COLOSTRININ™ INCREASES THE LIFE-SPAN AND NEUROLOGICAL PERFORMANCE IN SENESCENCE ACCELERATED MICE

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ABSTRACT

Background and aims: Colostrinin™ (CLN), a uniform mixture of low-molecular weight proline-rich polypeptides extends the life-span of diploid fibroblasts, induces neurite outgrowth of pheochromocytoma cells, decreases mutation frequencies in both Chinese hamster and human cells and inhibits beta amyloid-induced apoptosis in human neuroblastoma cells. Most importantly, oral administration of CLN has shown a stabilizing effect on cognitive function in Alzheimer's patients measured by the Alzheimer's disease Assessment Scale-cognitive (ADAS-cog) and in Instrumental Activities of Daily Living (IADL). In this study, we investigated the effects of oral administration of CLN on the life-span and various behavior characteristics in senescence-accelerated mice. *Methods:* The battery of behavioral tests included: swim maze, locomotor distance, rotorod running, walking initiation, alley turning, bridge walking, wire suspension, and discriminated active avoidance tests. *Results:* Here we show that CLN administration to mice prolongs life-span (26% increase), improves age-associated locomotion, motor coordination, and learning/memory capacities. Increase in life-span and improved neurological performance correlated well with the levels of oxidative stress markers measured in various organs. In particular, we demonstrate an improved mitochondrial function, decrease in levels of 8-oxoguanine in nuclear and mitochondrial DNA and significantly reduced oxidative damage to proteins in brain and liver. *Conclusions:* These results support the view that this newly discovered characteristic of CLN underlines its utility in age-related neurodegenerative diseases, and the quality life improvement in the elderly.

INTRODUCTION

Colostrinin™ (CLN) is isolated from colostrum by various chromatographic steps, including ion exchange and affinity, and molecular sieving, combined with ammonium sulfate precipitation (Janusz and Lisowski, 1993; Janusz et al., 1974; Kruzel et al., 2001). It has been shown that CLN is an important immune-modulator, which induces maturation and differentiation of murine thymocytes (Janusz and Lisowski, 1993; Zimecki et al., 1984), promotes peripheral blood leukocyte proliferation, and induces various cytokines (Janusz and Lisowski, 1993; Stanton, 2001). We have recently shown that CLN decreases intracellular oxidative stress levels, reduces 4-hydroxynonenal (4HNE)-mediated cellular damage and suppresses 4HNE-induced cellular signaling in cultured cells (Boldogh et al., 2003; Boldogh, 2001). Most importantly, CLN induces delicate cassettes of signaling pathways common to cell proliferation and differentiation, and mediates activities that are similar to those of hormones and neurotrophins, leading to neurite outgrowth (Bacsi et al., 2005). CLN protects neuroblastoma cells from beta amyloid-induced apoptosis by inhibiting amyloid aggregation (Schuster et al., 2005). In a recent study, its administration to one-day-old domestic chicks significantly enhanced long-term memory retention in a passive avoidance model (Stewart and Banks, 2006). Remarkably, its administration to Alzheimer's patients resulted in improvement in cognitive functions and instrumental activities in daily living (Bilikiewicz and Gaus, 2004; Leszek et al., 1999).

We have recently showed that CLN significantly decelerates the senescence processes of cultured murine diploid fibroblast (MDF) cells and increases their population-doubling levels. CLN-increased lifespan of MDF was comparable to an increase in population-doubling levels at low oxygen environment. This action of CLN is associated with a decrease in the intracellular levels of reactive oxygen species, which may be due to senescence-associated mitochondrial dysfunction (Bacsi et al., 2007). These data suggest that CLN may delay the development of cellular aging at the level of the organism.

In this context, the purpose of the present study was to determine whether or not CLN intake improves cognitive-motor performance and increases life-span of a senescence-prone strain of mice (SAMP1). Accordingly, groups of CLN fed and mock-fed mice were tested for their ability to perform on 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 battery 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.

Results of these studies show that CLN increases life-span, improves cognitive-motor functions and effective in reversing preexisting age-related impairments of cognitive or motor function. Moreover, the current findings indicate that CLN may have beneficial effects on some brain functions therefore it may be used in therapeutic approaches aimed at improving symptoms of age-associated neurodegenerative diseases.

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MATERIALS & METHODS

Animals

The senescence-prone strain of mice, SAMP1, was obtained from Harlan Sprague Dawley, Inc (Madison, WI, USA) and subsequently maintained individually in clear polycarbonate cages (five-mouse units) in the University of Texas Medical Branch, Animal Care Unit, at Galveston, TX. The ambient temperature was maintained at 23 ± 1°C, under a 12 h light/dark cycle starting at 06:00 h. Mice had free access to food and water except during the testing sessions. Mice were fed with the standard NIH-31 formulation. The diets were formulated by Harlan Teklad (Madison, WI, USA) using NIH-31 formula as the base diet. Body weights were recorded monthly.

CLN Administration

CLN and all controls (colostrum, BSA hydrolysate) were administered via drinking water. The concentrations of test materials were set so that individual mice obtained 100 ng or 10 ng per kg per day. CLN and colostrum was obtained from ReGen Therapeutics, Plc. London, England. BSA hydrolysate was prepared locally as we described previously (Boldogh et al., 2003).

Spatial learning and memory

Spatial learning and memory were measured using a swim maze test as described previously (Foster et al., 1996, Sumien et al., 2004). On a given trial, the mouse was allowed to swim in a steel tank (110 cm diam × 60 cm deep), filled with opaque water (nontoxic white paint) maintained at 24 ± 1°C; an escape was provided by means of a small platform hidden from view 1.5 cm below the surface of the water. A computerized tracking system recorded the length of the path taken by the mouse to reach the platform as well as the swimming speed (San Diego Instruments, San Diego CA, Model SA-3). During the pre-training period, mice learned the motor components of swimming and climbing onto the platform, without learning its location in the tank. Subsequently, mice were tested for their ability to learn the location of the platform during three phases: acquisition (8 sessions), retention (2 sessions after a 2-day rest), and reversal (4 sessions with the platform at a different location). Each session consisted of three trials during which the mouse had to swim to the platform from a different starting point in the tank.

Locomotion

Walking initiation and alley turning latencies were recorded to a maximum of 60 s on each of three consecutive days. For walking initiation, the mouse was placed on an open table and the latency for both hind legs to leave a 10-cm-diameter circle was recorded. For alley turning, the mouse was placed backward in a 3.5-cm alley and the latency to turn and face the open end was recorded. Distance traversed was recorded automatically during a 16-min period after the mice had been isolated in 40.5 x 40.5 x 30.5 cm clear acrylic activity chambers (Forster & Lal, 1992, Sumien et al., 2004).

Motor coordination

Maximum running capacity was measured using a procedure described previously (Forster et al., 1996, Sumien et al., 2004). The apparatus was a motor-driven treadmill (model Omnirotor Treadmill; Omnitech Electronics) that consisted of a 3.2-cm diameter nylon cylinder mounted horizontally 35.5 cm above a padded surface. The cylinder was separated into 11-cm divisions by circular plastic flanges and could be made to rotate (via a microprocessor-controlled motor) with constant acceleration. On a given trial, the mouse was placed on the cylinder and the speed of rotation increased by 0.5 rpm/s until the mouse could no longer perform the running response and fell to the padded surface. The cylinder speed or elapsed time at which the mouse fell was recorded as the measure of running performance. The mice received twice daily sessions consisting of four trials (each separated by 10 min) until at least eight sessions had been completed and a performance stability criterion had been met (three consecutive sessions over which the four-trial mean latency to fall did not differ by more than 15%). Maximum running speed was the fastest daily average achieved during testing (typically, one of the last three training days).

For the bridge-walking test, each mouse was placed on the center of a 13-mm-square rod suspended between two safe platforms (60 cm apart) located 45 cm above a padded surface. The latency to fall was recorded on each of three trials (a maximum fall latency of 60 s was scored for mice which had reached the platform in less than 60 s) (Sumien et al., 2004).

The wire suspension test was performed twice on each of three test days. The mouse was placed by its front paws on a horizontal wire approximately 27 cm above a foam pad, and the latency to fall was recorded for up to 60 s (Sumien et al., 2004).

Active avoidance

This test involved one component (the first training session) of a more general procedure described previously (Forster, & Lal, 1992; Forster, et al.,1995). The apparatus was an acrylic T-maze with compartments in the stem and goal arms, each demarcated with a removable door. The maze was situated on a grid floor wired for scrambled shock from a commercial source (Model E13-08; Coulbourn Instruments, Allentown, PA). During a single session, mice were trained to leave the start box and run to a designated correct goal arm within 5 s following the opening of the start door. On the first trial, entry into the incorrect arm was forced (by briefly closing the door to the correct choice), whereupon the mouse received shock and was allowed to escape by entering the correct arm. Thereafter, mice received a series of trials (separated by a 1-min interval) in which the start door opened and the mouse could avoid shock by running to the correct goal within 5 s. If more than 5 s elapsed or the mouse made an incorrect turn at the choice point, shock was initiated and continued until the mouse entered the correct goal. Training continued until each mouse had made a correct avoidance response (entering the correct arm of the T-maze within 5 s) on at least four of the last five training trials.

Statistical analysis

The data from most of the measures were subjected to two-way analyses of variance, with age and supplementation as between-groups factors. Planned individual comparisons of CLN-fed versus control groups and between age-matched treatment groups were made using single-degree-of-freedom *F* tests and the error term for the two-way interaction. Swim maze and coordinated running data were subjected to three-way analyses, with repeated measures on the sessions or the trials factor

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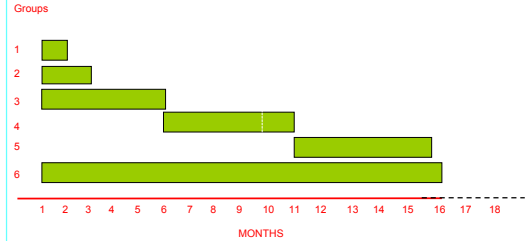
RESULTS

FIGURE 1

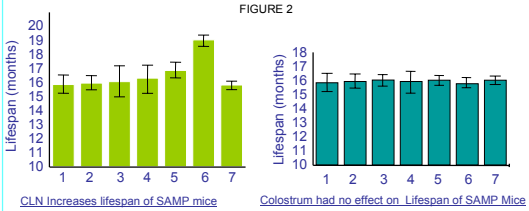


Treatment protocol

Group 1: CLN-fed for 1 ms: from age 1 month to 2 months old
Group 2: CLN-fed for 2 ms: from age 1 months to 3 months old
Group 3: CLN-fed for 5 ms: from age 1 months to 6 months old
Group 4: CLN-fed for 5 ms: from age 6 months to 11 months old
Group 5: CLN-fed for 5 ms: from age 11 months to 16.2 months old
Group 6: CLN-fed from 1 ms for their lifespan

Control groups B 1-6 were fed with Colostrum
Control groups C 1-6 were fed with BSA-hydrolysate

FIGURE 2



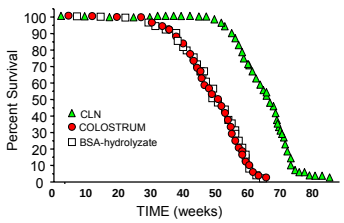
CLN Increases lifespan of SAMP mice

Group 1(n=42): CLN-fed for 1 ms: from age 1 to 2 months old
Group 2 (n=37): CLN-fed for 2 ms: from age 1 to 3 months old
Group 3 (n=39): CLN-fed for 5 ms: from age 1 to 6 months old
Group 4 (n=41): CLN-fed for 5 ms: from age 6 to 11 months old
Group 5 (n=46): CLN-fed for 5 ms: from age 11 to 16.2 months old
Group 6 (n=49): CLN-fed from 1 ms, for their life-span
Group 7 (n=37): BSA-hydrolyzate for their life-span

Colostrum had no effect on Lifespan of SAMP Mice

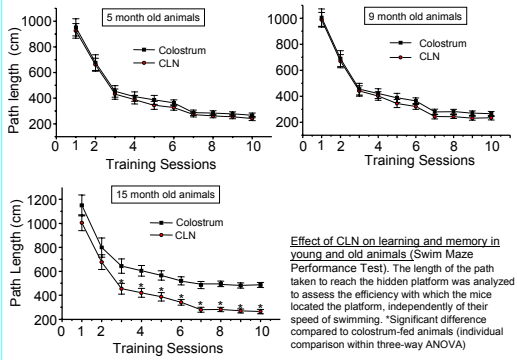
Group 1(n=38): COL-fed for 1 ms: from age 1 to 2 months old
Group 2 (n=41): COL-fed for 2 ms: from age 1 to 3 months old
Group 3 (n=40): COL-fed for 5 ms: from age 1 to 6 months old
Group 4 (n=37): COL-fed for 5 ms: from age 6 to 11 months old
Group 5 (n=44): COL-fed for 5 ms: from age 11 to 16.2 months old
Group 6 (n=42): COL-fed from 1 ms, for their lifespan
Group 7 (n=37): BSA-hydrolyzate, for their life-span

FIGURE 3



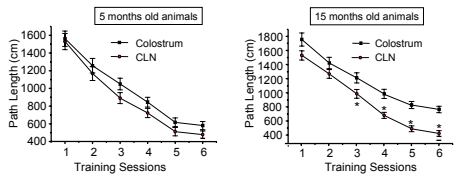
Life-span of CLN- colostrum and BSA-hydrolysate-fed mice. Mice were fed with CLN (in drinking water, 0.01 µg per kg) from age 1 month old for their entire life-span. CLN group: male mice (n = 50) and female mice (n = 45) were used to construct the survival curves. Colostrum group: male mice (n = 41), female mice (n = 40), BSA-hydrolysate group: male mice (n = 47), female mice (n = 44), were used to construct the survival curves.

FIGURE 4



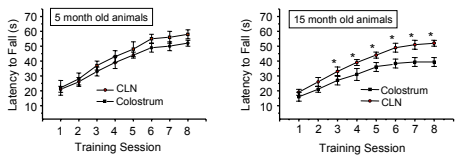
Effect of CLN on learning and memory in young and old animals (Swim Maze Performance Test). The length of the path taken to reach the hidden platform was analyzed to assess the efficiency with which the mice located the platform, independently of their speed of swimming. *Significant difference compared to colostrum-fed animals (individual comparison within three-way ANOVA)

FIGURE 5



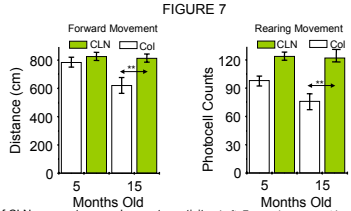
CLN improves learning and memory of aged animals shown by reversal phase swim maze test. During the reversal phase, for which the platform was moved to a new position, the CLN-fed aged animals tended to swim to the new platform position more efficiently than colostrum-fed animals. Each panel shows the mean path length (cm ± SE) on each of the six individual trials after the position of the hidden platform had been moved. *Significant difference (individual comparison within three-way ANOVA).

FIGURE 6



CLN increases motor learning and maximum performance of 15 month old mice. Each panel shows the mean latency to fall from the rotating cylinder in seconds (± SE) on each of the six training sessions. During the final two sessions (7 and 8) no further improvements occurred. *Significant difference from colostrum-fed (individual comparison within three-way ANOVA).

FIGURE 7



Effects of CLN on spontaneous locomotor activity. Left: Forward movement in centimeters within the activity chamber. Right: rearing behavior (standing on the hind limbs), measured by photocell counts (± SE) within a vertical plane 7.6 cm above the floor of the activity chamber. *Significant difference between control and CLN-treated (individual comparison within two-way ANOVA). Col: colostrum.

TABLE 1

CLN Supplementation improves on Motor Skills and Sensory Reactivity[1]

	5 months old		9 months old		15 months old	
	CNTRL	CLN	CNTRL	CLN	CNTRL	CLN
Motor Skills[2]						
Walking Initiation	2.1±0.25	2.2 ±0.2	2.3±0.15	2.2±0.3	3.1±0.25	2.3±0.2
Alley Turning	10.5±1.3	11.2±1.5	10.2±1.0	9.8±0.8	18.4±2.5	12.3±1.5
Negative Geotaxis	9.8±0.5	9.2±0.5	9.6± 1.5	8.8±1.0	14.9±1.0	10.1±0.5
Wire Grip	32.5±2.0	34.6±1.5	31.0±2.0	36.5±0.5	23.0±2.0	30.0±2.0
Bridge Walking	44.4±2.5	46.1±3.0	42.0±2.5	47.2±3.0	26.5±2.5	41.0±2.0
Sensory Activity						
Auditory Startle (force units)	4.7±0.5	4.4±1.0	5.1±2.5	5.3±0.5	2.3±0.5	4.4±1.2
Shock Startle (force units)	24.5±2.2	25.3±1.9	24.2±1.5	26.4±1.2	15.3±2.0	23.6±1.7
Reaction Time (ms)	46.2±2.5	42.1±1.2	45.4±2.0	40.1±2.5	59.8±2.0	46.1±0.2

1, All values are the group means ± SE; 2, Mean latency in seconds

CONCLUSION

♦ Mice receiving CLN (10 ng per kg per day) showed a 26% increase in median life span
♦There was no toxic effect observed in CLN-fed animals treated for their entire life-span.
♦CLN intake had no significant effect on performance at age 5 months and 9 months; however, at 15 months of age the performance during acquisition and retention phases were significantly better (p=0.011)
♦ During the sessions of the reversal phase, the aged CLN-fed mice swam to the new platform position more efficiently than any of the control groups, as reflected in a significant three-way interaction of CLN, age, and test session (p < 0.002)
♦ CLN improves spontaneous locomotor activity at young and old mice
♦ Analyses of variance on alley turning, wire grip, and bridge walking latency indicated significant effects of age (p < .001), reflecting age-related declines in performance, and there are apparent positive effects or interactions involving CLN supplementation
♦The startle responses of the aged mice to auditory stimuli were markedly increased in CLN-fed animals at all intensities when compared with controls
♦ Results of these studies show that CLN increases life-span, improves cognitive-motor functions and effective in reversing pre-existing age-related impairments of cognitive function. These findings indicate that CLN have beneficial effects on brain functions therefore it may be used in therapeutic approaches aimed at improving symptoms of age-associated neurodegenerative diseases

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