Anti-ageing potential of carnosine: approaches toward successful ageing

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Ageing is characterised by a wide variety of physiological changes and, as a consequence, an anti-ageing compound must fulfil a wide variety of roles to be effective. Carnosine is an antioxidant, antglycating and neuroprotective compound with well-studied clinical benefits. It is becoming a clinically accepted nutritional supplement with uses across a considerable spectrum of chronic diseases, from senile cataract to dementia. In this review, the benefits and actions of carnosine are discussed in the light of current research findings.

Introduction
When two Russian researchers first described carnosine in 1900, little they realised that the hitherto unknown dipeptide was to eventually be credited with a wide variety of benefits in several apparently unrelated areas of health. Carnosine (β-alanyl-L-histidine) is a naturally occurring compound, found in excitable tissues such as the myocardium, skeletal muscles and brain, but it is normally destroyed by the enzyme carnosinase. Congenital lack of this enzyme can cause carnosinemia with serious clinical implications, a fact that caused some ill-informed members of the public to express concerns about the use of carnosine as a supplement. However, in normal people with an intact carnosinase activity, no significant side effects of carnosine supplementation have ever been reported.

Normally, carnosinase acts fast and reduces the serum levels of carnosine, thus diminishing its clinical effectiveness. Nevertheless, its metabolites (alanine and histidine) are also considered clinically useful. For example, beta-alanine has a wide range of benefits itself [1]. There have been attempts at stabilising carnosine against carnosinase degradation, for example, by combining it with vitamin E derivatives [2]. These attempts have been partly effective in the clinical sense, although research in this area is continuing.

Over the past 20 years carnosine has been studied extensively and was confirmed to have several actions [3,4] such as cytosolic buffering, immunomodulation, neurotransmission, antioxidant and anti-glycating properties, benefits on the digestive system (as zinc-carnosine, or polaprezinc), and benefits in cataract and other degenerative diseases [5].

More recently [6], carnosine has also been studied in relation to neuroprotection, calorie restriction and apoptosis. Commercially, carnosine has been used for improving athletic endurance, as a skin protecting/wrinkle preventing agent including wound management, as a biomarker in nutrition [7] and a general anti-ageing supplement.

Antioxidant actions
It is now well known that carnosine is a strong antioxidant substance [8–10]. It scavenges reactive oxygen species (ROS) and products of lipid peroxidation such as unsaturated aldehydes. There are several newly published clinical and experimental papers describing the antioxidant properties of carnosine. For example, carnosine pre-treated rats which were subsequently given oral ethanol were protected against
the increase of lipid peroxidase and serum transaminase in the liver [11].

Used in association with other commonly used antioxidants such as co enzyme Q10, vitamin C, vitamin E, selenium, ginkgo biloba and vitamin B complex, carnosine exhibits evidence of neuroprotection, specifically in relation to Alzheimer’s dementia. Patients treated with the antioxidant combination plus the cholinesterase inhibitor donepezil, experienced a significant clinical improvement in mental test evaluation, compared to patients who were treated with donepezil plus placebo. The benefit is thought to be due to a combination of antioxidant activity and homocysteine reduction [12].

Carnosine, as a zinc-chelate (Polaprezinc), has proven activities against ROS generation, with particularly well-studied effects in the small intestinal mucosa [13]. This ROS-quenching effect of carnosine is believed to be responsible for anti-inflammatory benefits such as a reduction of cytochrome C, and caspase-3 [13]. During hypoxia, carnosine protects vascular endothelium via a reduction of lactate dehydrogenase levels [14]. Several other potential antioxidant mechanisms of carnosine are still under investigation.

**Anti-glycation**

During the process of glycation that happens with ageing, certain aldoses or aldehyde molecules attach to proteins (or to DNA) causing cross linking, that is, abnormal protein-to-protein or protein-to-DNA bonds. This process is facilitated by carbonyl groups. These abnormal proteins then may accumulate forming AGEs (Advance Glycation End-products) that may, in turn, react with free radicals to cause chronic degenerative diseases associated with ageing. Apart from its antioxidant activities discussed above, carnosine has been found to possess significant anti-glycating properties, interfering with the glycation processes at several steps [15,16].

It is known that carnosine supplementation decreases malondialdehyde (MDA) and protein carbonyl (PCO) groups, which are known to be involved in glycation [17]. An important anti-glycating activity of carnosine is its carbonyl scavenging properties. In general, carnosine is believed to function as nucleophilic trap for reactive carbonyl groups and thus reduce the formation of AGEs. In one study, Zucker obese rats were given a daily dose of 30 mg/kg of carnosine for a period of 24 weeks. At the end of the study it was shown that a possible carbonyl quenching mechanism was responsible for a significant reduction of the risk of hypertension, dyslipidemia, renal disease and obesity [18]. It has been suggested that methylglyoxal (MG), which is a glycatign agent that causes aberrant polypeptides, is specifically inactivated by carnosine [19]. This, in association with antioxidant actions, may account for the protective ability of carnosine against chronic age-related damage.

With regard to the question whether carnosine is predominantly an antioxidant or an antiglycating agent, some evidence exists that supports the former. It has been suggested, for example, that carnosine inhibits AGE formation primarily via an antioxidant mechanism and secondarily via a carbonyl trapping mechanism [20]. Nevertheless, carnosine is perhaps better known as an anti-glycator, and has been clinically used in association with other anti-glycating agents such as aminoguanidine and metformin.

**Metal binding**

One of the first-studied actions of carnosine is its ability to bind certain heavy metals, for example, copper(II). It has been shown that at pH 7 and 9, carnosine exists in equilibrium between the two tautomeric forms [21]. At neutral and basic pH there is a formation of the dimer Cu(2)L(2)H(–2)(0), where carnosine coordinates copper via the N (amino), O (carboxylate), and N (amide) donor atoms while the N(tau) nitrogen atoms of the imidazole ring bind to the copper ion. However, it may be the case that the metal ion chelation action of carnosine plays only a minor role during oxidation in vivo [22]. The ability of carnosine to buffer protons in muscular tissue has been exploited by athletes during resistance and endurance activities. In a recent double blind, placebo-controlled trial it was shown that supplementation with 2 g of carnosine and anserine for 30 days attenuates the exercise-induced elevation of epinephrine, norepinephrine and growth hormone [23].

**Apoptotic modulator**

Apoptosis is an important phenomenon in anti-ageing research. Normally, during apoptosis any damaged cells that are beyond repair are eliminated in an orderly and precise manner by the organism. However, the process of apoptosis becomes progressively less well regulated with advancing years. As a result, any damaged cells that could be repaired, or even any healthy cells, are targeted for apoptosis. This may be the basis of certain chronic age-related diseases such as heart disease and neurodegeneration where there is an excessive loss of functional cells. On the contrary, if the rate of apoptosis is reduced, there could be accumulation of damaged cells that could result in malignant tumour formation. It is therefore important to regulate the process of apoptosis in an ideal manner, preventing healthy cells from being eliminated and promoting elimination of malignant or otherwise damaged cells. There have been attempts at characterising certain substances that could act as apoptotic modulators [24]. For example, histidine analogues [25] (including carnosine) were found to prevent apoptosis in neural tissues by reducing caspase activity. The anti-apoptotic and neuroprotecting benefits of carnosine are being progressively elucidated, and there has been an increased research interest regarding this during the past two or three years. It is known for example that carnosine protects against neuronal cell loss via an attenuation of oxidative stress, but more recently an anti-apoptotic effect has also been described [26].
The carnosine-zinc chelate (Polaprezinc) was found to decrease apoptotic markers such as p51, p21 and Bax, protecting against apoptotic cell death following irradiation of the gastrointestinal mucosa [27]. In addition, it was found to prevent hepatocyte apoptosis following ischaemia-reperfusion injury [28]. The hepatoprotective effect of carnosine is not only due to its anti-apoptotic actions but could be due to its antioxidant profile with wide-ranging liver protective actions [29].

**Neuroprotection**

Studies performed primarily over the past two years have suggested that carnosine has significant neuroprotective actions. These are not merely based upon its antioxidant properties but, in addition, may be related to other properties such as membrane receptor stabilising actions and anti-apoptotic actions. It has been shown that carnosine protects the bioelectrical activity of neurones during stroke models [30]. Other researchers have shown antidepressant and neuromodulatory actions of beta-alanine (one of the active metabolites of carnosine) and it is known that supplementation with b-alanine increases carnosine concentration both in the cerebral cortex and in the hypothalamus. In addition, it increases brain-derived neurotrophic factor (BDNF) concentration in the hippocampus. [31]. Another possible way carnosine exerts its neuroprotecting action may be due to a reduction of excitotoxicity, for example by regulating glutamate levels. Glutamate is a neurotransmitter exhibiting a dose–response relationship (i.e. it is beneficial at low doses but deleterious at higher doses) and it can cause hyperactivity of neuro-excitation mechanisms. However, carnosine acts as a glutamate modulator, reducing the risk of excitotoxicity and regulating glutamate levels within ideal limits [32].

**Caloric restriction mimetic**

The long-term consumption of approximately 30–50% fewer calories than the amount consumed ad libitum (calorie restriction – CR), is perhaps the only widely accepted method of extending maximum lifespan in most animals studied so far. Not surprisingly, humans are not always willing to undergo a life-long CR diet, even if this may possibly mean an extension of the currently maximum human lifespan of around 110–120 years. CR affects several genes, molecules, hormones, and other parameters, and during the past few years, there has been an attempt to identify compounds that may have biological activities similar to those of CR. Several such compounds have been identified and have been classified as Calorie Restriction Mimetics (CRM) [33]. It has been hypothesized [34] that CR may exert some of its benefits via suppression of glycolysis, thus reducing the formation of reactive oxygen species (ROS), and reducing the formation of glycat ing agents such as methyglyoxal (MG) [35]. As carnosine also reduces MG and ROS it may be considered as a CRM, mimicking several other physiological actions of CR itself [36].

Together with other compounds that exhibit a biphasic dose response relationship (low dose activation, high dose inhibition), carnosine activates certain signalling pathways that regulate survival proteins in case of stress (sirtuins e.g.). Both CR and carnosine may thus influence sirtuins which depend on redox-sensitive intracellular pathways, resulting in increased neuroprotection [37]. In any case, the role of carnosine as a CRM may not be clinically significant, as there exist other, more powerful CRMs such as resveratrol and metformin.

**Cataract**

The acetylated form of carnosine (N-alpha-acetyl-carnosine, or NAC) has been used in several clinical trials against senile cataract [38]. It is known that the crystalline molecules within the lens of the eye become progressively glycated with age resulting in visual impairment [39]. Carnosine and NAC act as anti-glycators [40] thus protecting the crystallines against cross linking [41,42].

Apart from anti-glycating action within the lens, carnosine and NAC seem to also affect telomere length in the lens epithelium [43]. The authors of this study report that treatment with NAC eye drops results in the improvement of visual acuity and glare sensitivity in older people. NAC eye drops have been used in several proprietary preparations (e.g., Can-C or Bright Eyes) with unconfirmed reports of clinical effectiveness [44]. Despite lack of negative critical reviews of NAC, the majority of clinical eye specialists do not consider NAC eye drops as an alternative treatment for cataract and choose conventional methods instead. A statement by the mainstream Royal College of Ophthalmologists (UK) is as follows:

‘The evidence for the effectiveness of N-acetyl carnosine eye drops is based on experience on a small number of cases carried out by a Russian researcher team. To date, the research has not been corroborated and the results replicated by others. The long-term effect is unknown. Unfortunately, the evidence to date does not support the ‘promising potential’ of this drug in cataract reversal. More robust data from well conducted clinical trials on adequate sample sizes will be required to support these claims of efficacy. Furthermore, we do not feel the evidence base for the safety is in any way sufficient to recommend its use in the short term. More research is needed’. Amoaku, W. (2008). ‘N-Acetyl Carnosine for Cataracts’. Royal College of Ophthalmologists. http://www.rcophth.ac.uk/docs/publications/published-guide lines/N_ACETYLCARNOSINE_FOR_CATARACTS.pdf.

**Other uses**

As research into apparent and real benefits of carnosine becomes more widespread, new or hitherto unconfirmed potential clinical uses are being suggested:

**Anticancer**: Recent tentative research shows promising results with regards to its anti-tumour actions [45]. One mode of
action may be by blocking energy (ATP) availability to cancer cells [46].

**Autism:** Carnosine has been used by many children with Attention Deficit Hyperactivity Disorder (ADHD) [47], autism [48] dyslexia and learning difficulties [49]. It is believed to increase socialisation and vocabulary in autistic children. **Cosmetics:** Creams containing carnosine have been used as a cosmetic, applied directly on the skin, to prevent or treat wrinkle formation [50]. In other cases, carnosine cream has been used against age-related skin damage such as solar keratosis. **Polaprezinc:** The use of the chelate zinc-carnosine is relatively widespread in Japan, where most of the research regarding this compound is emanating. It has been shown that Polaprezinc protects mucosal epithelium (oral and gastric) [51] perhaps through a mechanism of anti-inflammatory action, as it reduces plasma nitric oxide, and tumour necrosis factor [52] as well as through induction of Heat Shock Proteins [53].

**Conclusions**

It appears that carnosine is an effective general anti-aging compound [54], affecting a wide range of age-related processes and functions. Some of its actions are more significant than others, however it can be classified as a true pluripotent compound.

Commercially, it is easily available and used by the general public, although questions about the dosage, purity and stability of commercial preparations remain. Because carnosine is found in meat, vegetarians are believed to be exposed to sub-optimal levels of dietary carnosine. However, it is not known if this has any detrimental health effects on vegetarians, and in particular, it is unknown whether the rate of ageing is accelerated in vegetarians.

As far back as in 1990 we were the first to suggest carnosine in oral form specifically for its antiaging properties. Some of our original patients are still taking the oral form of carnosine (a total of 20 years, at a dose of 100 mg a day) with no side effects reported. These patients subjectively report a general improvement in well-being and appearance, compared to their cohort. Unfortunately, lack of properly performed placebo-controlled trials reduces the credibility of these claims. As carnosine is a natural molecule it is difficult, if not impossible for any commercially driven attempt to patent a proprietary preparation to succeed. Nevertheless, on the whole, both experimental and clinical findings regarding the use of carnosine are positive, and the general lack of side effects of this compound suggests that a more widespread human use is warranted for helping to achieve successful ageing.

**References**

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