The importance of carnosine to erythrocyte rheology

S. Aydogan

Erçiyes University, Faculty of Medicine, Department of Physiology, Kayseri, Turkey; aydogans@erciyes.edu.tr

Abstract
Carnosine is a naturally occurring multifunctional dipeptide made up of a chemical combination of the amino acids beta-alanine and L-histidine. It commonly presents in mammalian tissues, in especially high levels in long-lived cells (e.g. nerve and muscle), but it declines with age. Its main physiological function is uncertain although many possibilities have been proposed including physiological buffer, wound healing agent, antioxidant, free radical scavenger, metal ion chelator, immunomodulator, antitumor agent and anti-aging compound. Carnosine is a naturally occurring antioxidant that has been shown to be an anti-glycating agent. It also has the ability to suppress advanced glycation end products (AGEs) and inhibit the formation of reactive oxygen species. On the other hand, red blood cells experience continuous oxidative insult by being exposed to endogenous and exogenous reactive oxygen species. Although the red cell contains an extensive antioxidant defense system, oxidative damage of membrane proteins and lipids contributes to the senescence of normal red cells and results in a shorter life span for pathological RBCs. Oxidative damage in RBC is one of the well established mechanisms of mechanical impairment. RBC deformability is an important determinant of blood rheology and normal RBC deformability is essential for proper tissue perfusion and oxygenation, as well as for the normal survival of RBC in the circulation. Therefore, maintaining normal RBC mechanical properties should be an important objective in therapeutic approaches to some hematological and other disorders. Carnosine, as an antioxidant molecule, protects the erythrocyte from oxidative and peroxidative damage and also protects against oxidative stress induced (e.g. by H_2O_2, SNP and Glucose) impairment of RBC deformability. It shows rejuvenating effects in erythrocytes and it can be used to improve RBC quality and as a potential therapeutic agent for hemorheological pathologies.

Keywords: Carnosine, erythrocyte rheology, oxidative stress, antiaging

1. Introduction
Carnosine is a naturally occurring multifunctional dipeptide made up of a chemical combination of the amino acids beta-alanine and L-histidine. It is found both in food and in the human body and is commonly present in mammalian tissue, in particular in skeletal muscle cells. It is formed by carnosine synthetase and is kept in equilibrium by the carnosinase. High concentrations of carnosine are present in long-lived cells, particularly in muscle (myocytes) and brain. Its concentration in muscles correlates with lifespan, decreasing with age. Carnosine was discovered, and its structure determined, in the very beginning of the 20th century by the Russian scientist W.S.Gulewich. It was the first and simplest example of a biologically active dipeptide. In 1953, another Russian scientist, S.E.Severin, showed that carnosine effectively buffered lactic acid produced by working muscles. Widespread interest in carnosine has increased markedly over the last years. There are several other related dipeptides such as carcinine, anserrine, homocarnosine and ophidine, all of which are naturally-occurring. These are believed to be buffering agents, helping to maintain the homeostatic equilibrium [1, 2].

Carnosine has been shown to have a wide repertoire of beneficial effects in the body. Its mechanisms of action are explained in terms of proton buffering (maintaining pH balance in the muscles in heavy exercise), heavy metal chelating (especially copper and zinc), free-radical and active sugar molecule scavenging (prevents glycation and carbonylation of proteins), preventing the modification of biomacromolecules thereby keeping their native functionality under oxidative stress, proteosome protection and suppression of the proinflammatory and carcinogenic cytokine IL-8 [1, 2, 3]. There are age-related conditions that carnosine may be useful for: neurological degeneration (Alzheimer’s, Parkinson’s, epilepsy depression, schizophrenia, mild cognitive impairment, dementia and stroke), Autistic spectrum disorders,
cellular senescence in general, cross-linking of the eye lens (cataracts), cross-linking of skin collagen (skin ageing), formation of advanced glycation end products (AGEs), accumulation of damaged proteins, muscle atrophy, brain circulation deficit (stroke), cardiovascular conditions, diabetes and its complications. Carnosine as a multi-functional dietary supplement is a relatively novel discovery. It is a remarkable physiological and completely natural super-antioxidant with numerous biological roles including (in addition to those above mentioned): universal and versatile antioxidant activity, supporting muscle vitality, increasing muscle strength and endurance, speeding up recovery after sprints and inhibiting cellular damage caused by alcohol, acting as neurotransmitter or message-carrying chemical, in the brain and nerves.

2. Biological significance of carnosine as a rejuvenating agent (antiaging effect of carnosine)

Oxygen free radicals are thought to be the most important factor in determining biochemical and physical changes during the senescence process. In a remarkable series of experiments, scientists at an Australian research institute have shown that carnosine rejuvenates cells as they approach senescence [4, 5]. The scientists cultured human fibroblasts (connective tissue cells) from the lung and the foreskin. Fibroblasts that went through many rounds of division, known as late-passage cells, displayed a disorganized, irregular appearance before ceasing to divide. Fibroblasts cultured with carnosine lived longer, retaining youthful appearance and growth patterns. But when they transferred the fibroblasts back to a medium lacking carnosine, the signs of senescence quickly reappeared. The scientists switched late-passage fibroblasts back and forth several times between the culture media. They consistently observed that the carnosine culture medium restored the juvenile cell phenotype within days, whereas the standard culture medium brought back the senescent cell phenotype [4, 5].

The carnosine medium also increased life span, even for old cells. Carnosine's revitalizing effects on cultured fibroblasts may explain why it improves post-surgical wound healing.

A Russian study tested the effect of carnosine on life span and indicators of senescence in senescence-accelerated mice [6, 7]. Half the mice were given carnosine in their drinking water, starting at two months of age. Carnosine extended the life span of the treated mice, by 20% on average, compared to the mice not fed carnosine. Carnosine did not alter the 15 month maximum life span of the senescence-accelerated mice strain, but it did significantly raise the number of mice surviving to old age. Carnosine distinctly improved the appearance of the aged mice, whose coat fullness and color remained much closer to that of young animals. The current knowledge about the mechanisms involved in the aging process and the defence mechanisms indicate that two phenomena are of particular concern: The deleterious effects of reactive oxygen species and the formation of reactive carbonyl compounds, related to the glycation reaction. The powerful action of carnosine is effective against all the elements that trigger the aging process and against all the phenomena that contribute to its propagation and amplification. Carnosine shows different functions at different steps of the whole aging process. It stops the oxidative damage by acting as an antioxidant agent, a ROS (reactive oxygen spcies) scavenger agent, metal ions chelating agent and by expressing a SOD-like activity. It inhibits the glycation reaction, by quenching carbonyl compounds and AGEs. It prevents macromolecular cross-linking [8, 9].

3. Antioxidant properties of carnosine as a free radical scavenger and metal chelator

Carnosine is widely believed to be an antioxidant which stabilizes and protects the cell membrane. The anti-oxidant and oxygen free radical-scavenging activities of carnosine have been demonstrated in many studies [10–12].

Specifically, as a water-soluble free radical scavenger it prevents lipid peroxidation within the cell membrane [9]. It is thought to be a natural counterpart to lipid-soluble antioxidants such as vitamin E. Many antioxidants are aimed at preventing free radicals from entering the tissues, but have no effect after this first line of defense is broken. Carnosine is not only effective in prevention, but it is also active after free radicals react to form other dangerous compounds. So, it protects the tissues from these damaging 'second-wave' chemicals. For example, a highly reactive lipid peroxidation end-product called malondialdehyde (MDA)- a deleterious product of a free radical reaction- is blocked by carnosine [10-14]. MDA, if left uncontrolled, can cause damage to lipids, enzymes and DNA, and plays a part in the processes of atherosclerosis, joint inflammation, cataract formation, and aging in general. Carnosine, by reacting and inactivating MDA,
sacrifices itself in order to protect the amino acids on the protein molecule [15]. Carnosine has also the ability to reduce concentrations of thiobarbituric acid reactive substances (TBARS). Interacting with aldehydic lipid oxidation products, carnosine protects biological tissues from oxidation, since aldehydes can form adducts with DNA, proteins, enzymes, and lipoproteins causing harmful alterations in their biological activity [16]. Many studies have demonstrated at tissue, cell and organelle levels that carnosine may prevent peroxidation of many model membrane systems and also biological cell membranes, including those of erythrocytes [3]. Carnosine inhibits lipid oxidation by a combination of free radical scavenging and metal chelation. In animal models of ischaemic injury, it is also thought that carnosine has protective properties against free-radical damage in brain[17]. Carnosine, as a dietary supplement, seems to have all the same chelating properties as EDTA, and it offers a possibility for an inexpensive oral chelation therapy. Carnosine has an ability to chelate pro-oxidative metals, such as copper, zink and toxic heavy metals (lead, mercury, cadmium, nickel) [18].

4. Antiglycosylation effect of carnosine

The body is made up largely of proteins. Unfortunately, proteins tend to undergo destructive changes with age due largely to oxidation and interactions with sugars or aldehydes. These interrelated protein modifications include oxidation, carbonylation, cross-linking, glycation and advanced glycation endproduct (AGE) formation. They figure prominently not only in the general processes of aging but also in its more familiar signs such as in the skin, cataracts and neurodegeneration. Studies show that carnosine is effective against all these forms of protein modification. Perhaps the most important action of carnosine is its antiglycosylation effect [8]. One of the cardinal processes of aging, apart from free-radical damage, is the process of glycosylation (or glycation). Glycation is the reaction between reducing sugars and proteins. During normal, everyday metabolism, sugar aldehydes may react with the amino acids on the protein molecule. The result is the formation of AGEs. AGEs are very toxic for the cells, as they are very rich in double bonds, which can react irreversibly with biological substrates leading to a loss of their physiological functions. Crosslinking with proteins also causes loss of their biological functions. These are abnormal, cross-linked oxidized products which are thought to cause extensive damage to the organism. Carnosine blocks this deleterious reaction, protecting against cross-linking of proteins, cross-linking of proteins to DNA molecules, and formation of other abnormal proteins; all of which are fundamental features of the aging process. Other anti-glycators such as aminoguanidine may also protect against glycosylation but not as effectively as carnosine. Some amino acids (arginine or lysine) are also able to combine with glucose in order to eliminate dangerous AGEs, but the endproduct of this reaction is mutagenic (i.e. it may cause cancer). Specifically, carnosine reacts with and inactivates aldehydes and ketones, reducing protein glycosylation and the formation of AGEs. It also binds to already formed AGEs and inactivates them. Normally, AGEs are removed by scavenging macrophages (immune system cells) which carry special receptors called RAGEs. Carnosine facilitates this process of elimination by helping macrophages to better recognize the AGE molecule. Because of its anti-glycosylation actions, carnosine may be useful in treating or preventing diabetic complications such as cataract, neuropathy and kidney failure [8].

5. Other benefits

Carnosine plays a part in neurotransmission, it is a heavy metal binder (chelates ionic metals) and modulates enzymatic activities. Other actions, some of which have not been extensively studied include: anti-neoplastic properties, which make it a potentially beneficial agent for use in cancer prevention, immune booster (it stimulates maturation of immunocompetent cells), and reducing inflammation, wound healing and protection against radiation damage. Laboratory animals treated with carnosine were found to have faster and better wound healing compared to controls. This has potential applications to treating burns, wounds following surgery, or during nutritional preparation for surgery, to reducing gastric ulceration (particularly when the ulcer is related to stress), both by preventing the formation of the ulcer and by healing it [14].

6. Aging, oxidative stress and antioxidant defense systems in erythrocytes

Mature, circulating RBCs have a finite lifespan. Aged erythrocytes are ultimately removed from
circulation by phagocytic cells. Each day, slightly less than 1% of circulating red cells are destroyed and replaced by a similar number of new cells [19]. The molecular mechanism that determines removal of cells from the circulation remains unknown, but probably involves recognition of senescence antigens by phagocytes. It has been proposed that the major senescence antigen in aged erythrocytes is derived from the band 3 protein, the main transmembrane glycoprotein in erythrocytes. The erythrocyte aging process is a multifactorial event, and understanding the interrelationship between various cellular changes is essential to define the complex process of senescent cell recognition. Free radical theory is a widely accepted chemical theory of aging. Free radical theory treats aging as the result of cumulative oxidative damage to biomolecules such as proteins, lipids and nucleic acids. Other possible mechanisms for red cell aging include mechanical fatigue, ATP depletion, calcium accumulation, and the generation of ROS. ROS, which damage proteins and initiate lipid peroxidation, can be generated either inside erythrocytes through the hemoglobin oxidation pathway or outside. The ROS theory of red cell aging has been widely accepted, yet it lacks direct supporting evidence [20]. On the other hand, red blood cells experience continuous oxidative insult by being exposed to endogenous and exogenous ROS. Although the red cell contains an extensive antioxidant defense system, oxidative damage to membrane proteins and lipids contributes to the senescence of normal red cells and results in a shorter life span for pathological RBCs. The major source of intracellular ROS in the red cell is autoxidation of oxyhemoglobin, which generates superoxide and produces H2O2. Catalase and GSHPx scavenge most of the H2O2 generated in the cells. It has been reported that degradation of the heme moiety takes place in conjunction with the reaction of H2O2 with Hb. In addition, it has also been demonstrated that even small concentrations of H2O2 generated during the autoxidation of oxyhemoglobin contributes to heme degradation. Heme degradation is, therefore, expected to take place in the red cell when the antioxidant enzymes are not able to eliminate all the H2O2 [20, 21, 22]. Oxidative damage in RBC’s is one of the well established mechanisms of mechanical impairment. RBC deformability is determined by cellular geometry, cytoplasmic viscosity of RBC (Hb concentration) and viscoelastic properties of the RBC membrane. Membrane viscoelasticity is in turn determined by RBC membrane skeleton, which is mainly a spectrin network attached to the integral proteins. Oxidative reactions that start in the lipid components (i.e. lipid peroxidation) lead to the formation of cross-linkages within the membrane skeletal proteins or hemoglobin, increasing membrane viscosity. Additionally, oxidative damage may affect transport processes through the RBC membrane, affecting the cell geometry and cytosolic viscosity [21, 22]. Red cells from newborns, especially premature infants, have previously been shown to be more sensitive to peroxidative damage in vitro than RBC’s from adults, due in part to deficiencies of antioxidant capacity [23].

7. Erythrocyte rheology and carnosine experiences

RBC deformability is an important determinant of blood rheology, either in bulk flow conditions or in the microcirculation. Normal RBC deformability is essential for proper tissue perfusion and oxygenation, as well as the normal survival of RBC in the circulation. Therefore, maintaining normal RBC mechanical properties should be an important objective in therapeutic approach to some hematological disorders and also red blood cell rheology.

The significant role of L-carnosine in maintaining RBC physiology under various conditions, (i.e. under oxidative stress) has been demonstrated previously by Aydogan et al [24]. In this study, L-carnosine significantly improved RBC deformability which had been impaired by H2O2-induced oxidative stress under in vitro conditions. This study provided the first evidence for the importance of L-carnosine for maintaining normal RBC properties and to protect them from oxidative damage in the circulation. It also suggests that L-carnosine supplementation can be used to improve RBC quality [24]. After this in vitro study, Aydogan et al. investigated the protective effects of carnosine on sodium nitropruside (SNP)-induced peroxidative damage of red blood cell under in vivo conditions [25]. The results have also suggested that in vivo carnosine supplementation can be used to protect the RBCs from oxidative or peroxidative damage, to improve their survival in the circulation and to reduce the oxidative effects of SNP, which is a potent hypotensive agent used to control hypertension. Because L-carnosine suppressed lipid peroxidation, MDA levels as an index of lipid peroxidation were elevated in SNP-treated animals, but this elevation was not observed in carnosine administrated animals. In addition, red blood cell elongation index was decreased in the SNP-induced group, but carnosine significantly increased it [25]. In another experimental study, Aydogan et al. (2010) also showed that carnosine has a dose dependent positive effect on red blood cell deformability and aggregability.
and that in the presence of carnosine, erythrocytes showed also an increased ability to resist haemolysis. These beneficial properties of the dipeptide appear to be rejuvenating or to improve erythrocyte quality and mechanical properties. L-carnosine supplementation also can be used to protect RBC's from various other sources of damage in the circulation [26]. A further recent hemorheological in vitro study of red blood cells that were incubated in glucose-rich media, showed similar results with the addition of carnosine [27]. In this study, hemorheological properties, which are easily modified by glucose-induced oxidation and glycation could be prevented by carnosine. Furthermore, carnosine reveals its rejuvenating effects in erythrocytes that are exposed to glucose-rich plasma [27, 28].

Red blood cell deformability, is a pivotal determinant of blood flow and function in the microcirculation. Several studies to explain impaired erythrocyte deformability have proposed factors including elevated blood glucose concentration and hyperosmolarity, hypoinsulinemia, alterations in erythrocyte membrane lipid composition, increased internal viscosity and increased erythrocyte membrane rigidity caused by glycation reactions [29].

On the other hand, it is known that oxidative stress and the glycation process play an important role in physiopathology of chronic complications in diabetes mellitus. Impaired red blood cell deformability is also a hemorheologic perturbation induced by diabetes mellitus. The effect that changes in erythrocyte deformability, and other hemorheological alterations, have on the microcirculation have also been implicated in the pathogenesis of diabetic vascular complications. A number of studies have provided evidence that impaired erythrocyte deformability is linked to AGEs accumulation. Glycation of several erythrocyte membrane proteins and hemoglobin molecules has been found in patients with diabetes mellitus [30,31]. This type of alteration of the erythrocyte is one of the factors that may account for rheologic properties of erythrocytes in diabetes and also the aging process[32]. It has been proposed that carnosine can inhibit generation of many of the protein alterations accompanying aging, especially those associatedwith diabetes mellitus and its complications.

Recently, Yapsilar and Aydogan (2011) have investigated the erythrocyte deformability indexes which are impaired in diabetes and the antioxidant effects of carnosine on these properties of erythrocytes in experimental diabetes in rats [33]. In these experiments, it was found that glucose and MDA levels of the diabetic group were significantly increased, but erythrocyte deformability indexes were decreased. But they also found that carnosine can significantly reverse the erythrocyte deformability effect and reduce the lipid peroxidation. It can be concluded that carnosine can recover microvascular circulation problems by increasing erythrocyte deformability, and it can be used as a multifunctional antioxidant and antiglycation agent in treatment of diabetes mellitus to prevent the complications of diabetes [33].

8. Conclusion

Our interest in using this compound on erythrocytes is that it is a natural and nontoxic compound, a powerful antioxidant and antiglycation agent, its lack of side effects, and the high bio-availability. Hemorheological properties which are easily modified by different oxidative stress factors (including hydrogen peroxide, sodium nitroprusside and high glucose) can be protected by carnosine. Carnosine can be used for maintaining normal RBC properties and to protect them from oxidative damage in the circulation, to improve RBC quality and also as a potential therapeutic agent for pathologies that involve hemorheological modification. Further experiments are in progress aimed at examining more widely the effects of carnosine on aging, diabetes and red blood cell rheology. It is expected that carnosine supplementation will become much more widespread during the coming years. It is believed that carnosine will play an ever increasing role in longevity medicine of the future.

References