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MINI-REVIEW

Use of Carnosine as a Natural Anti-senescence Drug for Human Beings

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Abstract—Carnosine is an endogenous free-radical scavenger. The latest research has indicated that apart from the function of protecting cells from oxidation-induced stress damage, carnosine appears to be able to extend the lifespan of cultured cells, rejuvenate senescent cells, inhibit the toxic effects of amyloid peptide (Aβ), malondialdehyde, and hypochlorite to cells, inhibit glycosylation of proteins and protein–DNA and protein–protein cross-linking, and maintain cellular homeostasis. Also, carnosine seems to delay the impairment of eyesight with aging, effectively preventing and treating senile cataract and other age-related diseases. Therefore, carnosine may be applied to human being as a drug against aging.

Key words: carnosine, anti-senescence drug

Carnosine is a non-enzymatic free-radical scavenger and a natural antioxidant as well [1, 2]. It is widely distributed in tissues and exists at a particularly high concentration in muscle and brain [3, 4]. Carnosine was discovered by the Russian scholars Gulewitsch and Amiradzibi in 1900 [5] and other Russian scholars, namely Severin and Boldyrev, made great contributions to research on the biological effects and medical application of carnosine. Generally, the biological properties of carnosine are not better known more than those of SOD and vitamins C and E, but scholars throughout the world have become very interested in its properties as an antioxidant, immunomodulator, and neuroprotector against free radicals [6-8]. Various studies have shown that carnosine could be used in the therapy of gastric ulcer and burns, to lower blood pressure, and to protect the brain from ischemia/reperfusion injury [1, 9]. The goal of this article is to elucidate experimental research and medical application of carnosine as an anti-senescence drug.

FREE RADICALS AND SENESCENCE

Since the American scholar Harman [10] suggested that free radicals are possibly one of the important factors causing aging and senile diseases, the theory has been gradually accepted by scholars all over the world. Many findings have strongly shown that the emergence and development of aging and senile diseases are intimately associated with oxygen free-radical-induced damage to cells, which leads to instability and malfunction of cells and consequently to the occurrence of aging and senile diseases. Such diseases as atherosclerosis, diabetes [11], Alzheimer's disease [12, 13], and senile cataract [14, 15] are all related to free radical induced damage. Further research on the biological effects of various free-radical scavengers and antioxidants has demonstrated that they have the ability of protecting cells from oxygen free radical modulated damage and a normalizing function on the metabolism of cells. Some known anti-oxidants like SOD, vitamin E, EGB761, and melatonin have already been taken into medical practices and positive results have been obtained. Thus, scholars all over the world now hold the shared hope of exploring and developing a more effective free radical scavenger in order to increase longevity.

EVIDENCE FOR THE ANTI-AGING EFFECT OF CARNOSINE

The biological effects and medical application of carnosine have even been investigated generally and intensely. Besides being an antioxidant and free radical scavenger, carnosine is inclined to enliven membrane enzymes, to inhibit non-enzymatic glycosylation of proteins, and to modulate immunoreactivity. Recent data

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report that carnosine has the ability of rejuvenating senescent cells [16], and our research on medical application also indicates that carnosine can delay eyesight impairment with aging while simultaneously exerting a remarkable effect on prevention and treatment of senile cataract.

Evidence for the anti-aging effects of carnosine from experiments *in vitro*. McFarland and Holliday [16] have examined the effects of carnosine on the growth, morphology, and lifespan of cultured human diploid fibroblasts. They have demonstrated that the cells grown in DMEM with 50 mM carnosine have a flat, spread-out appearance, with very uniform spacing of cells, and these cells continued to grow with unaltered morphology for most of their life span. They found that these late-passage cultures preserve a non-senescent morphology in the presence of carnosine. In the experiment, when control MRC-5 cells at population doublings (PDs) 55.3 and 55.1, which were showing characteristic signs of senescence, were transferred to medium containing carnosine, these cultures showed remarkable rejuvenation These cells continued to grow slowly for an additional 274 days and finally reached PD levels significantly greater than the control cultures.

McFarland and Holliday used MRC-5 and HFF-1 cells to examine the effects of carnosine on cell attachment and plating efficiency [17]. They observed that 20 mM carnosine in DMEM medium has a strong stimulatory effect on colony formation of young cells and even more striking effect on late-passage senescent cells. They also have found that the presence of carnosine clearly rejuvenates cells, allowing more colonies to appear and grow to a larger size and that the largest colonies are equivalent to about 15 PDs from a single cell. By additional experiments, they proved that the life span was increased by carnosine in a dose-dependent manner.

McFarland and Holliday observed that carnosine promoted the attachment and survival of cells over a long period and that cells held in medium containing carnosine for a long period also had a more normal phenotype than those kept in un-supplemented medium. Using three different approaches (microscopic, flow cytometric, and ELISA for one of the markers of DNA oxidative damage), Kantha et al. [18] studied in rat embryonic fibroblasts the effect of L-carnosine on retention of cell morphology during a nutritional insult. They found in microscopic observation that in contrast with cells grown in control medium, the fibroblasts subjected to the same degree of nutritional insult but grown in medium supplemented with 30 mM L-carnosine retained the normal morphology and that the integrity of the cells was preserved after four weeks. The results obtained by flow cytometric measurements suggested that the percentage of cell damage occurring in controls was noticeably higher in comparison to fibroblasts grown in DMEM with 30 mM L-carnosine. The cell viability profile of fibroblasts grown in 10 mM L-carnosine was intermediate between that of controls and those grown in 30 mM L-carnosine. 8-OH-dG is one of the major products of oxidative DNA damage. They reported that fibroblasts grown in control medium showed a significant (fivefold) increase ($p < 0.05$) in the release of 8-OH-dG after five weeks; cells grown in the medium supplemented with 30 mM L-carnosine did not show such a comparable increase in the release of 8-OH-dG. Their results showed carnosine could sustain the retention of cell morphology in continuous fibroblast culture subjected to nutritional insult.

The fibrillar form of Aβ is a defining feature of Alzheimer's disease (AD) [19]. Aβ is known to increase oxidative stress in endothelial cells and smooth muscle cells of cerebral blood vessels, which accumulate the peptide during AD [20]. It inhibits endothelial cell replication and is directly toxic to both peripheral and cerebral vascular endothelium. In the brain, Aβ could result in impairment of the blood–brain barrier. Preston et al. [7] studied the protection by carnosine from toxic effect of truncated form of Aβ on immortalized rat brain vascular endothelial cells (RBE4). Using a mitochondria dehydrogenase activity reduction assay, lactate dehydrogenase release, and glucose consumption, they found that addition of 20 mM carnosine immediately before Aβ treatment significantly protected the cells from toxic effects of 200 and 300 μg/ml of Aβ. They postulate that the mechanism of carnosine protection lies in its antiglycating and antioxidant activities, both of which are implicated in neuronal and endothelial cell damage during AD.

Medical application of carnosine. From our investigations, we have reported [21, 22] that eye drops containing 20 mM carnosine were used to treat 96 patients aged 60 years old having senile cataract of various degrees of maturity, with the duration of the disease from 2 to 21 years. The method is that after stopping the use of all other anti-cataract drugs, patients instilled 1-2 drops of the carnosine-containing solution in each eye 3-4 times each day for a period of treatment ranging from 3 to 6 months. The level of eyesight improvement and the change of lens transparency were considered as an evaluation index of the curative effect of carnosine. The result shows that carnosine gives a pronounced effect on primary senile cataract, the effective rate being 100%. For mature senile cataract, the effect rate is 80%, and positive effects were observed with other types of cataract. It is significant that no side effect has been found in the observed cases. During recent years, we have also applied carnosine drops containing the same content to nearly one thousand patients with senile cataract. Our research findings (ready to be published) show similar result.

In addition, we applied carnosine drops to patients aged 48-60 years with various degrees of eyesight

impairment but without symptoms of cataract. The course of treatment is from 2 to 6 months. The results demonstrate that carnosine appears to alleviate eye tiredness and comparatively improve eyesight (obviously improve eyesight, giving more clear vision). Subjects reported that carnosine could brighten and relax their eyes. It is an important point that all the above research on medical application of carnosine has statistical significance.

Therefore, carnosine has the potential of being a drug to prevent and treat senile cataract and to delay eyesight senescence, which is one of the manifestations of aging. The pharmacological effects of carnosine seem to due to its penetration into the lens [14, 23], then boosting the antioxidant ability of the lens, increasing the activity of Na,K-ATPase [24, 25], and regulating metabolism and other physiological reactions of the lens. It is reported in Australia and Russia that carnosine may be proposed to be used for skin care and smoothing wrinkles, and as a tonic remedy in Germany; our data is also proof that carnosine has anti-senescence effects. All these results provide valuable data for further efforts of making carnosine a drug against senescence.

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