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N-Acetylcarnosine Lubricant Eyedrops Possess All-In-One Universal Antioxidant Protective Effects of L-Carnosine in Aqueous and Lipid Membrane Environments, Aldehyde Scavenging, and Transglycation Activities Inherent to Cataracts: A Clinical Study of the New Vision-Saving Drug N-Acetylcarnosine Eyedrop Therapy in a Database Population of Over 50,500 Patients

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The antioxidant activity of L-carnosine (β-alanyl-L-histidine, bioactivated in ocular tissues) versus N-acetylcarnosine (N-acetyl-β-alanyl-L-histidine, ocular-targeted small dipeptide molecules) was studied in aqueous solution and in a lipid environment, employing liposomes as a model of lipid membranes. Reactive oxygen species (ROS) were generated by an iron/ascorbate promoter system for induction of lipid peroxidation (LPO). L-carnosine, which is stabilized from enzymatic hydrolysis, operates as a universal aldehyde and ROS scavenger in both aqueous and lipid environments and is effective at preventing ROS-induced damage to biomolecules. Second-generation carnosine analogs bearing the histidyl-hydrazide moiety were synthesized and tested versus L-carnosine for their ability to reverse the glycation process, also known as the Maillard reaction, and reverse the stable intermolecular cross-links, monitored in the glucose-ethylamine Schiff base model, ultimately resulting in the formation of the advanced glycation end products (AGEs) from nonenzymatic glycation, accumulating in numerous body tissues and fluids. The obtained data demonstrate the transglycation properties of the ophthalmically stabilized L-carnosine and L-carnosine histidyl-hydrazide derivatives tested and can be used to decrease or predict the occurrence of long-term complications of AGE formation and improve therapeutically the quality of vision and length of life for diabetes mellitus patients and survivors with early aging. Scientists at Innovative Vision Products, Inc. (IVP), developed lubricant eyedrops designed as a sustained-release 1% N-acetylcarnosine prodrug of L-carnosine. The eyedrops contain a mucoadhesive cellulose-based compound combined with corneal absorption promoters and glycerine in a drug-delivery system. Anti-aging therapeutics with the ophthalmic drug eyedrop formula including N-acetylcarnosine showed efficacy in the nonsurgical treatment of age-related cataracts for enrolled participants in the prospective, randomized, double-masked, placebo-controlled crossover clinical trial after controlling for age, gender, and daily activities. In a cohort in excess of 50,500 various patients seeking cutting-edge medical care, the N-acetylcarnosine topical eyedrops target therapy was demonstrated to have significant efficacy, safety, and good tolerability

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for the prevention and treatment of visual impairment in this older population with relatively stable patterns of causes for blindness and visual impairment. Overall, accumulated study data demonstrate that the IVP-designed new vision-saving drugs, including N-acetylcarnosine eyedrops, promote health vision and prevent vision disability from senile cataracts, primary open-angle glaucoma, age-related macular degeneration, diabetic retinopathy, and aging. N-acetylcarnosine eyedrop therapy is the crown jewel of the anti-aging medical movement and revolutionizes early detection, treatment, and rejuvenation of aging-related eye-disabling disorders. N-acetylcarnosine, as an innovative medical science tool and component of the home medicine and alternative medicine approaches, has the potential to alleviate visual impairment and its associated social, economic, and political woes for an aging population.

The real voyage of discovery consists not in seeking new landscapes but in having new eyes.
—Marcel Proust

Keywords: age-related ophthalmic diseases, aldehyde scavenging, cataracts, hydrazide carnosine derivatives, L-carnosine, N-acetylcarnosine lubricant eyedrops, transglycation

INTRODUCTION

The elderly population in the United States is increasing rapidly. By the year 2030, approximately 70 million Americans will be over 65 years of age. Loss of vision among the elderly is a major health care problem: approximately 1 in 3 elderly persons has some form of vision-reducing eye disease by the age of 65.[1] Vision impairment is associated with a decreased ability to perform activities of daily living and an increased risk for depression.[2] This article reviews the most common oxidation mechanisms of vision impairment in the elderly—age-related macular degeneration, glaucoma (Figure 6), cataract (Figure 1), and diabetic retinopathy—and the prospects of therapy for sight-threatening eye diseases with a patented formula of N-acetylcarnosine eyedrops that have been effective in restoring vision among the elderly.[3,4]

Oxidative mechanisms are believed to play an important role in the pathogenesis of cataracts, the most important cause of visual impairment at advanced age. Oxidant by-products of normal metabolism cause extensive damage to DNA, protein, and lipids.[5] The role of free radical–induced lipid oxidation in the development of cataracts has been identified.[5] Initial stages of the human cataract are characterized by the accumulation of primary lipid peroxidation (LPO) products (diene conjugates, cetodienes, dialdehydes), whereas in later stages there is a prevalence of LPO fluorescent end-products. The injection of LPO dialdehyde products into the vitreous has been shown to induce cataract as a model of cataract associated with retinal disorders.[6] It has been theorized that peroxide damage of the lens fiber membranes may be the initial cause of cataract development.[5–7]

Hyperglycemia causes the auto-oxidation of glucose, glycation of proteins, and activation of polyol metabolism. These changes accelerate generation of reactive oxygen species (ROS) and increases in oxidative chemical modification of lipids, DNA, and proteins in various tissues.[8] Oxidative stress may play an important role in the development of complications in diabetes such as lens cataracts, retinopathy, nephropathy, and neuropathy. Glycation reactions, especially Maillard reactions, occur in vivo as well as in vitro and are associated with the chronic complications of diabetes mellitus and aging and age-related diseases by increases in oxidative chemical modification of lipids, DNA, and proteins. In particular, long-lived proteins such as lens crystallines, collagens, and

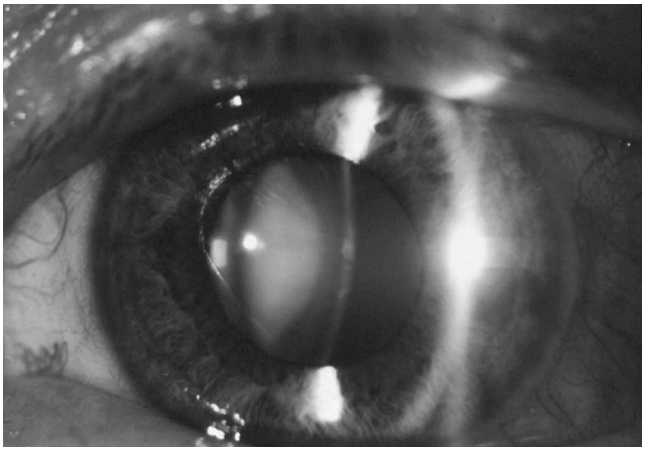

FIGURE 1. Cataract. Slit lamp image reveals marked lens opacity of the eye. The elderly patient complained of a slowly progressive, painless loss of vision.

hemoglobin may react with reducing sugars to form advanced glycation end products (AGEs). Free radicals have extremely short half-lives, and they readily oxidize lipids and initiate an autocatalytic chain reaction of lipid peroxidation, which leads to the formation of lipid peroxides. The lipid peroxides undergo degradation to form metastable lipid aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE). We have shown earlier that under hyperglycemia, lipid peroxides increase; and aldose reductase, an enzyme that reduces glucose to sorbitol, efficiently reduces HNE.[9,10] MDA is a deleterious end-product of lipid peroxidation. Recently, we have originally discovered that some natural compounds of a peptide character or their metal chelates may be among the most potent lipoperoxidase mimetics that have ever been characterized.[5,10] L-carnosine (β-alanyl-L-histidine), a naturally occurring dipeptide found in lens, brain, and innervated tissues at concentrations up to 20 mM, and its ophthalmic prodrug bioactivating N-acetylcarnosine, are part of this group of products.[11–13] It is proposed that nontoxic carnosine and related peptides might be explored as potential therapeutic agents for pathologies that involve protein modification mediated by MDA or another cytotoxic α,β-unsaturated aldehyde.[14] Two reaction products of carnosine were identified in a pH-dependent equilibrium: (a) the Michael adduct, stabilized as a 5-member cyclic hemi-acetal, and (b) an imine macrocyclic derivative. The adduction chemistry of carnosine to HNE appears to start with the formation of a reversible α,β-unsaturated imine, followed by ring closure through an intra-molecular Michael addition. The biological role of carnosine as a quencher of α,β-unsaturated aldehydes was verified by detecting carnosine-HNE reaction adducts in oxidized rat skeletal muscle homogenate.[14] These results prompted us to further investigate the carnosine-like properties of this new class of ophthalmic antioxidants, both in scavenging active and toxic aldehydes and in reversing the protein glycation process.

N-acetylcarnosine (NAC) has been shown to improve vision by partially reversing the development of the cataract, thus increasing the transmissivity of the lens to light.[3] In order to bioactivate an antioxidant status naturally, ocular tissue enzymes can modify the NAC molecule and deacetylate NAC, increasing the resistance of lens tissues and its cells to oxidative stress. The topical administration of N-acetylcarnosine in the developed and patented lubricant eyedrop formulation delivers pure L-carnosine and allows its increased intraocular absorption into the aqueous humor surrounding the lens, thus enabling significant improvements in anticataract efficacy.[3,15] This NAC formulation, including a mucoadhesive cellulose-based compound, lubricants, and corneal absorption promoters, also optimizes beneficial effects in a number of ocular degenerative age-dependent disorders.[3,15]

Strengths of this N-acetylcarnosine eyedrop clinical study design are the use of a randomized double-masked, placebo-controlled crossover trial involving patients with cataract and older subjects (including drivers or computer users) to determine the effect of medicine on vision, particularly with regard to occupation. This approach was ethical since *N*-acetylcarnosine has been an accepted and proven therapeutic modality of vision care available in the field of anti-aging medicine since 2002.[3,4,15–18]

MATERIALS AND METHODS

L-carnosine and N-acetylcarnosine were synthesized by Hamari Chemicals Ltd (Japan) per specifications proposed by Innovative Vision Products, Inc. (IVP). The remaining described carnosine derivatives (Figure 2) were synthesized at IVP-connected laboratories and patented by IVP for health care and ophthalmic applications.[10,19,20] The standard peptide chemistry procedures were employed for the synthesis of carnosine derivatives (Figure 2), and the obtained compounds were purified by liquid chromatography (LC) or high-performance LC (HPLC) to obtain pure specimens, as confirmed by nuclear magnetic resonance and mass spectroscopy.[10]

Peroxidation reaction system

The techniques for phospholipid extraction, purification, preparation of liposomes (reverse-phase evaporation technique), lipid peroxidation induction, and product measurement have been described previously in detail.[11,21] Reactive oxygen species (ROS) were generated by an iron/ascorbate promoter system for induction of lipid peroxidation (LPO).

^{13}C nuclear magnetic resonance experiments

Transglycation efficiency of L-carnosine and carnosine derivatives 2–7 (Figure 2) was assessed by following the Szwergold protocol,[39] using the Schiff base glucosyl–ethylamine (G–E) as a model of the first intermediate in the glycation process of side chain primary amines of proteins. ^{15}N-labeled ethylamine was used to minimize electric quadrupole moment and obtain a C-1 peak of glucose as a sharp doublet centered at 90.00 ppm. The kinetics of the transglycation reaction for the control reaction, for carnosine, and for related compounds were measured. For a better evaluation of the transglycation kinetics

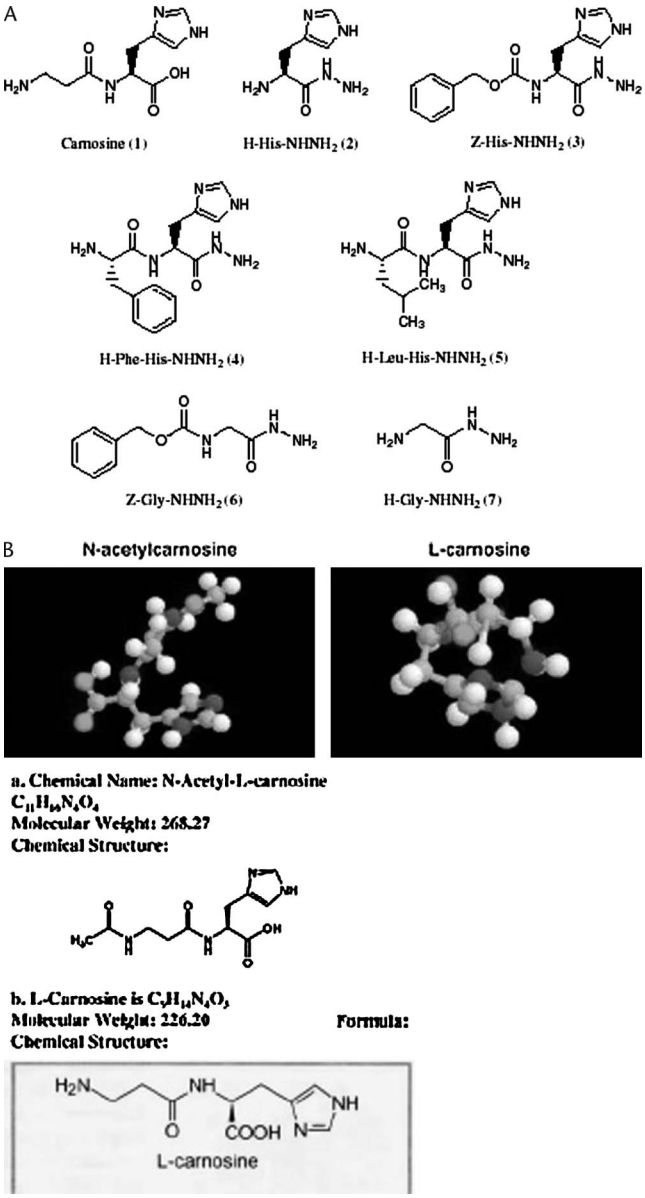

FIGURE 2. Carnosine derivatives were synthesized at IVP-connected laboratories and patented by IVP for health care and ophthalmic application.[10,19,20] The standard peptide chemistry procedures were employed for the synthesis of carnosine derivatives, and the obtained compounds were purified by liquid chromatography (LC) or HPLC to obtain pure specimens, as confirmed by nuclear magnetic resonance and mass spectroscopy.

of the compounds, for each ^{13}C spectrum the integral of the buffer Hepes signals (50–55 ppm range) was set as = 1, then the integral of the C-1 glucose peak at 90.00 ppm was measured and integration values, normalized and corrected for the natural decay of the G–E Schiff base (control curve), were plotted against time (Figure 5A).

Clinical studies

The first enrolled cohort of examined subjects consisted of 75 older adults with age-related uncomplicated cataracts in one or both eyes and 72 adult subjects who did not have cataract in either eye. Patients in these subsamples had different degrees of glare problems (Figs. 3A,B,C). Those with cataract ranged in age from 53 years to 83 years (mean ± SD, 69 ± 8 years); 48% were female and 100% were white non-Hispanic. The noncataract subjects ranged in age from 54 to 78 years (mean ± SD, 66 ± 8 years); 53% were female 100% were white. Subjects who were cataract free had to meet the same inclusion criteria as the subjects with cataract described previously.[4,16–18,22] All subjects with cataract were required to meet the following inclusion criteria: (1) cataract in one or both eyes, with best-corrected visual acuity of 20/40 or worse in one or both eyes, as indicated by the medical record; (2) no previous cataract surgery in either eye; (3) a primary diagnosis of cataract in the medical record; and (4) living independently in the community. Specific items were addressed if appropriate: (5) driving skills (licensed driving during the 5 years prior to enrollment) and (6) related general or eye health problems experienced during computer use, as related by subjects. Among participants, bilateral cataracts were present in 95% according to the medical record from the most recent eye examination (within 1 month of enrollment). In the right eye, 46% had nuclear sclerotic cataract, 8% had cortical cataract, 9% had posterior subcapsular cataract, and 38% had a combination of at least two types. The breakdown was similar in the left eye, with 49% nuclear sclerotic, 10% cortical, 7% posterior subcapsular, and 35% combination. Seventy-four percent of subjects with cataract had no additional ocular conditions other than refractive error; 6% had early nonexudative age-related maculopathy, 9% had primary open-angle glaucoma (POAG) associated with cataract, 3% had diabetic retinopathy, 1% had a combination of 2 of these problems, and 7% had another ocular condition. Subjects who were cataract-free had to meet the same inclusion criteria as the subjects with cataract, except that they were required to be free of cataract and to have a best-corrected visual acuity of 20/25 in each eye, according to medical record review. No cataract-free subjects had secondary eye conditions other than refractive error.

Patients with known or presumed hypersensitivity to any component of the ophthalmic preparations (active substances or excipients) and those treated with drugs that could interfere with this trial were also excluded from the study. The subjects were recruited and examined by ophthalmology practices of IVP,

FIGURE 3. Disability glare results when a light source reflects from or otherwise covers the visual task like a veil, obscuring the visual target, reducing its contrast, and making the viewer less able to see and discriminate what is being viewed. A, sun glare. Many suits have been brought in United States courts as a result of sun glare obliterating traffic control devices or oncoming vehicles or pedestrians from view. Multiple terms have been used to describe this situation, including disability glare, veiling glare, sun blindness, and sun dazzle. The effect is to 'wash out' the image on the retina with a bright, overwhelmingly dominant spot or pattern. Evaluation of sun glare requires factoring in latitude and longitude, road direction, weather conditions, vehicle size and type, driver position, time of day, windshield transmission, whether the driver was wearing sunglasses, and any other parameters that could affect line of sight. B, example of headlight disability glare. C, example of outdoor glare.

using the clinical procedures and design of a randomized study of patients recently described.[23] The study protocol was approved by the Corporative Review Board for Human Use. After the purpose of the study had been explained, each subject was asked to sign a document of informed consent before enrolling. Demographic data, driving status during the prior 5 years, and computer use at work were confirmed by interview (Table 1).

Procedures

After enrollment, subjects were computer-randomized into 2 groups according to the double-blind method: to receive treatment with *N*-acetylcarnosine 1% eyedrops (Can-C) or to receive placebo eyedrops (control group). The blinded testing was carried out by an independent medical worker, who handed out the eyedrops (NAC versus placebo) to the randomized members of the clinical groups. The enrolled subjects underwent follow-up examinations at baseline and after 9 months of enrollment. Test examiners were masked to the driving histories of all subjects. Two types of visual functions were assessed: visual acuity and glare sensitivity (disability glare). All acuity measurements were made while subjects wore the lens correction they typically used during the performance of everyday distance activities, including driving. Each eye was assessed separately. Distance acuity was measured as described before using the letter chart and its standard protocol and was expressed as log minimum angle resolvable.[4,16–18,36–38] For each eye, visual acuity measurements were grouped into four categories: 20/25 or better, 20/25–20/30, 20/35–20/50, and worse than 20/50. These cut points were chosen because they were the approximate quartiles of the acuity distribution and included the practically significant cut point for driving licensure in many countries (20/40 to 20/50). Some of the vision problems from this course will include computer-related work and the circumstances under which that work is performed.

Slit-lamp biomicroscopic examination or exampled photographic registration was performed after pupil dilation to a minimum of 6 mm with tropicamide.

Disability glare was defined with an optical instrument Halometer DG and by a previously described method for measuring susceptibility to glare of a human vision system.[4,16–18,22–24] A constant ''point''-like bright glare source is used to create the glare condition. The examining room was dark (less than 20 foot-candles), as typical when working with glare testers to ensure maximum contrast of the projected target. Tests were performed with the best correction in place. The indicator of optotypes on the front or back panels of the instrument indicated the tested optotype

Table 1. Demographic and ergonomic occupational characteristics of cataract and no-cataract adult subjects enrolled in the study.

	Cataract		No cataract	
Characteristic	n	%	n	%
Total	75		72	
Age group, years				
50–59	18	24	18	25
60–69	43	57	40	56
70–85	14	19	14	19
Sex				
Female	36	48	38	53
Male	39	52	34	48
Race: white	75	100	72	100
Driving exposure*				
Total	40	53	42	58
<150 km/wk	23	58	17	41
≥150 km/wk	17	42	25	59
Computer use†				
Any	47	63	51	71
Occasional	17	36	21	41
Moderate	18	38	16	31
Intensive	12	26	14	28

*Driving subjects were classified into 2 categories according to whether they drove more or less than the median number of km (150 km) driven per week, based on the distribution of all subjects. Although this was a self-report measure, prior studies indicate that older adults can provide valid estimates of driving exposure.[27]

†Occasional use: typically <3 hours per day; user tends to have an extensive variety of tasks (computer and other) and is unlikely to regularly spend extended amounts of time at the computer. Moderate use: typically 3–5 hours per day; user tends to have some variety in daily work tasks but may spend up to half the workday at the computer. Intensive use: typically >5 hours per day; user may have few or no noncomputer tasks and is considered to be at high risk of developing computer-related injuries if precautions such as appropriate workstation design, layout, and work practices are not addressed.

to the patient or clinician, respectively. The diagnostic block of a device contained source light window (glare source) and the moving indicator of the optotypes illuminated with red or green light. The back panel of the Halometer device facing the clinician was equipped with a chart/scale and with a moving indicator of the optotype transition. According to a special embodiment of the invention,[4,16–18,22,24] for the clinical testing of glare sensitivity of a patient we used an illuminated target with red or green color, which enabled assessment of the effect of wavelength on the scattered light.

Treatments with N-acetylcarnosine 1% lubricant eyedrops

N-acetylcarnosine (NAC) eyedrops (Can-C) contained a 1% solution of NAC[19,25,26] with a lubricant, 0.3% carboxymethylcellulose, in the isotonic ophthalmic formulation in borate buffer with preservative benzyl alcohol (corneal absorption promoter) and showed the increased intraocular absorption of the active principle (L-carnosine) in the aqueous humor compared to topical administration of a pure 1% NAC solution (Table 2).

The ophthalmic formulation thus creates a facility to examine the efficacy of treatment for improvements of vision during the short-term periods of administration of *N*-acetylcarnosine 1% eyedrops (9 months in the present study). The administration schedule was 2 drops instilled twice daily for patients assigned to NAC and those assigned to placebo (the same formulation without *N*- acetylcarnosine 1%) alone for 9 months. The use of other topical or nutritional antioxidants was not measured or evaluated between the two groups. The control groups and the treated group did not take any prescribed antioxidant vitamins that might have added to the antioxidant level. Neither the investigators nor the patients knew who was receiving NAC.

Statistical analyses

Statistical analysis was performed by Student's t test; $P = 0.05$ was taken as the upper limit of significance. To assess associations, correlation and linear regression analyses were used.

Repurchase earnings analysis

The major factors that led to the sharp increase in net earnings for the quarters of 2007 in comparison with the corresponding quarters of 2003 were a significant increase in selling and general and administrative expenses for the promotion of Can-C N- acetylcarnosine lubricant eyedrops to public. The launch of the Can-C ophthalmic formulation and its continuing significant sales as an anticataract and antiglare product contributed most of the sales growth in the United States and Europe. The Computer Based Facilities Inventory & Utilization Management Information Subsystem allowed the authors to analyze the trades that occurred and ongoing data from the fourth

Table 2. Recommended patented[19] topical ophthalmic formulation (lubricant eye drops Can-C) including 1% N-acetylcarnosine combined with corneal absorption promoters.

Deionized water	970 g
Glycerine, 1.0%	13 g
N-Acetylcarnosine,1.0%	10 g
Carboxymethylcellulose, 0.3%	3 g
Benzyl alcohol, 0.3%	3 g
Potassium borate	7.91 g*
Potassium bicarbonate	3.47 g*

*Or what is necessary to bring the solution up to a pH of ~6.3–6.8.

quarter of 2002 to the third quarter of 2007; they now have in place a publicly announced repurchase program that, after calculation of the number of sold eyedrops, generally supports enhanced patient compliance and improved safety of the drug candidate for subjects who may self-administer the IVP N-acetylcarnosine lubricant eyedrops prescribed for the reduction or treatment of cataracts, and every challenge opportunity is taken to promote self-care.

Patient compliance with the self-administered Can-C eyedrops was considered fine.

RESULTS

Antioxidant activity of N-acetylcarnosine versus L-carnosine in the liposome peroxidation system; metabolic bioactivating antioxidant activity of N-acetylcarnosine; scavenging activity of L-carnosine towards dialdehyde products of lipid peroxidation

The comparative antioxidant activity of NAC and L-carnosine was assessed in the LPO system (acting as oxidative lipid membrane substrate) catalyzed by Fe^{2+} + ascorbate (Figs. 4A and 4B). The accumulation kinetics of molecular LPO products such as MDA and liposomal conjugated dienes and trienes are shown in Figure 4 (A,B,C). The results demonstrate that the LPO reactions in the model system of lipid membranes are markedly inhibited by L-carnosine. The effective concentrations of L-carnosine are 10 and 20 mM. Data on the biological effectiveness of L-carnosine as an antioxidant preventing PC liposomal or linoleic acid peroxidation in physiological concentration ranges of 5–25 mM have already been published.[12,21,29] Figure 4A shows that the level of 2-thiobarbituric acid (TBA)-reactive substances (TBARS, dialdehydes) reached at the 5-min incubation decreases in the presence of L-carnosine (10 or 20 mM) at 10 min and at later time points (20 mM), which must be due to a loss of existing TBARS or peroxide precursors of MDA and not due to a decreased formation of peroxide compounds. From the data published by Babizhayev in 1989,[11] it follows that the addition of carnosine against a background of accumulated peroxide products (dialdehydes), determinable according to MDA, leads to a decrease in their concentration. Most data indicate that carnosine, in contrast with other "gold standard" antioxidants, at a concentration 15–50 mM, interacts directly with the already formed LPO products (dialdehydes) in the membrane structures, providing for neutralization of their injurious action. The β-alanine and L-histidine contained in carnosine, imidazole (chemically the most active part of the histidine molecule), as well as reduced glutathione (endogenous antioxidative substrate of the lens), have no eliminating effect on ascorbate-dependent LPO product accumulation.[11]

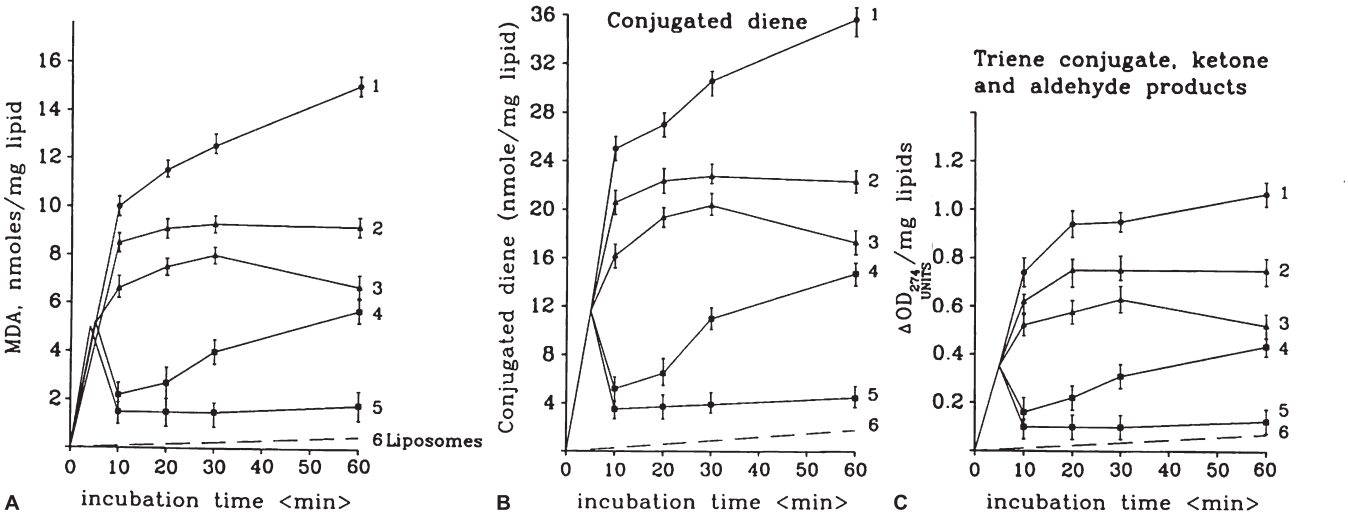

FIGURE 4. Accumulation of lipid peroxidation products (2-thiobarbituric acid [TBA]–reactive substances, or TBARS, measured as MDA). A, diene conjugates; B, triene conjugates and ketone and aldehyde products (274 nm absorbing material) in C, liposomes (1 mg/mL) incubated for 60 min alone (6, dotted line) and with addition of the peroxidation-inducing system of Fe^{2+} + ascorbate (1). Antioxidants N-acetylcarnosine (NAC) (10 or 20 mM) (2,3) or L-carnosine (10 or 20 mM) (4,5) were added at the fifth minute of the incubation period to the system containing the peroxidation inducers. Samples were taken at zero time and at time intervals indicated in the figures and were used immediately for measurement of TBARS (see Materials and Methods, Peroxidation Reaction System). A similar amount of sample was partitioned through chloroform and used for detection of conjugated dienes and trienes dissolved in 2–3 mL of methanol-heptane mixture (5:1 v/v).

Thus, in histidine and imidazole medium, the final content of LPO products (dialdehydes) does not differ from that observed in Tris-HCl buffer (control), whereas the initial rate of accumulation of peroxides (dialdehydes) was even higher than in the control.

The ability of the histidine-containing compound NAC to inhibit the (Fe^{2+} + ascorbate)-induced oxidation of PC liposomes was compared with that of equimolar concentrations of L-carnosine. The antioxidant activity of 10 and 20 mM NAC corresponded to 38% and 55% inhibition of LPO for the two concentrations after 60-min incubation. NAC exhibited less antioxidant protection than L-carnosine, corresponding to 60% and 87% of the equimolar (10 or 20 mM) L-carnosine inhibition percentage. However, because NAC can act as a time-release version metabolized into L-carnosine during its crossing through the cornea to the aqueous humor (but not with oral use), the bioactivating antioxidant activity of NAC converted to L-carnosine with in vivo application is significantly increased. Once released from NAC, L-carnosine in the aqueous humor might act against peroxidation of the lens during its target pharmaceutical use.

The prodrug N-acetylcarnosine approach has been utilized to enhance the ocular delivery of L-carnosine. The N-acetylcarnosine prodrug approach is one of the most promising in ophthalmology and one of the most viable strategies currently being investigated in this study for ocular drug delivery. Careful consideration and understanding of ocular tissue metabolic processes within the eye and site-specific cornea/conjunctiva tissues have important implications for controlling the activity of considered therapeutic peptide agents and for providing the potential for intraocular antioxidant bio-activation of certain N-acetylcarnosine prodrug formulations and designed codrugs, thus enabling significant improvements in efficacy and the minimization of local and systemic side effects.

Transglycation activities of L-carnosine derivatives

The data presented in Figures 5A and 5B show that the transglycating efficiency of the tested carnosine derivatives is generally lower than that of carnosine, with the exception of leucyl-histidylhydrazide (5), of which the transglycation activity is markedly higher than of carnosine in the tested objective G-E Schiff base decay system. Log *P* value and transglycating efficiency of the derivatives show good correlation ($R^2 = 0.38$) (Figure 5B). The hydrazide moiety of leucyl-histidylhydrazide (5) boosts the aldehyde scavenging efficiency of the compound,[10] and in combination with a free N^{α}-amino group, it concurs in the disruption of the Schiff base adduct G–E as a model of protein glycation. Further structure/activity relationship details the synergistic efficacy of leucyl-histidylhydrazide (5) in therapeutic applications.[20] The data are related to a sample supporting the IVP invention of the worldwide patented codrug formulation including N-acetylcarnosine (an ophthalmic prodrug of L-carnosine) and a revealed tripeptide peptidomimetic reversing the glycosylation (glucose-derived intermolecular) crosslinks in proteins (advanced glycation endproducts [AGEs]) and the Schiff bases for the next-generation treatment of ophthalmic complications of diabetes mellitus (DM), such as the development of visual impairment or blindness consequent to cataract formation, retinopathy, or glaucoma.[19,20] Diabetes affects the (outer) lens, middle (vitreous), and inner (retina) areas of the eye.

Randomized, double-masked phase II clinical trial of cataract patients treated with N-acetylcarnosine lubricant eyedrops (Can-C) compared with placebo treatment: clinical study to evaluate the safety and efficacy of treatment

Sample characteristics

Table 1 lists the demographic and ergonomic occupational characteristics of the cataract (n = 75) and no-cataract groups (n = 72). Those with cataract were similarly older on average than the noncataract group of subjects. Both groups were split evenly between males and females and matched in racial composition (white).

Table 3 lists the visual function for both groups enrolled in the study and the distribution of visual acuity and disability glare scores for subjects with cataract and those without. As would be expected by the case definition for cataract group membership, those in the cataract group had impairments in visual function as compared to the no-cataract group. This was true for both the ''worse'' and ''better'' eyes. In addition, visual acuity in the range of 20/35 to 20/50 and disability glare readings in the range of glare radius more than 12 mm were associated with driving difficulties (such as crash involvement) and computer-related injuries. Although not statistically significant, there was also a possible relationship between visual acuity worse than 20/50 and experiencing a crash during driving.[27] Disability glare (glare radius measured in millimeters) was correlated for statistical significance with visual acuity at red and green targets at baseline and a 9- month examination interval in the total samples of older subjects with cataract and in the no-cataract older adult subjects (Table 4).

Older subjects enrolled in the study were divided into two groups: the group treated with NAC and the

control group (Tables 5 and 6). Table 5 lists the analogous and adjusted analyses for the worse eye, for which generated results in the eyes with cataract upon treatment with NAC prodrug ophthalmic formulation are qualitatively similar to those for the better eye. None of the baseline differences between the different groups were significant. The two groups were similar in smoking history, sunlight exposure, and alcohol use. There was not any substantial difference in the use of sunglasses, where the patients lived, or occupational hazard exposure between the two groups.

Ophthalmic examinations indicated that the methodological variances of measurements were approximately equal. Correlations of glare sensitivity at red versus green targets were significant (Table 4). Intra-operator correlation coefficients obtained as repeated measurements for each combination of operator, eye (right or left), and glare radius (at red and green targets) were statistically significant and presented earlier.[4,16–18,22] Overall, the reproducibility for the one operator was good. Tables 3–7 summarize the effects of study treatment on VA and glare sensitivity over 9 months in older subjects with cataract and no-cataract. In the control placebo-treated group, comparison with baseline values showed some variability of data in gradual worsening of glare sensitivity at red and green targets and minimal VA changes over 9 months (Table 6). Glare sensitivity indicated mostly changes in lens clarity and confirmed the tiny changes in the optical media of the eye at the short-term follow-up examinations when slit-lamp-associated image analysis data and densitometric readings did not differ significantly with baseline.[4,16–18,22]

Table 3. Distribution of visual acuity and disability glare in the cataract and no-cataract groups of adult subjects enrolled in the study, at baseline examination.

	Adult subjects			
	Cataract group		No-cataract group	
Variable	n	%	n	%
Total	75		72	
Visual acuity of worse eye				
20/25 or better	9	12	50	70
20/25 to 20/30	14	19	13	18
20/35 to 20/50	48	64	6	8
Worse than 20/50	4	5	3	4
Disability glare readings (glare radius)				
At red target				
<12 mm	10	14	27	38
≥12 mm	65	86	45	62
At green target				
<12 mm	7	9	20	28
≥12 mm	68	91	52	72
Visual acuity of better eye				
20/25 or better	12	16	53	74
20/25 to 20/30	30	40	9	12
20/35 to 20/50	25	34	7	10
Worse than 20/50	8	10	3	4
Disability glare readings (glare radius)				
At red target				
<12 mm	29	38	40	55
≥12 mm	46	62	32	45
At green target				
<12 mm	18	24	24	34
≥12 mm	57	76	48	66

Note. Normal measures of glare sensitivity of young subjects (20–30 years) with best correction without cataracts are 3 ± 2 mm (mean SD) of at least 4 measurements at red and green targets in the daytime.[16]

In the NAC-treated group, 9-month follow-up generally showed an improvement in VA (according to the distribution score of distance acuities in worse and better eyes), and a significant improvement in glare sensitivity at red and green targets was documented in worse and better eyes with use of a critical cut point halometer score (Tables 5 and 7). VA was mostly improved in older subjects with cataract in worse and better eyes, and an improvement in glare sensitivity was found both in older subjects with cataract and no-cataract older adult subjects in worse and better eyes after 9 months of treatment with NAC. The exampled images of cataract reversal in older subjects are presented on the slit-lamp images just for note (Figs. 7 A,B,C).

The NAC-treated eyes had a statistically significant difference in VA and glare sensitivity compared with the control group ($P < 0.001$) at the 9-month timepoint of treatment, as supported by the overall *t*-test results of the ratio of the follow-up data to the baseline values (Table 7). The previously published data illustrate examinations over 24 months of the eyes treated with NAC to show that the effect of treatment is sustainable over more prolonged periods.[4,16–18] In the eyes of older subjects with different localization and grade of cataract and in no-cataract older adult subjects, short-term and prolonged treatment with NAC did not seem to result in a worsening of the visual outcome in this study and elsewhere.[4,16–18] Topical short- or long-term administration of 1% NAC to the eye was very well tolerated, with no ocular or systemic adverse effects, no hyperemia of conjunctival vessels, and no signs of allergy or other toxic manifestations being reported. No clinically significant changes from baseline, and no statistically significant differences between the treatment and control groups,

Table 4. Linear correlation coefficients (r) between the characteristics of cataract and no-cataract groups of older adult subjects, as measured by visual acuity (VA) and glare radius (GR at red and green targets) at baseline and at 9-month follow-up ophthalmic examinations.

	Baseline study			9 months		
Parameter	VA	GR red target	GR green target	VA	GR red target	GR green target
Older subjects with cataract (75 eyes examined)						
VA	X	−0.63*	−0.65*	X	−0.47	−0.45
GR red target		X	+0.83*		X	+0.94*
GR green target			X			X
Older adult no-cataract subjects (72 eyes examined)						
VA	X	−0.61*	−0.66*	X	−0.43	−0.46
GR red target		X	+0.81*		X	+0.91*
GR green target			X			X

*$P < 0.01$.

were observed regarding ocular comfort and ocular signs and symptoms (lack of burning and stinging, blurred vision, ocular dryness, superficial punctate keratitis, foreign body sensation, itching, ocular discharge, ocular pain, tearing, ocular inflammation, photophobia). All patients completed the study without any problems related to their allocated treatment.

Boards of directors analyzed the repurchase behavior of Can-C in open market programs. The product has been on the market since fall 2001, and it is well known according to records of how many boxes of Can-C are actually repurchased. The analyses in this article demonstrate the repurchase behavior of patients in more than 52,000 individual buyback programs. We find that repurchase announcements during 5 recent years of follow-up show the credible figure of 50,500 patients compliant in using their Can-C eyedrops for published therapeutic indications (including treatment of cataracts) and fine safety on a daily basis. On average, these patients repurchase more boxes of the drug than they have originally authorized over the latest 4 quarters following the announcement of greater sales, although there is considerable variation across patients' requests. We examined the factors influencing repurchase behavior and found that repurchases in the latest quarters are associated with a number of variables, including the efficacy of the product and its persistent safety for controlling the signs of glare sensitivity, cataract amelioration, and quality of vision during daily life activities. We also considered the past and current returns, profitability, and prior repurchase activity.

According to the records of repurchase behavior, the unique and patented N-acetylcarnosine lubricant all-in-one eyedrop formula Can-C can also provide beneficial results with the following eye-disorders:

- Presbyopia[19]
- Open-angle primary glaucoma (in combination with β-blockers) (Figure 6)
- Corneal disorders[15,16]
- Computer vision syndrome
- Driving and night vision disability glare; perceived driving disability at night[36]
- Eyestrain
- Ocular inflammation
- Blurred vision
- Dry eye syndrome
- Retinal diseases
- Vitreous opacities and lesions
- Complications of diabetes mellitus and other systemic diseases
- Contact lens difficulties, particularly with soft contact lenses (not only do the lubricants in the Can-C eyedrops help to make wearing contact lenses more comfortable, but also N-acetylcarnosine is believed to reduce the build-up of lactic acid in the eye, thus enabling the lens to be left safely in the eye for longer).

We also investigated how the sales outstanding change after repurchase program announcements. Over the last test-year period, the average increase in sales was about 80%, with only about 35% repurchases. For the most part, changes in sales of Can-C are influenced by the same factors affecting repurchases and in the predicted direction of warranted efficacy and safety of the product. The data regularly present examples, but most typical and standard are appreciable testimonials received from adult patients

Table 5. Visual function in the better and worse eyes after 9 months of treatment with *N*-acetylcarnosine 1% eyedrops (Can-C), versus baseline examination.

	Adult subjects			
	Cataract group		No cataract group	
	n	%	n	%
Total	39		37	
Baseline examination				
Visual acuity of worse eye				
20/25 or better	5	13	26	70
20/25–20/30	8	21	5	14
20/35–20/50	22	56	3	8
Worse than 20/50	4	10	3	8
Disability glare readings (glare radius)				
At red target				
<12 mm	6	15	15	41
≥12 mm	33	85	22	59
At green target				
<12 mm	4	10	11	30
≥12 mm	35	90	26	70
Visual acuity of better eye				
20/25 or better	5	13	25	68
20/25–20/30	12	31	8	22
20/35–20/50	17	43	2	5
Worse than 20/50	5	13	2	5
Disability glare readings (glare radius)				
At red target				
<12 mm	10	26	21	57
≥12 mm	29	74	16	43
At green target				
<12 mm	8	21	12	32
≥12 mm	31	79	25	68
After 9 months of treatment with *N*-acetylcarnosine 1% eyedrops				
Visual acuity of worse eye				
20/25 or better	9	23	27	73
20/25–20/30	16	41	7	19
20/35–20/50	13	33	2	5
Worse than 20/50	1	3	1	3
Disability glare readings (glare radius)				
At red target				
<12 mm	12	30	25	67
≥12 mm	27	70	12	33
At green target				
<12 mm	10	25	21	56
≥12 mm	29	75	16	44
Visual acuity of better eye				
20/25 or better	15	38	30	80
20/25–20/30	18	47	5	14
20/35–20/50	4	10	1	3
Worse than 20/50	2	5	1	3
Disability glare readings (glare radius)				
At red target				
<12 mm	18	45	30	81
≥12 mm	21	55	7	19
At green target				
<12 mm	19	46	21	57
≥12 mm	21	54	16	43

who have originally purchased Can-C. The N-acetylcarnosine lubricant eyedrops have been successfully used for medically oriented home health care, usually for helping seniors recover from visual impairment or illness including cataracts. It is important to note that most workers for home health care agencies, hospitals, or public health departments are licensed by the state.

DISCUSSION

The NAC 1% eyedrops seem to improve the vision of the older adult subjects regardless of whether they have cataracts or not, but the improvement in visual acuity was significantly better in the group of cataract subjects versus the older adult subjects in the matched noncataract group. The data on visual functions (VA, glare sensitivity) in older adult subjects and older subjects with cataract treated with 1% NAC showed significant improvement as contrasted with the control group, which showed generally no improvement in visual functions, with no difference from baseline in VA and glare sensitivity readings. In most of the patients the study treatment was well tolerated, and no ocular or systemic adverse events were reported. The data overall are an additional mode of evidence suggesting that carnosine applied in the form of NAC reverses and prevents lens opacity in humans.[3,4,16–19]

One of the important problems in modern ophthalmic therapeutics is the regulation of ROS in ocular tissues, to equilibrate between prevention of their damaging effects to the cells and the support of their signaling functions. The overproduction of ROS is known to result in the oxidative damage of a variety of cellular components, including nucleic acids, proteins, and lipids. However, lipophilic antioxidants are only partially protective. Utilizing the specific-purity (L)-isoform N-acetylcarnosine ingredient manufactured at the cGMP facility according to specifications developed by IVP, as a source of pharmacological principal L-carnosine, we have created an ophthalmic formulation that contains varying amounts of the actives, tailoring the enhanced intraocular absorption of the beneficial ingredient. When cataract was accompanied with primary open-angle glaucoma (POAG) (Figure 6), NAC was prescribed 15 min prior to the topical application of β-blocker, specifically used to decrease the intraocular pressure.[19] The improvement of visual functions in patients with cataracts associated with POAG was accompanied with a significant decrease in intraocular pressure, and there was an increase in the outflow facility in the eyes of patients with POAG treated with the indicated combined therapy.[19]

Table 6. Visual function in the better and worse eyes after 9 months of treatment with placebo (control group), versus baseline examination.

	Adult subjects			
	Cataract group		No-cataract group	
Variable	n	%	n	%
Total	36		35	
Baseline examination				
Visual acuity of worse eye				
20/25 or better	3	8	21	60
20/25–20/30	7	19	7	20
20/35–20/50	23	64	5	14
Worse than 20/50	3	8	2	6
Disability glare readings (glare radius)				
At red target				
<12 mm	9	25	17	49
≥12 mm	27	75	18	51
At green target				
<12 mm	6	17	11	31
≥12 mm	30	83	24	69
Visual acuity of better eye				
20/25 or better	11	31	25	72
20/25–20/30	17	47	5	14
20/35–20/50	6	17	5	14
Worse than 20/50	2	5	0	0
Disability glare readings (glare radius)				
At red target				
<12 mm	19	53	23	66
≥12 mm	17	47	12	34
At green target				
<12 mm	13	36	16	46
≥12 mm	23	64	19	54
After 9 months of treatment with placebo				
Visual acuity of worse eye				
20/25 or better	2	6	19	54
20/25–20/30	6	17	9	26
20/35–20/50	25	69	5	14
Worse than 20/50	3	8	2	6
Disability glare readings (glare radius)				
At red target				
<12 mm	8	22	16	46
≥12 mm	28	78	19	54
At green target				
<12 mm	5	14	8	23
≥12 mm	31	86	27	77
Visual acuity of better eye				
20/25 or better	9	25	25	71
20/25–20/30	17	47	5	14
20/35–20/50	8	22	5	14
Worse than 20/50	2	6	0	0
Disability glare readings (glare radius)				
At red target				
<12 mm	16	44	21	60
≥12 mm	20	56	14	40
At green target				
<12 mm	10	28	14	40
≥12 mm	26	72	21	79

Carnosine has been proposed to act as an antioxidant in vivo, and its activity in the crystalline lens can be related to the prevention of the free radical–induced inactivation of activity of the proprietary antioxidant enzymes in the lens, such as superoxide dismutase.[28] Similar protective action of carnosine toward telomerase,[40] an enzyme important for the protection of cellular machinery, was demonstrated recently. Besides enzymes, some membrane-bound receptors are also protected by carnosine. Carnosine exhibits an ability to inhibit LPO catalysts in addition to inhibiting free metals, scavenging OH· and lipid peroxyl (RO2·) radicals or donating hydrogen ions. In addition to inhibiting the generation of lipid peroxyl radicals, carnosine catabolyzes lipid hydroperoxides to their alcohols, both in aqueous medium and in a phospholipid system.[29] A possibility exists from our study that carnosine is reacting directly with MDA and other aldehydes/ketones. Indeed, carnosine has been shown to protect against MDA-induced crosslinking and toxicity, and a hydroxynonenal-carnosine adduct has recently been characterized, providing further evidence for carnosine's potential as an aldehyde scavenger.[10,14,30] The ability of L-carnosine to inhibit LPO reactions as well as to diminish the content of LPO-derived products (including aldehydes) makes N-acetylcarnosine applied with lubricant carboxymethylcellulose a prominent tool in therapy, especially for posterior subcapsular and cortical cataracts, whose mechanisms can be related to the toxic effects of LPO products.[6]

There is a rising evidence that carnosine prevents oxidation and glycation, both of which contribute to the crosslinking of proteins.[31] The imidazolium group of histidine of carnosine may stabilize adducts formed at the primary amino group.[31] Cellular aging is often associated with an increase in protein carbonyl groups arising from oxidation and glycation-related phenomena and suppressed proteasome activity. Dicarbonyl compounds have been identified as the predominant source for the formation of advanced glycation end products (AGE) in various tissues including the lens. In our studies advanced glycation end products (AGE) have been found to contribute to aging and cataract formation in the lens. Their roles in diabetic related complications have now been gained importance. Besides the increased levels in long term diabetics, both AGE and ALE (advanced lipoxidation end products) might be a factor for influencing progress of senile cataract. The ability of carnosine to react with protein carbonyls was recently reviewed by Hipkiss and Brownson.[32] They showed that carnosine protects protein molecules from carbonylation much more effectively than does lysine and that this may related

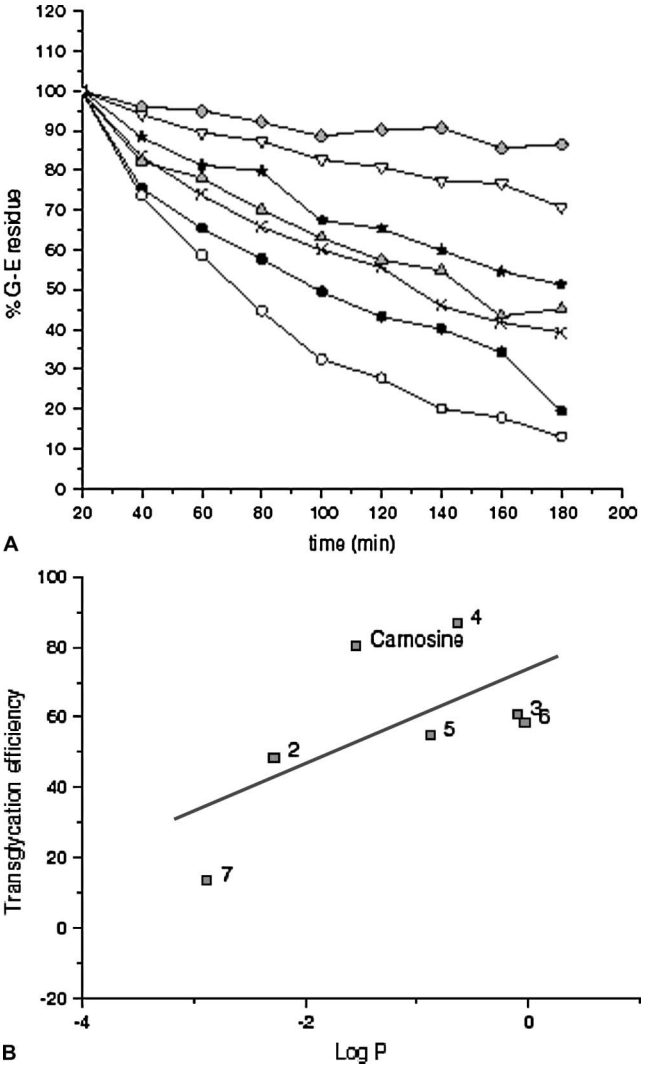

FIGURE 5. A, transglycation efficiency of tested compounds: carnosine 1(●); His-$NHNH_2$ 2 (★); Z-His-$NHNH_2$ 3 (◈); Phe-His-$NHNH_2$ 4 (▲); Leu-His-$NHNH_2$ 5 (○); Z-Gly-$NHNH_2$ 6 (▽); Gly-$NHNH_2$ 7 (×). The area of the G–E doublet at 90 ppm was plotted against time and corrected for the G–E Schiff base decay measured in the control experiment. B, logP/transglycation efficiency (% of G–E removal after 180-min incubation) correlation for carnosine and compounds 2–7. $R^2 = 0.38$.

to both rejuvenation of human fibroblast cultured (propagation of Hayflick limit) and correlation between life span of different species and content of carnosine in their tissues.[32] Glycation of lens α-crystallin occurs *in vivo* and may contribute to cataractogenesis. Anti-glycation compounds such as carnosine may be preventive, but interestingly carnosine reverses lens opacity in our human trials. The mechanism for this observation may involve carnosine's ability to disaggregate glycated protein. Seidler et al.[33] recently investigated this hypothesis using glycated α-crystallin as the *in vitro* model. Methylglyoxal-induced glycation of α-crystallin caused aggregation as evidenced by increased 90 degrees light scattering. After addition of carnosine, light scattering returned to baseline levels suggesting that the size of the glycation-induced aggregates decreased. These data support the hypothesis that carnosine disaggregates glycated α-crystallin. To distinguish between the carbonyl trapping and antioxidant activity of the advanced glycation end product (AGE) inhibitors, Price et al.[34] measured the chelating activity of carnosine by determining the concentration required for 50% inhibition of the rate of copper-catalyzed autoxidation of ascorbic acid. In their studies L-carnosine exhibited the anti-glycating activity with the estimated IC_{50} 4 μM for inhibition of copper catalysed oxidation of ascorbic acid that proposes this natural dipeptide as a potent inhibitor of glycation reactions in the lens proteins mediated by metal-catalyzed oxidation of ascorbate present in the aqueous humor.[34] The obtained in the present study data can be satisfactorily explained by the cited above ability of carnosine to protect cellular and tissue structures from aldehydes, which in excess may be toxic because of the non-enzymatic glycosylation of proteins. Glycation, preferentially modifies the ε-amino group of lysine residues in proteins, especially when proline neighbors lysine. It is likely that the structural similarity between lysyl-proline and β-alanyl-histidine allows carnosine and its synthetic histidyl-hydrazide derivatives assessed in this study to demonstrate their transglycation properties, e.g. to compete for the glycating agent, protecting proteins (lens crystallins) against modification. Actually, carnosine may prevent accumulation of Amadori products (forming after re-arrangements of products of primary glycation) within lens cells and tissues as well as cross-linking of biomolecules. At moderately high concentrations, carnosine also reverses protein-aldehyde cross-linking, a reaction that is difficult to reverse, thus providing a rejuvenating effect on the lens for vision.

AGEs are a class of complex, often unstable, reactive compounds formed in excess during aging and diabetes mellitus. According to the ''glycation hypothesis,'' accumulation of AGEs alters the structural properties of tissue proteins and reduces their susceptibility to catabolism. It has been generally known that the process of AGE formation is accelerated by hyperglycemia. Some of the protein alterations observed in diabetic patients resemble those in much older, nondiabetic patients, suggesting ''diabetes-induced early aging.'' Protein glycation and AGE

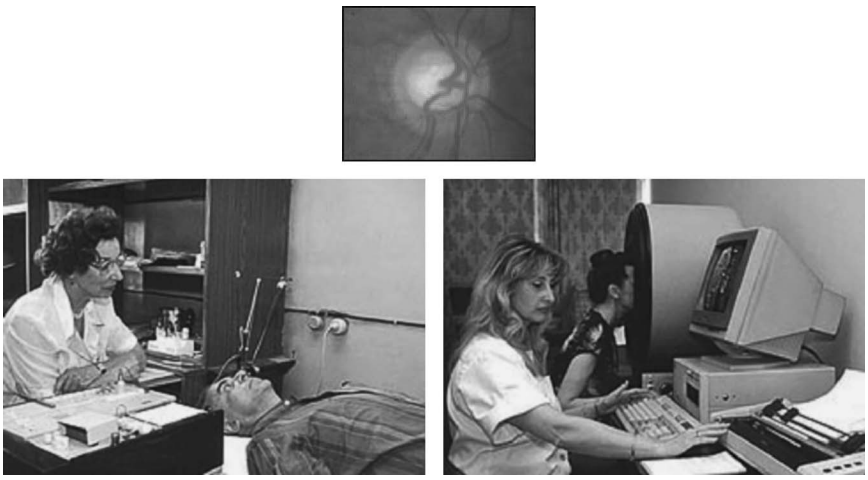

FIGURE 6. Studies of glaucoma at IVP Institute clinical facilities. Over 2 million Americans aged 40 years or older are affected by open-angle glaucoma. Open-angle is the most common type of glaucoma and one of the nation's leading causes of vision loss. Glaucoma occurs when the optic nerve is damaged. In most cases, increased pressure in the eye is a risk factor for this damage. The damage to the optic nerve causes loss of peripheral (side) vision, although people are often unaware that they have glaucoma. As the disease worsens, the field of vision gradually narrows and blindness can result. Growing evidence supports the involvement of oxidative stress as a common component of glaucomatous neurodegeneration in different subcellular compartments of retinal ganglion cells (RGCs). Oxidative stress also promotes the accumulation of advanced glycation end products in glaucomatous tissues. N-acetylcarnosine eyedrop therapy promises value for decrease of intraocular pressure in glaucomatous eyes and protection from the oxidative stress in glaucomatous neurodegeneration.

formation are accompanied by increased free radical activity that contributes to bimolecular damage in diabetes.[35] AGEs act as mediators and can initiate a wide range of abnormal responses in cells and tissues such as the inappropriate expression of growth factors, alterations in growth dynamics, accumulation of extracellular matrix, and initiation of cell death, through decreased solubility, elasticity, and enzymatic affinities in long-living proteins such as collagen.[35] Retinopathy may be associated with an upregulation of the receptor for AGEs (RAGE) in a proinflammatory axis, concomitant with increases in AGEs.[35] We propose that the worldwide-patented IVP codrug formulations of carnosine with histidine-hydrazide peptidomimetic derivatives, which effectively scavenge toxic aldehydes and glycotoxins, have the unique ability to reverse the glycation process according to their carnosine-associated mechanism of transglycation activity, and most intriguing, have the ability to reverse effectively (de-link) the already formed glycosylation (glucose-derived intermolecular) crosslinks in the tissue proteins via the established transglycation mechanism. The described tripeptide compounds and ophthalmic formulations thereof are useful as therapeutic agents for the treatment of complications arising from diabetes and originating from normal aging glycosylation processes.

CONCLUSIONS

NAC acts as a universal antioxidant both in the lipid phase of the cellular lens membranes and in their aqueous environment and protects the crystalline lens from oxidative stress–induced damage. Reducing the oxidative burden on the ocular structures with the N-acetylcarnosine therapeutics in the IVP-developed eyedrop formulations can have lasting effects to promote health vision and prevent a disabling condition from vision disability in senile cataracts (Figure 1), primary open-angle glaucoma (Figure 6), age-related macular degeneration, diabetic retinopathy, and aging.[19]

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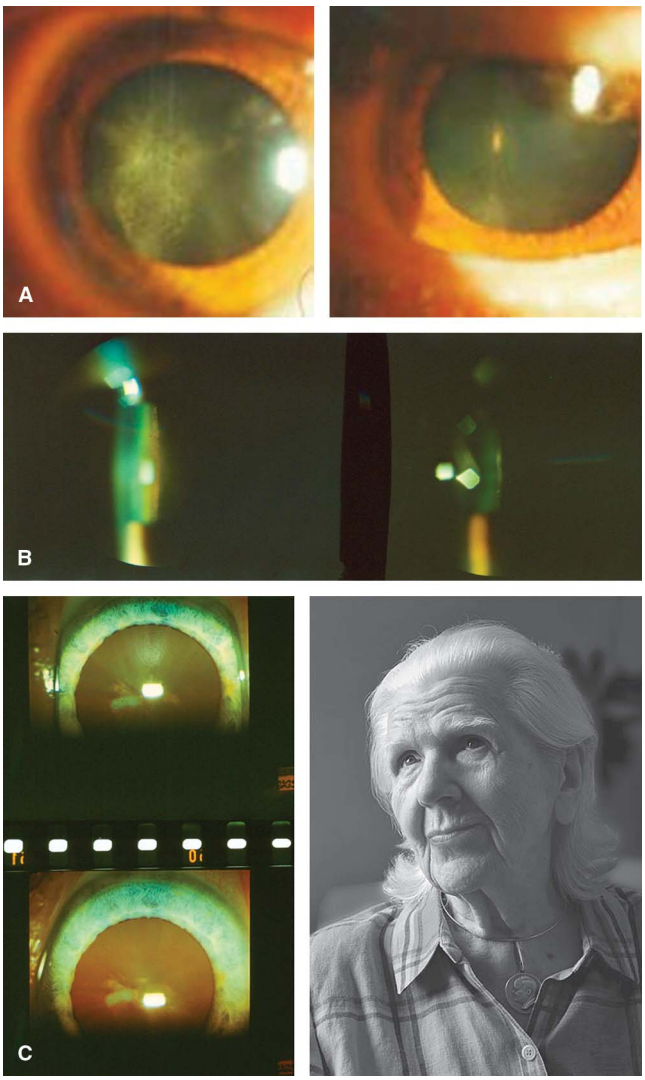

FIGURE 7. A, the treatment of cataract in an older subject with 1% Can-C eyedrops for 5 months. The left image shows the appearance of cataract, which resembles a bat in its form, and the right image shows that this opacity has disappeared after the cited period of treatment with n-acetylcarnosine is completed. The lens has become clearer. B, treatment of posterior subcapsular cataract in an older subject for 9 months with Can-C. Left image is before treatment; right image is after 9 months of treatment. The opalescent areas of lens opacity have been reversed and the lens has become clearer (dark greenish zones) in the right image. C, treatment of cortical cataract in the upper segment of the pupil image of an older woman for 9 months. Upper image shows the lens before treatment; lower image is the lens after 9 months of treatment with Can-C. The appearance of rose reflex in the lower image demonstrates that the lens has become clearer.

Table 7. Mean ± SD of changes (improvement) in visual functions.

Treatment group	Visual acuity	Glare radius
9-Month follow-up of older subjects with cataract		
Control group	0.90 ± 0.03 (n = 36)	1.53 ± 0.07 (n = 36)
NAC-treated group	1.54 ± 0.05*† (n = 39)	0.41 ± 0.05* (n = 39)
9-Month follow-up of older adult no-cataract subjects		
Control group	0.96 ± 0.03 (n = 35)	1.27 ± 0.05 (n = 35)
NAC-treated group	1.20 ± 0.04* (n = 37)	0.38 ± 0.05* (n = 37)

Note. The measure of visual acuity readings after 9 months of treatment was divided by the clinical baseline measure of visual acuity for each eye individually to get ratios, and then the average of those ratios through each clinical group of eyes was calculated. Similarly, with glare, the ratio of glare sensitivity at red and green target after 9 months of treatment to the baseline reading of glare sensitivity was calculated for each eye, and then the ratios were averaged through the whole groups of eyes. NAC, *N*-acetylcarnosine (Can-C).

*$P < 0.001$, compared to control group, who received placebo eyedrops.

†$P < 0.001$, where an improvement in visual acuity was statistically significantly better in the group of older subjects with cataract than in the group of older adult non-cataract subjects.

carnosine derivatives stabilizing carnosine from enzymatic hydrolysis. IVP is a pharmaceutical and nanotechnology development company with a focus on innovative chemical entities, drug delivery systems, and unique medical devices to target specific biomedical applications. Over the past decade, IVP has developed a track record in developing these technologies to effectively address the unmet needs of specific disease populations. The IVP Research Center is a state-of-the-art facility at which the greatest contemporary scientific minds conduct studies to discover the secrets of aging and devise novel, effective interventions to prolong quality and quantity of life. The IVP licensing strategies rapidly become the focal point of innovations in science-based healthcare that may be replicated in countries around the world, thereby making the advanced modalities of medicine available to all. IVP seeks benefactors who share our vision to realize anti-aging breakthroughs for the benefit of humanity, to support the projects. A.G. was supported by Consiglio Nazionale delle Ricerche, Progetto di Ricerca a Tema Libero DG.RSTL.019.009.

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