In contrast, none of the 31 patients from the selenium or placebo groups improved. Most importantly, in addition to significant improvements in disease, no serious side effects were reported during this study. In a subsequent trial, 33 patients with mild to moderately severe AD symptoms were enrolled for long-term CLN treatment (16–28 months) and evaluated by the Mini-Mental-State Examination (MMSE) scale [78]. Again, the results showed that CLN given orally (100 µg per every other day) resulted in stabilization of the disease with a trend of improvement. The adverse reactions (including anxiety, logorrhea, and insomnia) were remarkably mild.

The largest interventional study with CLN in AD was recently reported in the Journal of Alzheimer’s Disease [14]. The study was designed to confirm or negate findings from earlier trials demonstrating that CLN has potential for the treatment of mild or moderate AD. One hundred and five patients were recruited from six psychiatric centers in Poland. The trial consisted of a 15 weeks double-blind phase comparing CLN with placebo, followed by a second 15 weeks open-labeled phase when all patients received CLN. The dosage of CLN was 100 µg on alternate days for three weeks, followed by a 2 weeks drug-free period. This cycle was repeated three times for each phase. The primary outcome measures used were an AD assessment scale-cognitive portion and clinical global impression of change. Secondary outcome measures were instrumental activities of daily living (MMSE): the AD assessment scale, non-cognitive test and overall patient response. The main outcome measures were assessed at week 15 when active treatment was compared with placebo, but all parameters were evaluated at baseline between weeks 15 and 30. Two separate statistical analyses were undertaken, a full sample analysis, in which all missing values were replaced with the worst result observed and a valid for-efficacy analysis, in which those patients who had serious protocol violations were excluded. This resulted in 14 patients being excluded from the valid efficacy-analysis. The full-sample analysis at week 15 showed a significant (p = 0.02) stabilizing effect of CLN on cognitive functions in the AD assessment, scale-cognitive test and on instrumental activities of daily living. The overall patient response was also in favor of the active treatment (p = 0.03). Patients graded as mild on entry also showed a superior (p = 0.01) response, as measured by the AD assessment scale, cognitive test compared with more advanced cases. Evidence from this study indicates an early, beneficial effect on cognitive symptoms and daily function. Again, based on the current knowledge of CLN safety and toxicity, it seems reasonable to revise the original clinical protocol and consider a different drug regimen. In the context of this potential re-evaluation of the original protocol, it is worth mentioning a recent presentation entitled “Colostrinin increases the lifespan and neurological performance in senescence accelerated mouse model” [15]. In this study the beneficial effect was demonstrated for mice treated with CLN daily, at a dose equivalent to that used in human study for the entire lifespan. Thus, it seems apparent that the use of CLN is safe and no toxicity is associated with daily low dose administration of CLN for a prolong period of time.

**CONCLUSION**

Oxidative stress is believed to be one of the leading contributor to the aging processes and to age-associated diseases, such as AD, the most frequent form of demen-
...tia among the elderly. Oxidative damage occurs early the in brain of AD patients, before the onset of plaque pathology, precedes Aβ deposition, and is characterized by such neuropathological hallmarks as extracellular Aβ plaques and intracellular neurofibrillary tangles, composed of the abnormally hyperphosphorylated protein, tau. Tau and Aβ, as well as mitochondrial dysfunction, exhibit synergistic effects leading to deficient energy metabolism and accelerated neurodegeneration. When given orally, CLN has been shown to have a stabilizing effect on cognitive functions in improving the conditions of patients suffering from mild and moderate AD. In line with these findings, CLN resulted in a 100-fold enhancement of retention of memory and learning and improved various motor and sensory activities in animal models. These observations, supported by findings in cell culture models including modulation of cellular redox, prevention of mitochondrial dysfunction in oxidatively-stressed cells, as well as induction of cell differentiation/neurite outgrowth, are similar to those induced by hormones and neurotropins. Peptides in CLN bind to as-yet-unidentified membrane receptor(s), and induce signaling pathways that appear to be common to regulation of cell proliferation, differentiation and amelioration of chemokine cytokine production in inflammatory processes. Although extensive studies are required to understand the mechanism by which CLN exerts its biological effects, it clearly has a potential benefit in the prevention and treatment of neurodegenerative disorders, especially those of the central nervous system.

ACKNOWLEDGEMENT

This work was supported by ReGen Therapeutics, Plc, London, England. Dr. Kruzel serves as a consultant to ReGen Therapeutics, Plc. We are grateful to Mardelle Susman for scientific and editorial advice and corrections made in the manuscript.

References

hypersensitivity and allergic responses to common allergens, Int Arch Allergy Immunol (2008), In press.


