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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  $65.3 \pm 7.0$  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. © 2001 Elsevier Science Inc. All rights reserved.

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### 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 ( $\beta$ -alanyl-L-histidine) and related  $\beta$ -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 ( $\sim 10$  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, acetyl esterase (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

