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Review Article

Carnosine and Related Peptides: Therapeutic Potential in Age-Related Disorders

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ABSTRACT: Imidazole dipeptides (ID), such as carnosine (*β*-alanyl-L-histidine), are compounds widely distributed in excitable tissues of vertebrates. ID are also endowed of several biochemical properties in biological tissues, including antioxidant, bivalent metal ion chelating, proton buffering, and carbonyl scavenger activities. Furthermore, remarkable biological effects have been assigned to such compounds in age-related human disorders and in patients whose activity of serum carnosinase is deficient or undetectable. Nevertheless, the precise biological role of ID is still to be unraveled. In the present review we shall discuss some evidences from clinical and basic studies for the utilization of ID as a drug therapy for age-related human disorders.

Key words: imidazole dipeptides; biological activity; aging; children; serum carnosinase deficiency

Introduction

Imidazole dipeptides (ID) in biological tissues

The mammalian essential amino acid L-histidine originates compounds in biological tissues, including histamine [1], and the ID carnosine, homocarnosine, and anserine [2]. Liquid chromatography/electrospray ionization tandem mass spectrometry analyses performed in plasma or tissue homogenates from rats showed that the highest concentrations of carnosine and anserine are found in skeletal muscles, heart and brain. Homocarnosine was found only in the brain. No ID were detectable in plasma, liver, kidney and lung [3].

The homocarnosine biosynthesis from L-histidine and *γ*-aminobutyric acid (GABA) is favored by a ligase enzyme namely carnosine synthase (EC 6.3.2.11) [2]. Homocarnosine is present in human central nervous system (CNS) and is synthesized in specific GABAergic neurons [4–5]. Anserine is not present in human tissues [6], and it may be isolated from chicken pectoral muscle extracts [7]. This dipeptide is subject to cleavage to *β*-alanine and 1-methyl-L-histidine by the human serum

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carnosinase, also known as β-Ala-His dipeptidase (EC 3.4.13.20) activity [8].

Carnosine

Carnosine is a naturally occurring dipeptide (β-alanine and L-histidine), widely distributed in muscular and nervous tissues of vertebrates, whose concentrations may reach up to 20 mM [9–10]. The non-proteinogenic and precursor amino acid β-alanine is mainly synthesized by the liver degradation of uracil, from the conversion of β-ureidopropionate in β-alanine, ammonia, and carbon dioxide catalyzed by β-ureidopropionase (EC 3.5.1.6) [11]. Carnosine was first isolated from skeletal muscle extracts in the end of XIX century by Gulewitsch and colleagues [12–3].

Dietary carnosine and anserine uptake is carried out by an intestinal peptide transporter namely human H^+/peptide cotransporter 1 (hPEPT1) [14]. Carnosine biosynthesis from its precursor amino acids is catalyzed by carnosine synthase coupled to ATP hydrolysis [2]. It occurs in excitable tissues, such as skeletal muscle cells [15] and oligodendrocytes [16]. On the other hand, the cleavage of carnosine and homocarnosine is carried out by serum carnosinase [8], which is synthesized in brain and liver [17] and secreted into the cerebrospinal fluid (CSF) and blood [18]. Other dipeptidase able to cleave carnosine in biological tissues is the cytosol nonspecific dipeptidase (CNDP, EC 3.4.13.18). This protein is constitutively expressed in brain, kidneys, liver, spleen, gonads, lungs, and pancreas [17]. In the brain, CNDP is synthesized in histaminergic neurons of tuberomammillary nucleus, which is located in hypothalamus [19]. The products of ID hydrolysis are metabolized and excreted in urine [6].

Several biochemical properties have been assigned to ID by researchers since the past century, in animal and cell culture studies. Carnosine is the most studied compound in the group of imidazolic dipeptides and presents putative antioxidant [20], bivalent metal ion chelating [21], radioprotective [22], muscular proton buffering [23], and reactive carbonyl scavenger activities [24]. The latter property was also suggested to the D-enantiomer of carnosine [25].

Furthermore, many physiological effects were associated to carnosine and other peptides. For instance, evidence suggests that carnosine is distributed in dorsal horn of mice spinal cord; and presents antinociceptive activity [26]. Other physiological effect assigned to carnosine is the immunostimulant activity. Thus, Li and co-workers [27] showed that the number of lymphocytes in spleen and the level of plasma corticosterone presented no significant alterations in stressed animals receiving carnosine previously. The administration of this peptide also elicited antidepressant-like effects in rats subjected to forced swimming [28]. Since most of the mechanisms underlying the physiological effects of ID reported hitherto are unknown, the precise role of ID in biological tissues and fluids remains unclear.

Is the biological activity of ID associated to age of the subject?

It has been suggested that carnosine may be involved in the prevention and therapy of oxidative driven disorders, including neurodegenerative diseases and hypoxic-ischemic damage of CNS [29]. The major risk factor to neurodegenerative disorders is aging, and mitochondria were implicated in such phenomenon through mechanisms that include accumulation of mutations in mitochondrial DNA (mtDNA) and reactive oxygen species (ROS) generation [30].

Murphey and co-workers [31] reported carnosine accumulation in the serum (20–30 μmol/mL) and urine (60–200 μmol/24 h) of patients affected by a rare inherited metabolic disorder – serum carnosinase deficiency (SCD, OMIM 212200). The onset of the symptoms occurred during infancy and children presented progressive mental retardation, an unspecific symptom of such disorder. The present review gather information regarding the current state of ID utilization as drug therapy for age-related human disorders in clinical trials, as well as their safety.

Metabolic changes with aging

The explanation of the causes of aging remains somewhat controversial. However, the prime candidates seem to be environmental and endogenous factors which affect an organism's ability to survive by trigger genetic changes (e.g., DNA damage and telomere shortening), alteration in gene expression, oxidative stress, impairment of ATP provision, and accumulation of aberrant proteins [32]. According to the Free Radical Theory, aging and related neurodegenerative diseases are mainly assigned to oxidation of cellular components by free radicals, catalyzed by oxidative enzymes and traces of metal ions [33].

Mitochondria are the organelles responsible by ATP generation, Ca^{2+} uptake and storage, and generation and detoxification of ROS. The mitochondrial membrane potential ($\Delta\psi_m$) drives such functions, while many apoptotic signals are triggered by cytochrome *c* release into the cytoplasm [34]. Bioenergetics dysfunction is a more recent approach that may aid to understand the aging process. Respiratory chain complex I (EC 1.6.5.3) is thought to be mostly affected by oxidative damage, becoming strongly rate limiting for electron transfer [35]. Additionally, it was shown a significant decrease in the

state 3 of respiration and membrane potential in heart mitochondria of aged rats [36].

The oxidative damage and other age-induced alterations can be mitigated by long-term regimens of caloric restriction. The underlying mechanisms remain unknown, but caloric restriction protective effects may be mediated by the decrease in the mitochondrial proton leak [37], connecting disruption of bioenergetics to aging.

Mutations in mtDNA and decrease in the mtDNA copy number were associated to the pathogenesis of type 2 diabetes [38] linking, in part, this metabolic disorder to aging. However, the age-related energy dysfunction cannot be only attributable to changes in the sequence and copy number of the mtDNA. According to analyses carried out by Bakala and co-workers [39], liver mitochondria of aged rats present a decline in their capacity to generate ATP mainly due to decreased expression of oxidative phosphorylation complexes and glycation damage to β-oxidation and citric acid/urea cycle enzymes.

The ROS net production by the mitochondria may be the major source of oxidative damage that accumulates with age and it is detrimental to many mitochondrial functions. The contributing factors appear to include the intrinsic rate of proton leakage across the inner mitochondrial membrane, decreased membrane fluidity, and decreased levels and function of the cardiolipin, a membrane phospholipid that supports the functioning of many proteins of the inner mitochondrial membrane [40]. The oxidation of such compound seems to be involved in several age-related mitochondrial bioenergetics changes, such as electron transport chain impairment, substrate transport defects, activation of mitochondrial permeability transition, and cytochrome *c* release [41].

Other age-related metabolic changes in the lipid metabolism may be triggered. The physiological actions of the circulating hormone leptin decrease with aging, which is associated to inflammation of adipose tissue, decreased thermogenesis, and possibly impaired cognitive function [42]. In this way, aging increases cholesterol trafficking proteins in astrocytes and myelination in hippocampus of F344 rats. In fact, an early-adult metabolic changes favoring lipid/ketone body oxidation may trigger inflammatory degradation of myelin and result in excessive cholesterol that, in turn, activates its transport from astrocytes to remyelinating oligodendrocytes. Such processes may compete with neuronal pathways for bioenergetics supplies, thereby impairing cognitive function [43].

Other molecular and cellular signaling pathways were also suggested to underlie aging process. The nuclear factor-κB (NF-κB) triggers a pro-inflammatory environment with glycolytic metabolism, whereas the silent information regulator 1 (SIRT1) supports oxidative respiration and promotes the resolution of the inflammation. Increased NF-κB activation and subsequent inflammatory responses disturb bioenergetics driven by SIRT1, which could contribute to the aging process due to the potential generation of several age-related metabolic disorders, including type 2 diabetes and cardiovascular diseases [44]. Recently, it was proposed that ID might counteract many of the above-mentioned mechanisms associated to aging [45, 50, 58].

ID and aging: general mechanisms

The bulk of ID content synthesized in major vertebrates is present in excitable tissues, mainly in skeletal muscle [6]. HPLC analyses showed that the content of carnosine and anserine in skeletal muscle significantly decreases with age in SAMP8 senescence-accelerated mice. Oral creatine supplementation temporarily prevents such decline in muscle ID content and it is associated with attenuated muscle fatigue and enhanced recovery [46]. The proposed mechanisms for ID action include proton buffering [23] and sarcoplasmic reticulum Ca^{2+}-release channels modulation [47–8].

Antioxidant activity was assigned to carnosine [20], whose ability to react with superoxide, hydroxyl [9], and hypochlorite anions *in vitro* [49] was previously shown. Additionally, such dipeptide inhibits the non-enzymatic glycosylation and protein cross-linking induced by reactive aldehydes (e.g., MDA), the formation of protein-associated advanced glycosylation end products (AGEs) induced by MDA, and the formation of DNA-protein cross-linking induced by acetaldehyde and formaldehyde *in vitro* [49]. Carnosine administration (250 mg/kg, daily) for 1 month to aged rats (22 months old) resulted in significantly decreased levels of MDA and conjugated diene in serum, LDL + VLDL fraction, and erythrocytes, and increased GSH levels in erythrocytes [45].

Methylglyoxal (MG), a product of the spontaneous decomposition of glycolitic trioses phosphate, is a source of glycative damage to protein, mitochondrial dysfunction, and ROS generation. Carnosine reacts with MG and other reactive aldehydes, and scavenges various ROS, inhibiting ischemia and age-related mechanisms [50]. Additionally such dipeptide was showed to suppress the toxicity of protein carbonyls by reacting to form protein carbonyl-carnosine adducts [51] and inhibit glycation damage mediated by MG and glucose in *Escherichia coli* [52]. In fact, the putative anti-aging effect of carnosine seems more readily explainable by its ability to react with protein carbonyls than its antioxidant activity [51].

In attempt to examine the various mechanisms proposed to explain the anti-aging effects assigned to carnosine, studies searched for some of the carnosine

adducts that have been predicted following the dipeptide's putative reaction with reactive carbonyl compounds [53]. Evidence on quenching mechanism of ID towards α,β-unsatured aldehydes generated by lipid peroxidation (e.g., 4-hydroxy-*trans*-2-nonenal and acrolein) suggests that it occurs through a multi-step mechanisms, including: *i)* the formation of a reversible imine intermediate; *ii)* a key intramolecular Michael addiction between the histidinic imidazole ring and the acceptor β-carbon atom; and *(iii)* the hydrolysis of the imine group to give a final hemiacetal adduct (for more details, see reference 24). This was demonstrated *in vitro* through the reaction of carnosine and homocarnosine with acrolein, with further characterization of the formed adducts [54].

Another mechanism attributed to carnosine is modulation of glycolysis and inhibition the glycolysis-induced protein dysfunction. The underlying mechanisms are unclear, but the dipeptide may mimic the action of rapamycin, inhibiting the cellular respiration regulatory complex – mammalian target of rapamycin (mTOR) [55]. Alternatively, carnosine may activate the gluconeogenic enzyme fructose-biphosphatase (FBPase, EC 3.1.3.11), as shown in rabbit muscle [56]. If carnosine activates FBPase *in vivo* by chelating the allosteric regulator Zn^{2+} [57], this would create a futile ATP-consuming cycle since the ATP-utilizing enzyme phosphofructokinase-1 (EC 2.7.1.11) converts fructose 6-phosphate into fructose 1,6-bisphosphate. This cycle would decrease ATP levels and synthesis as well as the supply of carbon skeletons for amino acid synthesis, impairing the growth of senescent cells by intensive glycolytic process [58].

Microscopy, flow cytometry, and ELISA analysis performed by Kantha and co-workers [59] showed that high carnosine concentrations (30 mM) significantly reduce the generation of the DNA oxidative damage marker 8-hydroxy-2'-deoxyguanosine in rat embryonic fibroblasts subjected to oxidative damage. Carnosine (20 mM) also decreases the shortening rate of telomeres in cultured human fetal lung fibroblasts, possibly preventing these structures from oxidative DNA damage [60]. These findings may aid to explain the anti-aging and life-extension effects attributed to carnosine, as shown in cultured HFF-1 and MRC-5 human fibroblasts [61–2], mice of SAMP1 strain [63], and male *Drosophila melanogaster* [64].

ID and age-related human disorders

Cataract

According to the World Health Organization (WHO)'s Prevention of Blindness and Visual Impairment, cataract is the clouding of the eye lens, which prevents clear vision. Most cases of such disorder are related to aging process. Cataract is the leading cause of blindness, accounting for 51% of the world incidence (20 million people approximately) in 2010. The risk factors include smoking, prolonged ultraviolet light (UV) exposure, *diabetes mellitus*, and high body mass index [65]. Other possible causes include reduced dietary antioxidant intake, severe dehydration, and side effect of drugs such as corticosteroids [66].

Cataract is associated with conformational changes and unfolding of proteins in the lens, which could arise from post-translational modifications induced by the Maillard reaction [67]. This process is defined as a non-enzymatic modification of amino acids by reaction with sugars, cyanide or steroids. The unfolded proteins of the lens are susceptible to oxidative damage, characterized by the linkage of polypeptide chains through disulphide bonds and formation of high molecular weight water-insoluble aggregates. Such molecules account for the increased scattering of light, resulting in the opacity of the lens typical of the cataract [66].

In this scenario, an *in vitro* study showed that the dipeptides *N*-acetylcarnosine – a pro-drug version of carnosine (*N*-acetyl-*β*-alanyl-L-histidine) - and anserine, but not carnosine itself, significantly suppressed UV-induced aggregation of the β-L-crystallin – a protein associated to the lens. An inhibition at the initial stages of protein photochemical damage and a decrease in size of protein aggregates were also detected in the presence of both dipeptides [68]. *In vivo* and *ex vivo* studies also showed the antioxidant activity of the carnosine, *N*-acetylcarnosine, and *β*-alanyl-L-histamine. These compounds significantly prevented biological molecules from lipid peroxide-induced damage, including proteins, enzymes, unsaturated fatty acids, and membrane phospholipids, which may occur in the lens cells and predispose to cataract [13]. Importantly, the proposed mechanism of action for *N*-acetylcarnosine is the anti-lipid peroxidation [66], representing a modified form of carnosine resistant to hydrolysis by the human serum carnosinase [58].

Ophthalmic formulations containing *N*-acetylcarnosine may be promising for treating many ocular diseases mediated by lipid peroxide- and ROS-induced damage, including cataract [69], since such disorder is associated with the lack of a reductive detoxification system of phospholipid hydroperoxides in the lens cell membranes and biomolecules [70]. Telomere shortening and increased oxidative stress in human lens cells may be the result of the peroxidative damage to such cells, which is associated with the etiology of cataract. Therefore, telomere shortening rate as well as damages to telomere of the lens cells could be reduced by the systemic administration of carnosine (protected from enzyme

hydrolysis), *N*-acetylcarnosine eye drops, and ophthalmic formulations [71].

By convention, a suitable clinical trial of any drug should be performed with a large number of volunteers and by a long-term as a multicentre, randomized, double blind, and placebo-controlled trial [66]. In spite of the promising evidences, the clinical studies performed to date with *N*-acetylcarnosine eye drops [72–8] present certain limitations, including a limited number of patients. Further independent research of clinical effectiveness is needed to validate research claims of *N*-acetylcarnosine that have potential conflicts of interest in humans [66, 79].

Alzheimer's disease (AD)

AD is a neurodegenerative disease and the most common cause of dementia in elderly [80], characterized by a progressive cognitive decline. This disorder may be triggered by the formation of senile plaques primarily composed by amyloid-*β* peptide (A*β*), and neurofibrillary tangles mainly composed by hyperphosphorylated tau (τ) protein in the brain. About 5–10% of cases are inheritable, occurring in an autosomal-dominant manner. Three proteins are acknowledged be associated with such familial cases: the amyloid precursor protein, which is enzymatically cleaved to produce A*β*, and the presenilins 1 and 2 [30].

As showed in *in vitro* experiments, A*β* aggregation may be accelerated by cross-linking mediated by AGEs and carnosine, and other AGE-inhibitors were showed to attenuate this process [81–2]. Recently, a study was performed investigating the effects of carnosine on the formation of peptide fragment A*β*1–42 aggregates. Atomic force microscopy and thioflavin T assays showed an inhibition of A*β*1–42 fibrillogenesis *in vitro* and differences in the aggregation state of A*β*1–42 small pre-fibrillar structures in the presence of carnosine [83], suggesting that carnosine may be effective against the A*β* aggregation in AD brain.

Pretreatment of rat brain endothelial cells with carnosine (20 mM) also protected them against the toxicity mediated by an A*β* peptide fragment (residues 25–35) [49, 82]. Similarly, a set of *in vivo* experiments showed that 3xTg-AD mice receiving carnosine orally exhibited strong reduction of the hippocampal intraneuronal accumulation of A*β* and improvement of mitochondrial dysfunction related to aging and AD: recovery of the complexes II and IV activities in hippocampus and recovery of the complexes I and IV activities in cerebral cortex [84].

Carbonic anhydrase (CA, EC 4.2.1.1) activity is significantly decreased in the brain of AD patients [85]. In this scenario, kinetic and X-ray crystallographic studies performed by Temperini and co-workers [86] showed that both carnosine and its methyl ester acted as more efficient activators of three human CA isoforms than L-histidine, indicating that carnosine and its derivatives may have some therapeutic potential to mitigate the AD evolution through this mechanism.

Carnosine and homocarnosine present significant quenching ability towards acrolein [54], a lipid peroxidation product that was shown to be increased in AD brain [87]. The same authors detected different reaction products between acrolein and carnosine/homocarnosine, affording further insights on the involvement of such dipeptides in normal and pathological brain. MDA is also significantly increased in the brain of AD patients [88] and carnosine diminished MDA-induced cell damage, as shown by a significant decrease in protein cross-linking and ROS generation, as well as prevention of the $\Delta\psi_m$ dissipation in cultured rat cortical neurons [89].

In a double-blind placebo-controlled study addressing the therapeutic potential of carnosine in AD, it was assessed the efficacy of a combination of carnosine plus other antioxidants (formula F) in volunteers receiving donepezil (n = 26), in comparison to patients receiving donepezil plus placebo (n = 26) [90]. Using the Mini-Mental State Examination II (MMSE II) score, Cornelli [90] observed a significantly improvement of cognition in the group receiving donepezil plus formula F, as compared to those receiving donepezil plus placebo.

Parkinson's disease (PD)

PD is a neurodegenerative disorder clinically characterized by progressive rigidity, bradykinesia, and tremor. The pathological findings include loss of pigmented dopaminergic neurons in the *substantia nigra* and the presence of distinctive cytoplasmic inclusions immunoreactive for α-synuclein and ubiquitin, *i.e.*, the Lewy's bodies [30]. It was shown that carnosine, homocarnosine, and anserine significantly inhibited aggregation and carbonylation of α-synuclein mediated by ceruloplasmin and H_2O_2 *in vitro* [91].

In PD animal models, carnosine supplementation (0.5, 1, and 2 g/L, in drinking water) significantly decreased the oxidative stress, inflammatory damage, and dopamine depletion induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). On the other hand, reduced glutathione (GSH) levels, carnosine content, and mRNA levels and activities of the glutathione peroxidase (EC 1.11.1.9) and superoxide dismutase (EC 1.15.1.1) were found increased in the same animals. Furthermore, it was also found a decrease in the levels of interleukin 6, tumor necrosis factor-α, NO, as well as the mRNA level and activity of the inducible nitric oxide synthase (EC

1.14.13.39) in striatum of mice receiving MPTP and supplemented with carnosine [92].

Increased levels of the neurotoxin 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol) are detected in the CSF of PD patients [93]. An *in vitro* study showed that carnosine and *N*-acetylcarnosine prevented salsolinol-mediated oxidative modification of the ferritin, reflected as a decrease in ferritin aggregation and inhibition of the carbonyl and dityrosine formation [94]. This latter finding may be substantiated by the antioxidant [20] and carbonyl-scavenger [24] activities assigned to ID, as previously discussed.

Hyperhomocysteinemia is also present in PD patients [95–6]. Homocysteine activates cells expressing *N*-methyl-D-aspartic acid (NMDA) receptors (e.g., neurons) and elicits Ca^{2+} and ROS intracellular accumulation [97]. Carnosine significantly prevented the cells expressing NMDA receptors from excitotoxic damage mediated by NMDA and homocysteine [98], and it cannot be ruled out that this effect may be, at least in part, mediated by antioxidant and chelating ability of carnosine towards ROS and Ca^{2+}. Other possible mechanisms for the biological activity of carnosine in PD may be acknowledged in the review performed by Hipkiss and co-workers (2013), which emphasizes the putative role of the MG in the pathogenesis of such disorder. As previously discussed, carnosine counteracts MG and other cytotoxic aldehydes [50], which in part could substantiate its neuroprotective effect in PD patients.

A pilot study performed by Boldyrev and colleagues [99] assessed the efficacy of carnosine (1.5 g, daily, by 30 days) as a complement to DOPA drug therapy in PD patients. It was concluded that the combination of carnosine with basic therapy for certain PD patients may be a reasonable way to improve the protocol of PD treatment and to decrease the possible toxic effects of the overloading of DOPA-containing drugs [99]. Due to the small number of volunteers and the short duration of this study, a larger and long-term trial is needed to confirm the benefit of carnosine combined to DOPA drug therapy.

Other disturbances potentially age-related

Apart from cataract and neurodegeneration, research claims on potential age-related conditions against which carnosine could be explored therapeutically include diabetes and diabetic complications, ischemia, osteoporosis, deafness, slow wound healing, hypertension (raised blood pressure), and heart disease. However, it is noteworthy that further studies are required to verify these research claims [32].

Recent HPLC and computational studies performed by Vistoli and co-workers [24] with 40 ID that are more stable to enzyme hydrolysis revealed that the peptides L-Lysyl-L-Histidine-OMe and L-Tyrosyl-D-Histidine-OMe showed a carbonyl quenching activity slightly weaker, but significantly more selective than that of carnosine. These peptides may represent promising candidates for the design of improved carnosine derivatives.

ID in SCD: evidence that the biological activity of such compounds may be detrimental to younger subjects

SCD or carnosinemia is an autosomal-recessive inherited metabolic disease with an estimated prevalence of 1:500,000 newborns [100]. The serum carnosinase gene (*CNDP1*) is located in the region 18q22.3 [101]. SCD is biochemically characterized by carnosine accumulation in the serum and urine of affected patients due to low activity of serum carnosinase [31]. On the other hand, serum ID concentration in healthy subjects under fasting is undetectable [102]. The main clinical manifestations assigned to SCD include intellectual deficiency, growth delay, seizures, twitching, lethargy, generalized muscle wasting [31], and hypotonia [103].

To date, the most of the SCD patients reported were children with neurological dysfunction [31, 103–8]. Homocarnosinosis (EC 236130), which was considered as a distinct disorder, may be a severe serum carnosinase deficiency, since the presence of the enzyme homocarnosinase was not shown in human tissues [109]. The phenotype of SCD patients is very variable and it was suggested that the enzyme deficiency is not pivotal to the neurological dysfunction [108, 110].

However, it cannot be ruled out that the ID accumulation in the earlier stages of development of SCD patients may be detrimental to some tissues and organs. In the brain, ID-induced potential damage may aid to explain the neurologic disturbances observed in patients. Since metal ions are related to neurotransmission and synaptic plasticity modulation [111], it would be reasonable to infer that the dysfunction may be assigned to a detrimental chelating activity elicited by increased ID concentration in CNS [21].

Serum carnosinase activity is undetectable in newborns and young children, increasing with age and reaching the adult range at about 10 years [31, 112]. Consequently, carnosine levels in the human skeletal muscle [113], as well as the homocarnosine concentration in human CSF [114] significantly decrease with age. A recent paper published by Macarini and co-workers [115] corroborates such proposition. It was showed a significant decrease in the mitochondrial respiratory chain complexes I–III and II activities in skeletal muscle of young rats (30 days old) receiving carnosine (100 mg/kg body weight) acutely, as compared to animals receiving vehicle.

Therefore, carnosine accumulation could damage tissues in the earlier stages of development in SCD

patients and possibly in newborns and children, whose serum carnosinase activity is undetectable or deficient. A putative mechanism whereby the carnosine accumulation could induce damage in tissues is the previously discussed modulation/inhibition of glycolysis. Such process would occur in growing young cells with high demand for ATP and for carbon skeletons to the synthesis of amino acids through this metabolic pathway [58]. Another possibility is the impairment of ID utilization as precursors of GABA, β-alanine, and L-histidine, which may act as a contributing factor to neurologic dysfunction [116].

Conclusion

Aging is a physiological process whose precise underlying mechanisms are unclear, although it may include DNA damage and telomere shortening, changes in gene expression, induction of oxidative stress, impairment of ATP synthesis, and accumulation of altered proteins. Many physiological effects observed in experimental studies with cell culture, animals and humans are assigned to ID. In this scenario, carnosine is thought to counteract several mechanisms associated to aging process due to its presumable biochemical properties, including antioxidant, bivalent metal ion chelating, muscular proton buffering, anti cross-linking, and reactive carbonyl scavenger activities.

At present, ID seem to represent potential therapeutic agents in studies performed both *in vitro* and *in vivo*. Additionally, preliminary clinical studies with these compounds have also shown promising results, as observed to cataract, AD and PD, as well as other age-related human disorders.

Even though only beneficial effects are assigned to carnosine [99, 117] and other peptides, it cannot be ruled out that there are patients prone to present supraphysiological and potentially toxic ID concentrations. Therefore, experimental studies are needed to support or refute the presented hypothesis, and possibly its underlying mechanisms. The data raised by these studies may aid to shed new light on the role of ID in biological tissues in an age-specific manner.

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