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N-Acetylcarnosine, a natural histidine-containing dipeptide, as a potent ophthalmic drug in treatment of human cataracts

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Abstract

A study was designed to document and quantify the changes in lens clarity over 6 and 24 months in 2 groups of 49 volunteers (76 eyes) with an average age of 65.3 ± 7.0 enrolled at the time of diagnosis of senile cataracts of minimal to advanced opacification.

The patients received N-acetylcarnosine, 1% sol (NAC) (26 patients, 41 eyes = Group II), placebo composition (13 patients, 21 eyes) topically (two drops, twice daily) to the conjunctival sac, or were untreated (10 patients, 14 eyes); the placebo and untreated groups were combined into the control (reference) Group I. Patients were evaluated upon entry, at 2-month (Trial 1) and 6-month (Trial 2)-intervals for best corrected visual acuity (b/c VA), by ophthalmoscopy and the original techniques of glare test (for Trial 1), stereocinematographic slit-image and retro-illumination photography with subsequent scanning of the lens. The computerized interactive digital analysis of obtained images displayed the light scattering/absorbing centers of the lens into 2-D and 3-D scales.

The intra-reader reproducibility of measuring techniques for cataractous changes was good, with the overall average of correlation coefficients for the image analytical data 0.830 and the glare test readings 0.998. Compared with the baseline examination, over 6 months 41.5% of the eyes treated with NAC presented a significant improvement of the gross transmissivity degree of lenses computed from the images, 90.0% of the eyes showed a gradual improvement in b/c VA to 7–100% and 88.9% of the eyes ranged a 27–100% improvement in glare sensitivity. Topographic studies demonstrated less density and corresponding areas of opacification in posterior subcapsular and cortical morphological regions of the lens consistent with VA up to 0.3. The total study period over 24 months revealed that the beneficial effect of NAC is sustainable. No cases resulted in a worsening of VA and image analytical readings of lenses in the NAC-treated group of patients. In most of the patients drug tolerance was good. Group I of patients demonstrated the variability in the densitometric readings of the lens cloudings, negative advance in glare sensitivity over 6 months and gradual deterioration of VA and gross transmissivity of lenses over 24 months compared with the baseline and 6-month follow-up examinations. Statistical analysis revealed the significant differences over 6 and 24 months in cumulative positive changes of overall characteristics of cataracts in the NAC-treated Group II from the control Group I.

The N-acetylated form of natural dipeptide L-carnosine appears to be suitable and physiologically acceptable for nonsurgical treatment for senile cataracts.

Keywords: Antioxidant; Natural product dipeptide; N-acetylcarnosine; L-carnosine; Ophthalmic drug; Lens; Cataract; Ocular administration

1. Introduction

Cataract is the leading cause of blindness worldwide, accounting for over 50% of the world's blind population, affecting some 17 million people [36]. Although surgical extraction of the involved lens is effective, there is a considerable interest in identifying the risk and protective factors involved in cataractogenesis [35]. Age-related cataract is a multifactorial disease, and different risk factors appear to play a role for different cataract types. Numerous studies postulate that oxidative stress to the lens mediated by reactive oxygen species and lipid peroxides produced in the crystalline lens can initiate the process of cataractogenesis [2,13,18,22,23,31,34]. It is established that superoxide an-

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ion radical, hydroxyl radical, hydrogen peroxide, singlet oxygen and lipid peroxides can be generated by photochemical reactions in the lens surroundings triggering the development of different forms of cataract [7,11,30,33,38] and that the use of antioxidant supplements appears to be protective against cataract [29]. Peroxide damage to the lens plasma membranes may lead to disturbance of their permeability for ions, loss of thiol groups of the membrane-bound crystallins and the appearance of new fluorophores and also large protein aggregates with low solubility (scattering matrix) in the substance of the lens thus affecting the development of cortical (C), posterior subcapsular (PSC) and nuclear (N) cataracts [4,10,11,20].

L-Carnosine (β-alanyl-L-histidine) and related β-alanyl histidyl dipeptides (anserine and balenine) are generally found in mM concentrations in several mammalian tissues, potentially exhibiting different metabolic activities [14]. The previously published data suggest that L-carnosine has excellent potential to act as a natural antioxidant with hydroxyl radical, singlet oxygen scavenging and lipid peroxidase activities [14,21]. A striking effect of L-carnosine is its demonstrated ability to prevent, or partially reverse, lens cataract [3,19]. 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 enzyme that is present in blood plasma, liver, kidney and other tissues except muscle and probably lens [3,24].

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 L-carnosine in skeletal muscle (~10 mM) [27]. The pharmaceutical compositions containing N-acetylcarnosine aluminum salt have been reported for the treatment of gastric ulcers [28]. Among 29 dipeptides of the carnosine family tested as potential substrates for a highly purified human serum carnosinase preparation, N-acetylcarnosine and few other compounds were not hydrolyzed, [24] thus promising a prolongation of physiological responses to the therapeutic treatments. A knowledge of corneal and iris/ciliary body esterase activity, in particular, acetylesterase (EC 3.1.1.6) and, in addition to esterase, the identified N-acetyltransferase activities [1] prompted the development of a prodrug of L-carnosine in its ophthalmic application as antioxidant such as the chemically characterized N-acetylated form of the dipeptide [16]. Experiments with N-acetylcarnosine (NAC) (1% sol) topically administered to the rabbit eyes (instillation, subconjunctival injection, ultrasound-induced administration) revealed its penetration into the eye and accumulation of the native form of L-carnosine in aqueous humor within 15–30 min of administration extending in order of the indicated therapeutic modalities [6,8,16]. The NAC molecule showed a moderate inhibiting activity for catalysis of phosphatidylcholine liposomal peroxidation in vitro, less pronounced than that of L-carnosine [16].

The advantage of NAC to act as an in vivo universal antioxidant with physiological and therapeutic relevance deals with its ability to give efficient protection against oxidative stress in the lipid phase of biological membranes and in aqueous environment due to turnover into L-carnosine [6,8,16]. Due to relative hydrophobicity compared with L-carnosine, NAC might penetrate through the cornea gradually, thus maintaining longer the active therapeutic concentration of L-carnosine in aqueous humor of the treated eye [16]. Different techniques of ocular administration of NAC showed its excellent tolerability to the eye, safety and the lack of possible side effects [16]. The clinical study was designed to be a prospective evaluation of the lens opacities and visual function in cataractous patients who applied topically to the eye (eye drops) the physiologically acceptable solution of NAC [6,8].

2. Subjects and methods

2.1. Clinical design

The research was performed in agreement with the principles of Helsinki Declaration (ed. 1964 and following revisions) and the "Guidelines on the quality, safety and efficacy of pharmaceutical products used in European Community" (91/507/CEE). Each patient received verbal and written explanations about the object of the trial and the properties of the drugs which he would take. Each patient was also informed about his rights, particularly the right of withdrawing from the trial without any justification, and informed consent to the trial was obtained. All the patients were computer randomized concurrently in two clinical groups as to NAC-treated or placebo-treated cases and controls (Table 1) upon the entry in the study. The number of patients needed for each trial was chosen in order that the patient groups were well matched, with no significant differences in demographic and clinical characteristics. The sample size calculations depended on the accuracy of the monitoring method employed for any of the major types of cataract assessed.

A total of 49 elderly patients (76 eyes) completed the 6-month and the 2-year protocol. They were divided into following groups: I, control group representing untreated (10 patients, 14 eyes) or treated with placebo compositions (13 patients, 21 eyes); II, taking the composition of drops containing NAC (26 patients, 41 eyes), (Table 1). Twenty patients (34 eyes) with cataracts were enrolled into the study from the Consulting Division of Moscow Helmholtz Research Institute for Eye Diseases and follow-up examinations carried out every 2 months within a 6-month period (Trial 1). Twenty nine elderly patients with cataracts (42 eyes), supervised by the same observer, were enrolled from the Ophthalmic Division of Innovative Vision Products Inc. with ophthalmic examinations carried out every 6 months

Table 1
Population characteristics of patients

Parameter	Group I, control	Group II, treated with instillations of NAC, 1% sol
Trial 1 (Baseline study: 2 visits; Follow-up period: 6 months, 3 visits)		
No. (total)	10	10
No. eyes	16	18
Males	5	5
Females	5	5
Age-mean (years ± SD)	67.1 ± 7.1	67.0 ± 4.1
Epidimiologic status (N° eyes)	S (13); S_{compl}(3)	S (16); preS (2)
Trial 2 (Baseline study: 1 visit; Follow-up period: 24 months, 4 visits)		
No. (total)	13	16
No. eyes	19	23
Males	7	8
Females	6	8
Age-mean (years ± SD)	64.3 ± 6.7	64.0 ± 8.5
Epidimiologic status (N° eyes)	S (18); S_{compl} (1)	S (19); S_{compl} (4)

for 2 years (Trial 2). Neither the investigators nor the patients knew who was taking NAC.

The population characteristics are shown in Table 1. The groups were compared for sex composition, mean age of patients, severity of initial symptoms and presence of concomitant diseases: none of the baseline differences between the different groups was significant. The study did not measure or evaluate the use of other topical or nutritional antioxidant between the two groups. The control group and the treated group did not take any prescribed antioxidant vitamins that might have added to the antioxidant level. The two studied groups were similar in smoking history, as well as sunlight exposure and alcohol use. There was no any substantial difference in the use of sunglasses between the two studied groups. There was no difference where the patients lived, or occupational hazard exposure between the two examined groups.

2.2. Patient study

The examined eyes had cortical, nuclear, posterior subcapsular, or mixed lens opacities and varying degrees of nuclear color or brunescence. The patients had minimal to advanced lens cataractous changes. Eligibility criteria included a confirmed diagnosis of senile (S) cataract according to the medical history of disease, clinical observations and epidemiological study. Each patient met the following inclusion criteria: (1) availability for study of both lenses in each patient; (2) the presence of a cataract in at least one eye; (3) the cataracts were judged not to require surgery in the near future (2 years) based on the patients' visual needs and ocular symptomatology; (4) 52 to 80 yrs of age; (5) pupillary dilation could be done safely. Patients were not included if they had any other ocular disease such as glaucoma or clinically significant diabetic retinopathy, previous laser retinal photocoagulation, prior corneal or anterior segment surgery or corneal scars which would interfere with visualization or photography of the anterior segment, or mature cataract (VA less than 0.1) in both eyes, and would likely be candidates for cataract surgery within 1 year. Excluded were monocular aphakics and patients with secondary cataracts (e.g., cataracts associated with steroid intake, total body or local irradiation, local inflammatory or degenerative process and ocular trauma). Patients with known or presumed hypersensitivity to any component of the ophthalmic medications (active substances or excipients), or patients treated with drugs which could interfere with this trial were also excluded from the study together with the subjects wearing contact lenses or suffering from concomitant ocular diseases.

2.3. Topographic and 3-D visualization of human cataracts

The recently developed technique of lens photography and cataract grading and measurement permits an adequate assessment of cataracts in human longitudinal studies [15]. The clinical standardization system uses the serial images obtained by the stereocinematographic slit-image and retroillumination photography with the regular slit-lamp camera consecutively focused on the lens objects to overcome the problem of stereoscopy and depth of field. This system gives a topographic and 3-D volume visualization for nuclear, cortical and posterior subcapsular opacities in human cataracts supplemented with digital image analysis and 3-D computer graphics. The important image contents are discriminated from the serial negatives supplied with a standard density reference and the rigorous computer-based image processing digitally enhances the contrast of lens opacity features and structures the 3-D composite of the lens revealed from the optical scanning tomographic study of the anterior eye segment [26]. The measuring characteristics of

different types of cataract include the average lens area degree of clouding (M) computed from the retro-illumination images [12,17]. The light transparency histograms (H) represent the distribution of grey values in different layers of the cataractous lens captured optically in contrast and electronically displayed from the subsequent slit-lamp images of cataract [17]. Mean values of H correspond to the intensity of lens cloudings, whereas the SE values represent heterogeneities of opacities throughout the lens layers. The intra-reader reproducibility for image analytical characteristics derived from the stereocinematographic slit-image and retro-illumination photographs was good with the overall average of correlation coefficients 0.911 and high between observer kappa scores [15]. The method reduces the cost and complexity of epidemiological study of age-related cataract, including clinical trials of anticataract medications by reducing the number of participants while at the same time increasing the power of the study.

2.4. Evaluation techniques

The evaluation system used to diagnose and graduate the severity of lens opacities performed at each visit of a patient included (1) interviewing on patient's medical history; (2) measurement of best corrected (b/c) visual acuity (VA); (3) direct and indirect ophthalmoscopy; (4) glare test with optimal correction (for Trial 1); (5) stereocinematographic Zeiss photo-slitlamp examination and photography; (6) consecutive Zeiss photo-slitlamp retro-illumination photography: anteriorly focused and posteriorly focused; (7) quantitative interactive digital image analysis of obtained images from (5) and (6) with 3-D computer graphics; (8) clinical lens grading after maximum permitted mydriasis. All findings were recorded with drawings on standard documentation sheets.

2.5. Lens slit-lamp photography

All patients underwent standardized slit and retro-illumination lens photography on the Zeiss 30 SL photo-slit-lamp with the pupil maximally dilated. For this a narrow slit was imaged in the transparent system of the anterior ocular media in such a way that the observation and illumination axes formed as large an angle as possible and the ocular fundus remained dark. The slit beam was 0.3 mm wide × 9 mm long angled at 30° to the visual axis; the same image scale (magnification) of ×1.0 or 1.8 was selected in each set of photographs. For the retro-illumination photography either a broad, vertical beam was directed centrally through the dilated pupil at one side, or a narrow vertical beam, directed at a small angle (5°) to the central axis of the dilated pupil was used and the passage area of the illumination bundle was as far as possible from the object in the lens to be observed. Two photographs were taken with the slit beam on each side of the pupil during retro-illumination photography to allow the flash reflex [25]. The flash reflex could be eliminated later using the interactive measuring program. The flash intensity was set at 5, equivalent to 200 watt seconds (W-sec). Efforts were made in this study to reduce any variation in the photographs by using the same camera, the same film from the same batch, and using only one photographer. Furthermore, aperture setting and shutter speed was the same for all photographs. In all cases, film was developed using identical procedures. The opacities were recorded in focal illumination and the low depth of field made the surroundings appear unfocused. The neutral density step reference wedge was captured in the plane of focus of the camera at each negative film to calibrate the illumination of the slit aperture. This recording also took into account the non-linear logarithmic nature of film response. Serial black/white photos were taken during two-to-three subsequent settings of the focal plane of the optical section that made it possible to discriminate in contrast every optical feature of anterior segment of the eye from cornea to posterior lens capsule on at least one of the slit-lamp images (stereocinematographic principle). During the slitlamp examination the clinician made sure that only the important image contents were shown and all other unimportant details and sources of disturbance such as reflections, were eliminated. This precise "interactive" role of the operator was realized also upon the consecutive digital image analysis in computerized assembling of the composites of the crystalline lens.

2.6. Grading methods

Lens changes were graded according to anatomical location, severity and coevaluated with the visual acuity. The two- to-three black/white slit images, two retro-illumination images and the reference were used to grade the type and severity of cortical, nuclear, posterior subcapsular cataract and nuclear density. The nucleus was examined with a thin (~0.1 mm) slit beam of 8 to 9 mm in height set at an incident angle of reproducible slit orientation of 30°. The slit passed through the anatomic center of the lens (the embryonic nucleus) and the clarity of the optical section of the nucleus compared to the reference photograph of nuclear cataract and graded accordingly to the technique described by Taylor and West [32]. The densities of nuclear opacities as seen on clinical slit-lamp examination are graded in comparison with a set of standard photographs (Grades 0–4) with increasing nuclear opacity. In the presence of cortical opacities, which may cause intense light scatter, care was taken not to overcall the density of the nuclear opacity. In these cases, particular attention has been directed to the uniformity and distribution of the nuclear opacity. The reference photographs were 35 mm transparencies that are viewed in a hand-held, battery-operated slide viewer.

The cortex was examined using the reproducible optical plain setting in retro-illumination in terms of segments involved [32]. The slit-beam was relatively wide (0.5 to 1