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Biological activities of the natural imidazole-containing peptidomimetics *n*-acetylcarnosine, carcinine and L-carnosine in ophthalmic and skin care products

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Abstract

Apart from genetically programmed cell aging, different external aggressors related to oxidative stress and lipid peroxidation (LPO) can accelerate the skin aging phenomenon. Oxidative stress associated with the formation of lipid peroxides is suggested to contribute to pathological processes in aging and systemic diseases known as the risk factors for cataract. Despite the fact that L-carnosine-related peptidomimetics *N*-acetylcarnosine (*N*-acetyl-β-alanyl-L-histidine) (NAC) and carcinine (β-alanylhistamine) are metabolically related to L-carnosine and have been demonstrated to occur in tissues of many vertebrates, including humans, these compounds were shown resistant toward enzymatic hydrolysis. A series of related biocompatible imidazole-containing peptidomimetics were synthesized in order to confer resistance to enzymatic hydrolysis and ex vivo improvement of protective antioxidative properties related to L-carnosine. The included findings revealed a greater role of *N*-acetylcarnosine (NAC) and carcinine ex vivo in the prolongation and potentiation of physiological responses to the therapeutical and cosmetics treatments with L-carnosine as antioxidant. 3-D molecular conformation studies proposed the antioxidant activity of peptidomimetics (carcinine, L-prolylhistamine, *N*-acetylcarnosine, L-carnosine) for metal ion binding, quenching of a number free radicals, and binding of hydroperoxide or aldehyde (including dialdehyde LPO products) in an imidazole-peroxide adducts. NAC can act as a time release (carrier) stable version of L-carnosine during application in ophthalmic pharmaceutical and cosmetics formulations which include lubricants. Carcinine, L-prolylhistamine show efficient deactivation of lipid hydroperoxides monitored by HPLC and protection of membrane phospholipids and water soluble proteins from the lipid peroxides-induced damages. This activity is superior over the lipophilic antioxidant vitamin E. The biologically significant applications of carnosine mimetics were patented by Dr. Babizhayev and the alliance Groups (WO 2004/028536 A1; WO 94/19325; WO 95/12581; WO 2004/064866 A1).

Keywords: Natural peptidomimetics; Carcinine; *N*-acetylcarnosine; L-prolylhistamine; L-carnosine; Ophthalmic and skin care; Cataract; Oxidative stress; Lipid peroxidation; Lipid hydroperoxides; Protein cross-linking; SOD-like activity; Peptidase hydrolysis; Universal antioxidants

"There is nothing stronger in all the armies in the world.....than an idea whose time has come!!"

Introduction

Multilateral biological activity of histidine-containing dipeptides in combination with their high content in human and animal tissues have long been a serious challenge to biologists, pharmacologists, physiologists and clinicians. There is presently a large body of literature on the variety of biological effects of carnosine (β-alanyl-L-histidine) in various pathological states in experimental animals and in clinics.

Carnosine and related dipeptides have been postulated to have numerous biological roles including pH buffering, regulation of enzyme activity and inhibition of oxidative reactions. Among antioxidant mechanisms reported for carnosine are its ability to inactivate reactive oxygen species, scavenge free radicals, and chelate prooxidative metals (Boldyrev et al., 1988; Kohen et al., 1988; Decker et al.,

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1992; Aruoma et al., 1989). The dipeptide L-carnosine has beneficial effects on cultured human fibroblasts. The continuous growth of these cells in carnosine not only increases their lifespan in population doublings and period of growth, but also can reverse the normal features of senescent cells (McFarland and Holliday, 1994, 1999). A rather unusual reported antioxidant property of carnosine was its ability to reduce concentrations of thiobarbituric acid reactive substances (TBARS) when added to previously oxidized lipids (Boldyrev et al., 1988; Aruoma et al., 1989). A potential mechanism for this observation would be the ability of carnosine to interact with aldehydic lipid oxidation products through Schiff base or Michael addition-type reactions (Aldini et al., 2002). The adduction chemistry of carnosine to 4-hydroxy-nonenal (HNE) thus appears to start with the formation of a reversible alpha,beta-unsaturated imine, followed by ring closure through an intra-molecular Michael addition. If carnosine could interact with aldehydic lipid oxidation products, this could potentially help protect biological tissues from oxidation, since aldehydes can form adducts with DNA, proteins, enzymes, and lipoproteins, causing alterations in their biological activity. Previously published data suggest that L-carnosine has excellent potential to act as a natural antioxidant with hydroxyl-radical- and singlet oxygen-scavenging and lipid peroxidase activities (Babizhayev et al., 1994; Babizhayev, 1989; Dahl et al., 1988). However, exogenous carnosine entering the organism intravenously, intraperitoneally, with food or topically to the eye, is not accumulated by the tissues but is excreted in the urine or destroyed by carnosinase, a dipeptidase present in blood plasma, liver, kidney and other tissues, except muscle and, probably, lens (Jackson et al., 1991; Lenney et al., 1985; Babizhayev et al., 1996).

The *N*-acetyl derivatives of histidine, carnosine and anserine exist in the cardiac and skeletal mammalian muscles and the total concentration of these imidazoles may lie within the measured range of that of L-carnosine in skeletal muscle (i.e. ~10 mM) (O'Dowd et al., 1988). The level of carnosine in tissues is controlled by a number of enzymes transforming carnosine into other carnosine related compounds, such as carcinine, *N*-acetylcarnosine (NAC), anserine or ophidine (by decarboxylation, acetylation or methylation, respectively) or its cleavage into the amino acids, histidine and β-alanine. Hydrolysis is mainly due to tissue carnosinase (EC 3.4.13.3) which is widely distributed among different subjects (Jackson et al., 1991; Lenney, 1976) or serum carnosinase (EC 3.4.13.20), obtained in brain and blood plasma of primates and humans (Kunze et al., 1986; Lenney, 1990). Both carnosinases are characterized by higher activity toward carnosine compared with anserine or homocarnosine (Murphey et al., 1972; Lenney et al., 1982). Comparative study of hydrolysis of carnosine and a number of its natural derivatives by human serum and rat kidney carnosinase was carried out (Pegova et al., 2000). The rate of carnosine hydrolysis was 3–4-fold higher then for anserine and ophidine. The rate of homocarnosine, *N*-acetylcarnosine and carcinine hydrolysis was negligible by either of the enzymes used. Thus despite the fact that carcinine and *N*-acetylcarnosine are metabolically related to carnosine, they have not observed to be a substrate for carnosinase or other dipeptidases (Pegova et al., 2000; Fitzpatrick et al., 1989). Therefore, both carcinine and *N*-acetylcarnosine may play a greater role in the prolongation and potentiation of physiological responses to the therapeutical treatments with carcinine and *N*-acetylcarnosine as antioxidant. It has been shown during the ophthalmic applications that due to its relative hydrophobicity compared to L- carnosine, *N*-acetylated form of carnosine might cross the cornea of the treated eye gradually and maintain longer the concentration of the active principle (carnosine) reaching the aqueous humor (Babizhayev et al., 1996). We propose that *N*-acetylcarnosine can act as a time release version of L-carnosine during its external topical application to the ocular and probably skin tissues. In the present study we have examined the prospects of applications of the bioactive natural imidazole-containing compounds carcinine, L-carnosine and *N*-acetylcarnosine against phospholipid hydroperoxides and toxic aldehydes involved in the development of cataracts and cutaneous ageing. In the same way, it was also necessary to design new experimental models for the evaluation of the protective efficiency of carnosine related compounds.

Materials and methods

Carcinine (Decarboxy carnosine˙2HCl), L-prolylhistamine and *N*-acetyl-β-alanylhistamine were synthesized by Exsymol S.A.M. (Monaco, Principaute de Monaco). L-Carnosine and *N*-acetylcarnosine were synthesized by Hamari Chemicals Ltd (Japan) per specifications proposed by Innovative Vision Products, Inc.

Molecular modeling

Low-energy 3-D conformations of carnosine, carcinine and *N*-acetylcarnosine were derived using the PM_3 method of the MOPAC 6.0 program (Stewart MOPAC Air Force Academy: Boulder, CO 80840). The precise energy minima conformations were determined by semi-empirical Quantum mechanics. This technique structures a pool of energetically accessible shapes especially suitable for dipeptides comparative to large protein molecules. The program is supplemented with ZINDO/1 computer software for estimation of chelating properties of dipeptides and related compounds. The conformational geometry optimization was carried out using the revised computer program (Stewart, 1989a,b).

Pharmacokinetics of topical *N*-acetylcarnosine application

Formulations and animals

Grey Chinchilla rabbits (male) aged 3–4 months weighing 2–3 kg were used. Animal experiments conformed to the guidelines of the ARVO Resolution on the Use of Animals in Research. Thirty minutes prior to the ocular incision right eyes of rabbits were instilled with 80 μl of formulation A containing 1% *N*-acetylcarnosine (NAC) and the control right eyes of the