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Efficacy of N-Acetylcarnosine in the Treatment of Cataracts

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Abstract

Purpose: To evaluate the effects of 1% N-acetylcarnosine (NAC) solution on lens clarity over 6 and 24 months in patients with cataracts.

Trial design: Randomised, placebo-controlled study.

Participants: 49 subjects (76 affected eyes) with an average age of 65.3 ± 7.0 years with a diagnosis of senile cataract with minimum to advanced opacification in various lens layers.

Methods: 26 patients (41 eyes) were allocated to topical NAC 1% eyedrops twice daily. The control group consisted of 13 patients (21 eyes) who received placebo eyedrops and 10 patients (14 eyes) who did not receive eyedrops.

Main outcome measures: All patients were evaluated at entry and followed up every 2 months for a 6-month period (trial 1), or at 6-month intervals for a 2-year period (trial 2), for best-corrected visual acuity and glare testing. In addition, cataract was measured using stereocinematographic slit-images and retro-illumination examination of the lens. Digital analysis of lens images displayed light scattering and absorbing centres in two- and three-dimensional scales.

Results: The overall intra-reader reproducibility of cataract measurements (image analysis) was 0.830, and glare testing 0.998. After 6 months, 90% of NAC-treated eyes showed improvement in best corrected visual acuity (7 to 100%) and 88.9% showed a 27 to 100% improvement in glare sensitivity. Topographic studies indicated fewer areas of posterior subcapsular lens opacity and 41.5% of treated eyes had improvement in image analysis characteristics. The overall ratios of image analysis characteristics at 6 months compared with baseline measures were 1.04 and 0.86 for the control and NAC-treated group, respectively ($p < 0.001$). The apparent benefits of treatment were sustained after 24 months' treatment. No treated eyes demonstrated worsening of vision. The overall visual outcome in the control group showed significant worsening after 24 months in comparison with both baseline and the 6-month follow-up examination. The overall clinical results observed in the NAC-treated group by the 24-month period of examination differed significantly ($p < 0.001$) from the control group in the eyes with cortical, posterior subcapsular, nuclear or combined lens opacities.

Tolerability of NAC eyedrops was good in almost all patients, with no reports of ocular or systemic adverse effects.

Conclusion: Topical NAC shows potential for the treatment and prevention of cataracts.

Cataract is the leading cause of blindness worldwide, accounting for over 50% of the world's blind population and affecting some 17 million people.[1] Worldwide, 28 000 new cases of cataract are reported daily.[2,3] In the United States, over 1.3 million cataract operations are performed annually at a cost of $3.5 billion.[4] However, in developing countries, there are simply not a sufficient number of surgeons to perform cataract operations. Therefore, a significant number of people are permanently blind with cataract.

Cataract is found primarily in older individuals. It is the leading cause of functional impairment among the elderly in the United States.[5] Forty-three percent of all visits to ophthalmologists by Medicare patients are associated with cataract. [4] Approximately 25% of the population over 65 years of age, and about 50% over 80 years of age, have serious loss of vision because of cataract. Since the population over 55 years of age is expected to increase 4-fold worldwide and significantly in the United States,[6] both in terms of the numbers of people involved and of economic impact, cataract is a major disease.

It is apparent that it will not be possible to eliminate the overall blindness caused by cataract by increasing the number of surgeons, as there are too many people presenting with maturity-onset cataract.

The large and growing number of people blind with cataract and the significant complication rate has motivated a search for pharmacological treatment of cataracts. The considerable discomfort experienced by patients as their vision diminishes and the complete loss of accommodation resulting from the removal of the lens should also be recognised. Besides the risk of complications, an artificial lens does not have the overall optical qualities of a normal lens.

Age-related cataract is a multifactorial disease, and different risk factors appear to play a role in 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.[7-14] It is established that superoxide anion radical, hydroxyl radical, hydrogen peroxide, singlet oxygen and lipid peroxides can be generated by photochemical reactions in the lens surroundings, triggering the development of cataract,[11-18] and that the use of antioxidant supplements appears to be protective against cataract.[19] Peroxide damage to the lens plasma membranes may lead to disturbance of their permeability to ions, loss of thiol groups of the membrane-bound crystallins, and the appearance of new fluorophores, together with large protein aggregates with low solubility (scattering matrix) in the substance of the lens. These events promote development of cortical (C), posterior subcapsular (PSC) and nuclear (N) cataracts.[12,20-22]

L-Carnosine (β-alanyl-L-histidine) and related β-alanyl histidyl dipeptides (anserine and balenine) are generally found in millimolar concentrations in several mammalian tissues, potentially exhibiting different metabolic activities.[23] 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.[23,24] A striking effect of L-carnosine is its demonstrated ability to prevent, or partially reverse, lens cataract.[25,26]

Exogenous carnosine entering the body intravenously, intraperitoneally, with food or via 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.[23,27] The level of serum carnosinase increases with age.[27]

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. approximately 10mM).[28] Pharmaceutical preparations containing N-acetylcarnosine (NAC) aluminium salt have been evaluated for the treatment of gastric ulcers.[29] Among 29 dipeptides of the carnosine family tested as potential substrates for a highly purified human serum carnosinase preparation, NAC and a few other compounds were not hydrolysed.[27] Consideration 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,[30] prompted the development of NAC, a prodrug of L-carnosine, as an ophthalmic antioxidant.[31]

NAC 1% solution topically administered to rabbit eyes (instillation, subconjunctival injection, ultrasound-induced administration) penetrated into the eye and the native form of L-carnosine accumulated in aqueous humor within 15 to 30 minutes of administration.[31-33] NAC showed moderate inhibition of catalysis of phosphatidylcholine liposomal peroxidation *in vitro*, less pronounced than that of L-carnosine.[31]

NAC has potential as an *in vivo* universal antioxidant because of its ability to give efficient protection against oxidative stress in the lipid phase of biological membranes and in aqueous environment, by turnover into L-carnosine.[31-33] Because of its relative hydrophobicity compared with L-carnosine, NAC might be expected to penetrate through the cornea gradually, thus prolonging the active therapeutic concentration of L-carnosine in the aqueous humor of the treated eye.[31] NAC was well tolerated when applied by different techniques of ocular administration.[33]

In this clinical study we prospectively evaluated the effects of a topical solution of NAC on lens opacities and visual function in patients with cataract.[32,33] Previously we reported the data of the matched clinical study with major emphasis on the clinical techniqes for monitoring of anticataract activity.[34,35]

Subjects and Methods

Patients were enrolled into the study from the Consulting Division of Moscow Helmholtz Research Institute for Eye Diseases and received follow-up examinations every 2 months within a 6-month period (trial 1) or were enrolled from the Ophthalmic Division of Innovative Vision Products Inc. and underwent follow-up every 6 months for 2 years (trial 2). All patients were supervised by the same observer.

Selection of Patients

Eligibility criteria included a confirmed diagnosis of senile (S) cataract according to the medical history, clinical observations and epidemiological study. Inclusion criteria were: availability for study of both lenses; presence of a cataract in at least one eye; cataracts judged not to require surgery in the near future (2 years) based on the patients' visual needs and ocular symptomatology; 52 to 80 years of age; pupillary dilation could be performed safely.

Patients were excluded 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 that would interfere with visualisation or photography of the anterior segment, or mature cataract (VA less than 0.1) in both eyes, and were likely to be candidates for cataract surgery within 1 year. Other exclusion criteria were patients with monocular aphakia or secondary cataracts (e.g. cataracts associated with steroid intake, total body or local irradiation, local inflammatory or degenerative process or ocular trauma).

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, as were

subjects wearing contact lenses or having concomitant ocular diseases.

Study Conduct

After enrolment, patients were computer-randomised into two groups: to receive treatment with NAC 1% eyedrops, or to a control group who received placebo eyedrops or no ocular treatment. Pure NAC has been synthesised and purified according to a cGMP 'know-how' process and technology owned by Innovative Vision Products, Inc., Delaware, USA. NAC eyedrops contained a 1% solution of NAC[32,33] in phosphate-buffered saline (PBS) and showed good stability. The eyedrops were prescribed freshly prepared without a preservative. The administration schedule was two drops instilled twice daily, for patients assigned to NAC and those assigned to placebo (PBS alone),[32,33] for 6 months (study 1) or 2 years (study 2). The use of other topical or nutritional antioxidants was not measured or evaluated 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. Neither the investigators nor the patients knew who was receiving NAC.

At baseline, the following data were recorded: demographic information, medical history, resting pulse rate and blood pressure, visual acuity (VA), slit-lamp biomicroscopy examination findings. Intraocular pressure and cup/disk ratio were assessed when necessary to exclude presence of glaucoma. Patients were questioned about their ocular and general comfort. The number of patients needed for each trial was chosen so 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.

The research was performed in agreement with the principles of the Declaration of Helsinki (1964 edition 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 objectives of the trial and the properties of the drugs, and informed consent was obtained. The study plan was approved by the appropriate Ethics Committee.

Patient Evaluation

The recently developed technique of lens photography and cataract grading and measurement permits an adequate assessment of cataracts in human longitudinal studies.[34,35] The authors utilised the subsequent individual slit images to obtain a volumetric representation of the lens for analysis and the measuring system dealt with limitations of alignment, focusing and sampling errors. The clinical standardisation system processes the serial images obtained by the stereocinematographic slit-image and retro-illumination 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 visualisation 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.[35]

The measuring characteristics of different types of cataract include the average lens area degree of clouding (M) computed from the retro-illumination images.[36,37] 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 the cataract.[36] Mean values of H correspond to the intensity of lens clouding, 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 an overall average of correlation coefficients of 0.911 and high reproducibility was cited between observer kappa scores.[34,35] The method reduces the cost and complexity of epidemiological study of age-related cataract, by reducing the number of participants required, while at the same time increasing the power of the study.

The evaluation system used to diagnose and graduate the severity of lens opacities performed at each visit included: (1) interview regarding patient's medical history; (2) measurement of best corrected (b/c) VA; (3) direct and indirect ophthalmoscopy; (4) glare test with optimal correction (trial l); (5) stereocinematographic Zeiss photo-slitlamp examination and photography; (6) consecutive Zeiss photo-slitlamp retro-illumination photography: anteriorly 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 as published previously[34,35] after maximum permitted mydriasis. All findings were recorded with drawings on standard documentation sheets.

Quantitative Assessment and Image Analysis

The opacities were digitised and the intensity values of individual image pixels were used for the numerical determination of their areas and densities. For technical realisation of the image analytical procedures, the IBAS Interactive Image Analysis System (Zeiss, Germany) additionally equipped with a Semi-Automatic Evaluation Unit, Array Processor and Printer (OK1 DP-125) was used.

A fully automatic measuring programme provided valid measurements of the light intensities and densities in the lens image and comparisons with the step wedge image, regardless of any change in flash output, and also compensated for changes in development of the film, which affects the density curve (γ) of the negative.

The film used for photography was Micrat 200 (Tasma, Kazan, Russia) monochrome film, chosen because of its extended density range and fine grain. Negatives of the lens images were back-lit with the uniformly diffused source 'TL' 13W/33 (Philips, Germany) from a day-light lamp when positioned on an observer (look-up) table and the anterior surface of the negative faced the camera. Images were captured by an RCA TV camera (USA) with a Plumbicon tube (625 lines interlaced, 25 frames/sec, short persistence time), which ensured a highly linear transfer function between light intensity and electrical signal, with a photo-objective SMC PENTAX Macro (×10) [Japan]. The degree of enlargement of the negatives was adjusted so that the image of the pupil became equal in diameter to a circle of set size on the TV screen.

The analytical measurements of retro-illumination photos and the serial subsequent slit-lamp images of the lens made adjustments for variations in background caused by variation in reflection from the fundus. The light intensities passed through the lens image elements (I_{ij}) were automatically divided (normalised) by the background illumination intensity measured in the free image pixels (I_o) to obtain a transmission index (I_{ij}/I_o) or its logarithmic value [lg (I_{ij}/I_o)] and compose the matrix of density in homogeneities in the 2-D scale.

The lowest detectable density was set to correspond to that of the retro-illumination background in an area of the lens unaffected by cataract. Measurements were provided in each of the two captured retro-illumination photos used to subtract the corneal reflex by the software interactive filtering. The lens retro-illumination images were sectioned into up to several 'equidensities'.[36,37] Individual transmittance values for each pixel were acquired on a calibrated linear scale, where 0 and 255 correspond to 100% ('black') and 0% ('white') transmittances, respectively. Each video frame was stored in real time on a memory according to the format matrix 512 × 512 pixels. Final images were stored on disk after subtraction and

equalisation of the background optical and electronic noise.

Software enabled the selection of an individual area of the cortical and other types of cataract as a measuring field that was calculated with the help of the circular mask selection, whose position was under the control of the analyser and observer. The analytical measurements were carried out in automatic order using the packages of the IBAS-2 programmes and individual authorised supplementary programme DIAMORPH.[36] The data on grading the retroillumination photos were expressed as the average area degree of clouding[36,37]

$$\{M = (OD_{ij}. A_{ij})/(\Sigma_{ijL\ ODij.} A_{ij})\}$$

with the standard deviation about the mean, where OD_{ij}, A_{ij} are the corresponding measured values of optical density and area for the raster pixels.

The analysis performed for the different comparisons included: standardisation of the image input, including image averaging, shading correction, image filtering, contrast enhancement, copying of the image sections, suppression of unwanted features within the image, reading and writing of images on hard disk; calibration of the image features and identification of measurement data and editing of the light intensity distribution along a selected profile. Also included was discrimination of the image features chosen for analysis within different grey thresholds after the image was scanned electronically, and the density at each coordinate stored in the computer for further analysis.

Using the serial subsequent slit-lamp images of the lens, 2-D and 3-D pictures of the cataracts were obtained.[34,35] The original lens image, which included optical heterogeneities from images focused at various depths along optical sections, was reconstructed in a 3-D scale and displayed from different angles (figure 1). The detailed structural parameters of the lens were rearranged from the light intensity measurements and included volume intensity, size, fractions of the opacity within the lens areas, average distance of their separation, density and difference between the normal or opacified lens units and their environment. Quantitative assessments of nuclear opacities and distribution of opacities (light transparency histograms) in adjacent morphological layers of the lens (H) were undertaken, by determination of relative areas with different absorptive (transmittance) values based on the use of standard slitlamp photos with subsequent scanning and image processing. Posterior subcapsular opacities were measured in retroillumination and focal slit images using the discriminated area and density analysis.

Visual Acuity

VA testing was performed to obtain the best distant VA, with optical correction when required. VA was measured using projection screens (Carl Zeiss, Germany) with acuity lines in the following steps: 0.1 to 0.4 in 0.05 steps and 0.4 to 1.0 in 0.1 steps (trial 1). The standard Snellen charts with standard lighting were also used to monitor Snellen VA (trial 2).

Glare Test

The contrast-diminishing effect of glare is exaggerated in patients with opacities of ocular media, and VA is reduced. A method for measuring susceptibility to glare of human vision is schematically presented in figure 2. The examining room was dark (less than 20ft candles) to ensure maximum contrast of the projected target. Tests were performed with the best correction in place. The viewing distance was 300mm for an acuity target and a single-dot glare light source of preselected intensity sufficient to generate glare in the vision of a subject was used. The target was set in the same tangential plane at different distances from the glare source. The target consisted of self-luminous optotypes (figures or Landolt rings) displayed on an electronic table of 5×5mm with a light-sensitive diode, or incandescent lamp-transilluminated optotypes of red or green. The resulting target-glare source distance measured (mm) when the patient could just identify an optotype during illumination of the eye with the glare source was

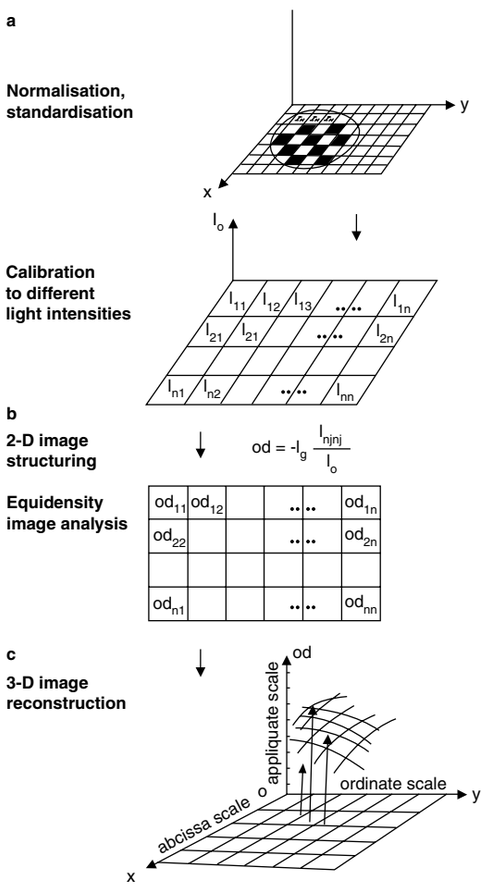

Fig. 1. Image analytical procedures provided with the aid of an automatic measuring program. Functions included 2-D structuring of an image, measurements of the surface density, specific area, mean linear dimension and curvature, and determination of 3-D volume densitometric parameters and geometric transformation functions with data stored at each coordinate.
Ig = logarithmic value index ?; **Inj** = light intensity in each pixel (lens image element); **nj** = nomination of any pixel at each coordinate; **Io** = illumination intensity measured in the free image pixels; **OD** = measured value of optical density.

assessed as a threshold measure of glare sensitivity in the tested eye.

The principle of glare test is based on the measurements of glare radius (r,mm) as a reading of glare sensitivity.

The halometer technique is based on the measurements of glare radius (defined as a target plane image projection for indicatrix of light scatter $I = I_0\cos^2\varphi$) when the glare source is activated. Because a patient cannot recognise an optotype-target when it enters into the glare area (because of 'halo' formation), a significant change in the glare radius value indicates a change in intraocular light scattering (and therefore lens clarity). An increase or decrease of the glare radius by 4mm with a SE (n = 4) ± 1mm is usually regarded as significant worsening (increased light scattering) or improvement (decreased light scattering), respectively. The input of the light scatter wavelength was estimated using the coloured (red or green) modes of the target.

Significant increases and decreases of the value parameter were assessed to indicate deterioration or improvement that indicated changes in lens clarity towards opacification (increased light scattering) and clarification (decreased light scattering). The increased or decreased figure of visual acuity indicated improved or deteriorated vision. The unchanged value of the parameter indicated stationary unchanged type of testing.

Statistical Analyses

Statistical analysis was performed using Student's t-test; p = 0.05 was taken as the upper limit of significance. Correlation and linear regression analysis were used to assess associations.

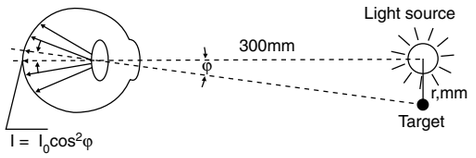

Fig. 2. Principle of glare test based on the measurements of glare radius (r,mm) as a reading of glare sensitivity.
I = an observation angle; **r** = glare radius in mm.

For determining intra-operator correlation coefficient, the measurements from two visits (trial 1, baseline) were considered as repeated measurements. The second photographs and glare test readings of the same patient by the same operator were taken at least 1 week apart after the first visit prior to the treatment allocation. The coefficient was computed by comparing the extra variation on repeated measurements by the individual operator with the existing variation between 20 patients. The clinical and photographic analyses related to image analytical procedures were additionally evaluated by statistical testing of mean values, variance, skewness, excess and distribution analysis (scatter diagram, linear and logarithmic distribution) as described earlier.[36] The structuring of the image included surface density, specific surface area, mean linear dimension, mean linear distance, curvature, diameter characteristics, stereology programme for the determination of volume densitometric parameters and provision of the geometric transformation functions with the data stored at each coordinate.

Results

A total of 49 elderly patients (76 affected eyes) completed the 6-month and the 2-year protocol. They were divided into following groups: group I – control group representing untreated patients (10 patients, 14 eyes) or placebo recipients (13 patients, 21 eyes); group II – treated with NAC eyedrops 1% (26 patients, 41 eyes) [table I]. Twenty of these patients (34 eyes) were enrolled into trial 1 and 29 (42 eyes) were enrolled into trial 2.

The population characteristics are shown in table II. The groups were compared for gender, 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 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.

The distribution of cataracts in the examined patients is shown in figures 3 and 4. Most of the lens changes in the baseline-examined cases were early and moderate (cortical, posterior subcapsular, mixture of grades 1 to 3), with the exception of corticonuclear mixed and nuclear colour brunescence cataracts with more pronounced opacities.

There was a good concordance in the severity of cataract as assessed by slit-lamp, photograding,

Table I. Characteristics of patients included in the study

Parameter	Group I, control (untreated or placebo)	Group II (treated with NAC 1%)
Trial 1 (6 months)		
No. of patients	10	10
No. of eyes	16	18
Male	5	5
Female	5	5
Age (mean ± SD) [years]	67.1 ± 7.1	67.0 ± 4.1
Epidemiological status (no. of eyes)	S; (13) S_{compl} (3)	S; (16) preS (2)
Trial 2 (24 months)		
No. of patients	13	16
No. of eyes	19	23
Male	7	8
Female	6	8
Age (mean ± SD) [years]	64.3 ± 6.7	64.0 ± 8.5
Epidemiological status (no. of eyes)	S; (18) S_{compl} (1)	S; (19) S_{compl} (4)

NAC = N-acetylcarnosine**; preS** = pre-senile; **S** = senile; S_{compl} = complicated; **SD** = standard deviation.

Table II. Linear correlation coefficients (r)[a] between the characteristics of patients with cataract measured by visual acuities, glare radius and photoslit-lamp image analysis at baseline and at 6-month follow-up ophthalmic examinations (trial 1)

Parameter	Baseline study					5-6 mos				
	VA	M	H	GR_{Red} target	GR_{Green} target	VA	M	H	GR_{Red} target	GR_{Green} target
VA	X	–0.83*	–0.52*	–0.60*	–0.62*	X	–0.80*	–0.62 ($p < 0.01$)	–0.55 ($p < 0.01$)	–0.63 ($p < 0.01$)
M		X	+0.57*	+0.38**	+0.42**		X	+0.59 ($p < 0.01$)	+0.43 ($p < 0.02$)	+0.62 ($p < 0.01$)
H			X	+0.08 (NS)	+0.27 (NS)			X	+0.31 ($p < 0.1$)	+0.39 ($p < 0.05$)
GR_{Red} target				X	+0.83*				X	+0.92 ($p < 0.01$)
GR_{Green} target					X					X

a Number of eyes examined = 34.

$GR_{Red/Green}$ targets = characteristics of glare disability test at red and green targets; **M, H** = characteristics of image analysis; **VA** = visual acuity; **NS** = not significant. * $p < 0.01$, ** $p < 0.05$.

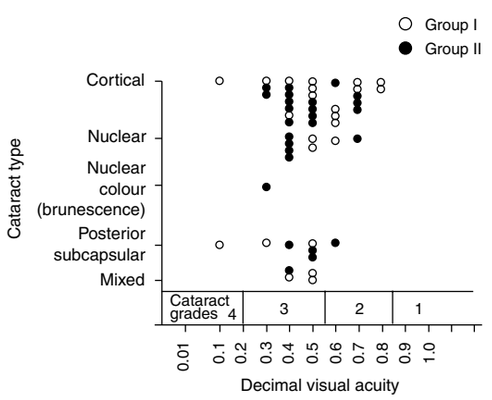

Fig. 3. Scattergraph outlining the distribution of cataract types, grades and best corrected decimal visual acuities of patients in trial 1.

glare test readings and the b/c VA results (table III). Linear correlation coefficients (r) between VA and parameters of the glare test and image analytical grading for 34 examined eyes ranged from –0.83 to –0.52 at baseline and from –0.80 to –0.55 at the 5- to 6-month follow-up. Ophthalmic examinations indicated that the methodological variances of subjective and objective systems of measurements were approximately equal. Correlations (r) of M versus H and glare test readings, as well as correlations of glare sensitivity at red versus green targets were significant.

Figure 5 shows a senile cataract with cortico-nuclear opacities (grade 4) that was used for further computerised editing and analysis of cataract. The individual areas in the optical section, seen in the photographs as the discontinuity zones selected with a slit, were digitally made sharply visible during automatic analysis. During analysis of the retro-illumination images (figure 5c,d) the transi-

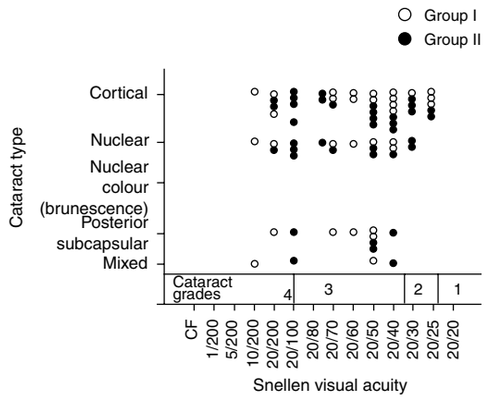

Fig. 4. Scattergraph outlining the distribution of cataract types, grades and best corrected decimal visual acuities of patients in trial 2.

tion area influenced by flashlight is subtracted from quantitation (equalised to the black background by suppression operation and image filtering functions).

Topographic structuring with image analysis of the selected equidensity lens areas and strict antero-posterior alignment of the lens layers were conducted to show the whole lens affected by more than one type of opacity. 3-D structuring provided the geometric transformation with the data from the light intensity measurements stored at each coordinate. This demonstrated the textures of the lens inhomogeneities in the axonometric image projection unidentifiable in the 2-D image analysis (figure 6). Pixel statistical analysis for individual lenses provided the gross quantitative degrees in the retro-illumination images and presented the spatial distribution of grey values in consecutive lens layers assembled from the digital optical sections (see data for M and H in the figures and tables).

Intra-operator correlation coefficient was obtained as repeated measurements for each combination of operator (1), eye (right or left), image analytical characteristic (M and H), and glare radius (at red and green targets). All correlation coefficients for the image analytical characteristics were between 0.554 ($p < 0.05$) and 0.953 ($p < 0.01$). The correlation coefficients for glare test values approached 0.998 ($p < 0.01$). Overall, the reproducibility for the one operator was good. The gross average of correlation values is 0.830 for the image analytical characteristics and 0.998 for the glare test readings. Since the difference in values between the two visits was small, the intra-operator results were averaged and mean, between-subject variance and extra variance from repeated readings were calculated (table IV).

Figure 7 and and tables IV and V summarise the effects of study treatment on VA, glare sensitivity and image analytical measurements over 6 months (trial 1) and on VA, photoslit-lamp and image analytical assessment over 24 months (trial 2). In the control group, comparison with baseline values showed some variability in densitometric readings, gradual worsening of glare sensitivity and minimal VA changes over 6 months, and a decrease in VA and increase in image analytical characteristics after 24 months. Glare sensitivity indicated changes in lens clarity when densitometric readings obtained at the 5- to 6-month follow-up examination did not differ significantly with baseline (figure 7).

In the NAC-treated group, 6-month follow–up showed an improvement in VA (7 to 100%) in 37 of the 41 treated eyes and a significant improvement in glare sensitivity at red and green targets (27 to 100%) was documented in 16 of the 18 eyes tested (table V). A significant improvement in lens clarity was found in 17 of 41 eyes (trials 1 and 2), as documented by a significant decrease of M and H characteristics during image grading.

The NAC-treated eyes had statistically significant differences in VA, glare sensitivity and

Table III. Summary statistics for intra-operator correlation

Eye (right/left)	No. of eyes examined	Image analytical/ glare test characteristic	Mean	Between eyes measured variation	Extra variation	Correlation
Right	19	M	0.450	0.070	0.021	0.953*
Left	15	M	0.460	0.061	0.024	0.943*
Right	19	H	137	40	30	0.869*
Left	15	H	150	20	32	0.554**
Right	19	GR_{Red} target	22	15	1	0.998*
Left	14	GR_{Red} target	18	11	1	0.997*
Right	18	GR_{Green} target	21	15	1	0.999*
Left	14	GR_{Green} target	19	9	1	0.997*

$GR_{Red/Green}$ targets = characteristics of glare disability test at red and green targets; **M, H** = characteristics of image analysis.

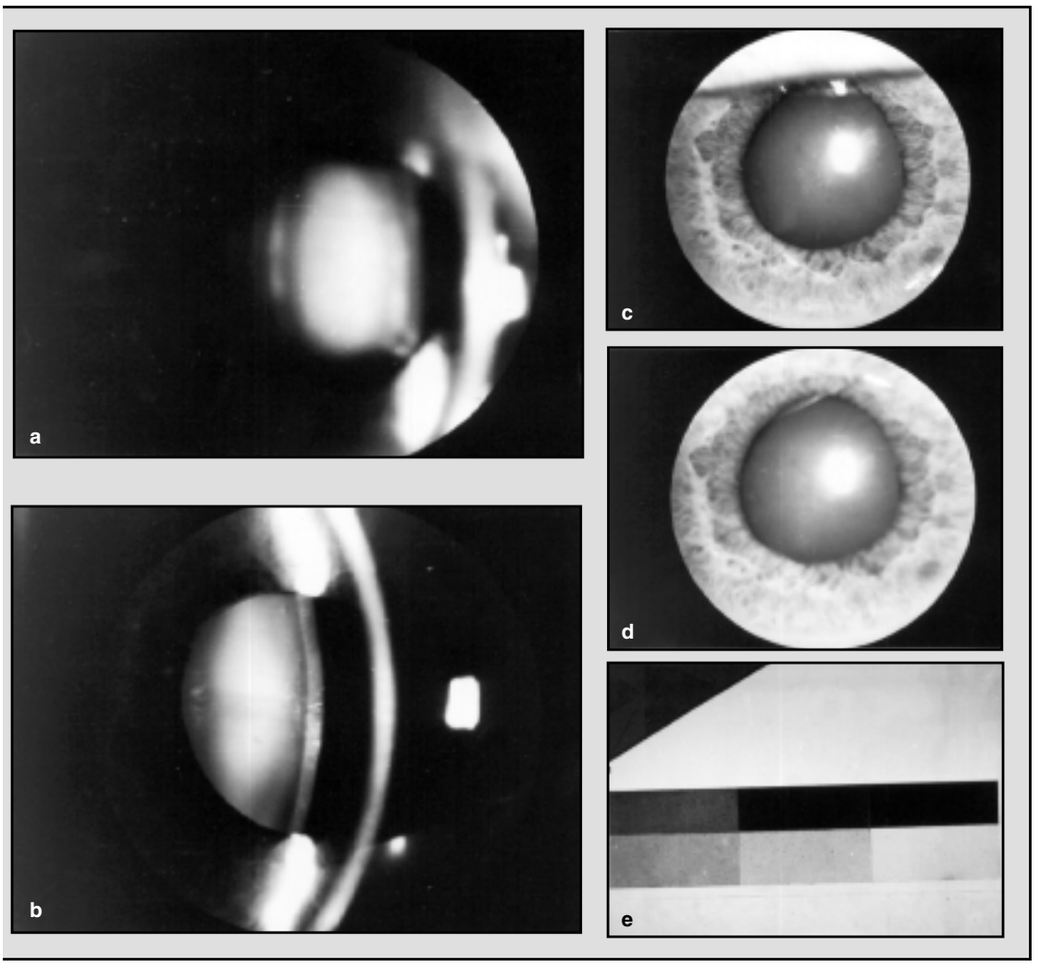

Fig. 5. Images of lens with a senile cataract (corticonuclear opacities, grade 4, age 75 years, female), and the subsequent slit images in optical section documenting in the focal plane: (**a**) marked light scattering in the nucleus and posterior cortical region outlined by the lens optical scanning with the focal plane movement inside the lens thickness; (**b**) light scattering in the anterior subcapsular, anterior cortical and nuclear regions of the lens; retro-illumination lens images with a focal plane positioned (**c**) onto the iris and (**d**) on the posterior lens layers. Opacities in the cortical layers are demonstrated as the white background inclusions in the boundary of the pupil locally masked by the flash light output. (**e**) The neutral density step reference wedge captured in the plane of the camera focus and allowing correction for variations in film development and flash light output.

characteristics of image analysis compared with the control group ($p < 0.001$) at this timepoint, as supported by the overall t-test results of the ratio of the follow-up data to the baseline values (table I).

3-D computer graphics showed a lens with cataract before and after treatment with NAC for 4 months, as displayed by the spatial technique and co-evaluated with the optical wedge scale (figure 6). The digitised constitution of the lens opacities

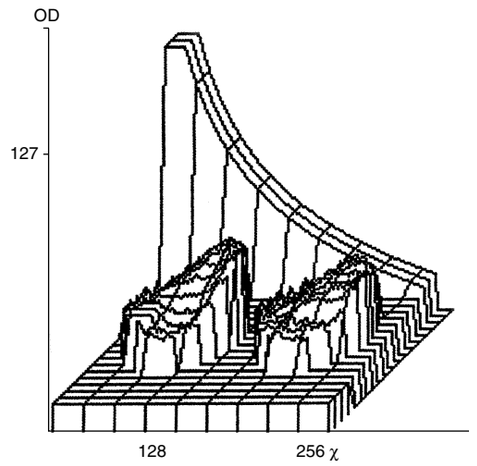

Fig. 6. Image analysis of a lens with cataract (opacities in cortical and nuclear regions, grade 1-2, age 66 years, female) before **(left image)** and after (**right image**) 4 months' treatment with 1% N-acetylcarnosine, showing a significant ($p < 0.01$) decrease in optical density (opacity) in posterior cortical layers after treatment. 3-D computer graphics represents opacities in the lens layers reconstructed from subsequent slit-lamp images and analytical processing. The image functions and quantitation are co-evaluated to the linear scale of the optical wedge.
Ordinate (y-axis): 0-200 grey levels (optical density: 0.0-2.0 units); abscissa (x-axis): number of characteristic linear densitometric pixels, 0-255; applicate (z-axis), antero-posterior direction: number of lens morphological layers from anterior cortex through the nucleus to the posterior cortical layers (1-10). The displayed densitograms are means (SD ± 3%) of the normalised data (4 measurements) in each pixel.

demonstrates a smooth significant decrease of optical heterogeneities in the lens posterior cortical region after treatment with NAC. Layer-by-layer computerised analysis of the lens opacities, permitting the proportional assignment of illumination intensities in the image pixels, documented the real changes in opacity densities during the topographic assembling, independent of an increased masking of the deeper lens layers by an apparent increase in opacity of the middle layers.

The data obtained by the same observer in trial 2 illustrate examinations over 24 months of the 23 eyes treated with NAC to show that the effect of treatment is sustainable over more prolonged periods (table V). Twenty of the 23 examined eyes showed a 12 to 67% improvement in VA, and the remaining three eyes were unchanged at the 24-month follow-up. Image analytical characteristics showed a significant improvement ranging from 11 to 49% in 11 eyes and 12 eyes were unchanged. In 41 eyes with different localisation and grade of cataract, prolonged treatment with NAC did not seem to result in a worsening of the final 24-month visual outcome.

The overall clinical results observed in the NAC-treated group in the 24-month study differed significantly ($p < 0.001$) from the control group (table V). Control group patients showed a 17 to 80% deterioration in VA in 17 of the 19 eyes examined and significant deterioration (13 to 44%) in the gross analytical characteristics (M) and (H) of the lens opacities occurred in 9 of 19 eyes. The overall visual outcome in the control group showed significant worsening after 24 months in comparison with both baseline and the 6-month follow-up

Table IV. Mean ± SD ratio of values at 6 or 24 months to baseline, averaged across the number of eyes examined in each group, for glare test (GR at red and green targets) and image analytical readings (M and H).

Treatment group	Visual acuity improvement	Glare test improvement	Image analysis improvement
6-month follow-up (trial 1/ trial 2)			
Control group	0.93 ± 0.03 (n = 35)	1.37 ± 0.08 (n = 27)	1.04 ± 0.01 (n = 70)
NAC-treated group	1.43 ± 0.04* (n = 41)	0.50± 0.05* (n = 33)	0.86 ± 0.01* (n = 82)
24-month follow-up (trial 2 only)			
Control group	0.63 ± 0.05** (n = 19)	-	1.13 ± 0.02*** (n = 38)
NAC-treated group	1.31 ± 0.05* (n = 23)	-	0.84 ± 0.01* (n = 46)

NAC = N-acetylcarnosine.

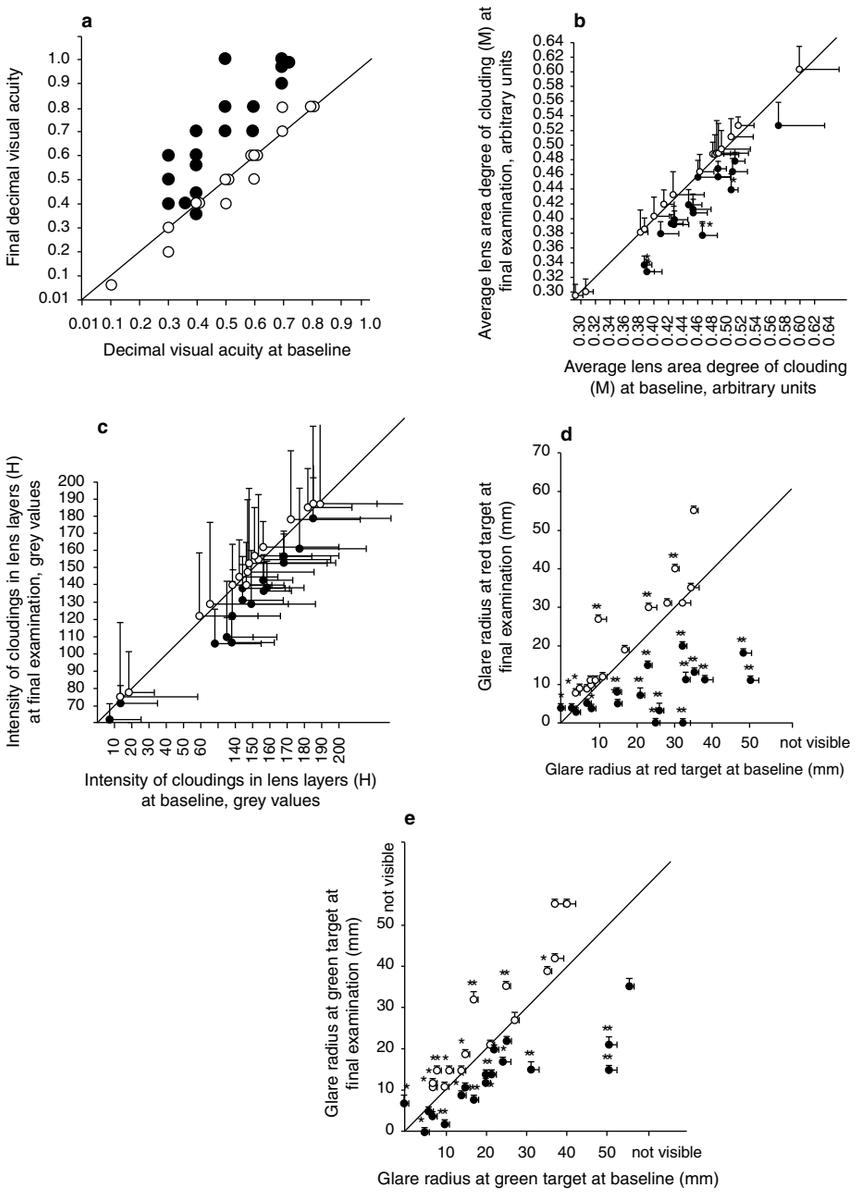

Fig. 7. Visual testing and eye examination findings in 34 eyes at baseline and at 5- to 6-month examination in trial 1. Open circles = patients from group I (control); solid circles = patients from group II (treated with instillations of 1% N-acetylecarnosine). * $p < 0.05$, ** $p < 0.01$ versus baseline. Image quantitative data are plotted as the mean with the bars (SD) computed from 5 to 10 measurements. The readings of glare test (mm) represent mean ± SD of four measurements. The bisector line indicates no change in all diagrams. (**a**) Scattergraph of visual acuity; (**b**) M-average lens area degree of clouding (arbitrary units) computed from the retro-illumination lens images; (**c**) H-intensity of cloudings (grey values) in the lens layers computed from slit-lamp images of cataract; (**d**) Glare sensitivity (glare radius) readings at red target; (**e**) Glare radius readings at green target.

Table V. Evolution of study parameters in patients with cataract after 6 and 24 months' follow-up [no. of eyes (%)]

Treatment group (n eyes)	Visual acuity			Glare test[a]			Image analysis		
	improve. [range]	unchang.	deterior. [range]	improve. [range]	unchang.	deterior. [range]	improve. [range]	unchang.	deterior. [range]
6-month follow-up (trial 1/ trial 2)								31(88.6)	
Control group (35)	2 (5.7) [14-60]	2 1 (60.0)	12 (34.3) [17-40]		7 (43.8)	9 (56.3) [11-170]			4 (11.4) [11-39]
NAC-treated group (41)	37 (90) [7-100]	4 (9.8)		16 (88.9) [27-100]	1 (5.6)	1 (5.6) [100]	17 (41.5) [12-50]	24 (58.5)	
24-month follow-up (trial 2 only)									
Control group (19)		2 (10.5)	17 (89.5) [17-80]					10 (52.6)	9 (47.4) [13-44]
NAC-treated group (23)	20 (87.0) [12-67]	3 (13.0)					11 (47.8) [11-49]	12 (52.1)	

Deterior. = deterioration; **improve.** = improvement; **unchang.** = unchanged.

examination. In the NAC-treated group, no significant differences in cumulative changes of lens opacities were noted between the 6-month and 24-month examinations.

Tolerability

Topical short- or long-term administration of 1% NAC to the eye was very well tolerated, with no ocular or systemic adverse effects, no hyperaemia 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, 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.

Discussion

In spite of the availability of an effective surgical treatment for cataracts, they are the leading cause of blindness worldwide. Although cataract surgery is generally recognised as being one of the safest operations, there is a significant complication rate. Between 30 to 50% of patients undergoing cataract extraction surgery in the USA tend to develop opacification of the posterior lens capsule within 2 years and subsequently require laser treatment. Since the number of cataract operations is so large, even a small percentage of complications represents a significant number of individuals. Other complications, including retinal detachment, corneal oedema, corneal transplantation and endophthalmitis, can also lead to re-hospitalisation and further treatment. Thus, aside from secondary cataracts, about 26 000 individuals develop serious complications as a result of cataract surgery annually in the USA alone.[4] Therefore, it is vitally important to address the problems of the large and growing number of people blind with cataracts and the significant complication rate.

Thus, although surgical extraction of the involved lens is effective, there is considerable interest in identifying the risk factors involved in cataractogenesis and possible protective strategies.[38]

We evaluated NAC 1% eyedrops in the long-term therapy of cataracts. NAC was used as a prodrug of an antioxidant that is resistant to hydrolysis with human serum carnosinase.[31] The L-carnosine liberated in aqueous humor can provide anti-

oxidant protection in the areas around the lens, and penetrate and accumulate in the lens tissue.[31]

The findings of the present study indicate agreement between the clinical data and results of applied quantitative techniques for measuring human cataracts. The developed light-scattering factor of glare sensitivity recorded with a coloured luminous target is shown to be a reproducible useful clinical index of lens transparency. The evaluation of contrast sensitivity under different conditions of stimulus luminance with and without glare was previously found not to be constant and thus did not represent a useful method.[39] The intra-reader reproducibility of the image analytical data in this study was good, with the overall average of correlation coefficients being 0.830.

After 6 months, 41.5% of eyes treated with 1% NAC showed significantly lower (12 to 50%) gross degrees of lens clouding as compared with baseline. The results of glare testing (improvement of 27 to 100% in 88.9% of eyes) shows a small decrease of light scattering. Glare sensitivity improvement was accompanied by improvement in VA (17 to 100%) in 83.3% of eyes. Less density and opacification area was observed in posterior subcapsular and cortical lens regions (grades 1-3) during treatment with NAC.

By contrast, the control group showed generally no improvement in visual function, with no difference from baseline in densitometric and area readings for cortical, posterior subcapsular, nuclear and combined opacities over 6 months. Glare sensitivity showed a significant deterioration in 56.3% of eyes over 6 months, indicating a slight opacification of the lenses.

In the 24-month study, there was a deterioration in VA of 17 to 80% in 89.5% of eyes in the control group. The level of visual improvement (overall) in the eyes treated with NAC over 24 months significantly differed from the results in the control group ($p < 0.001$). The results obtained over 24 months demonstrated improvement in VA of 12 to 67% in 20 (87%) of the NAC-treated eyes as compared with baseline, although no significant improvements in VA were observed between 6 and 24 months in the NAC-treated groups of patients (trials 1 and 2). The trial 1 study included a short-term (6-month) follow-up period not set up arbitrarily since the reference to the total study of 2 years (trial 2) revealed a sustainable effect of 1% NAC.

In most of the patients treated, study treatment was well tolerated and no ocular or systemic adverse events were reported.

Consistent with previous studies on the antioxidant activity of natural histidine-containing dipeptide in the lens,[25,26,31,34,35] the results of this study provide a substantial basis for further evaluation of NAC in the treatment and prophylaxis of senile and age-related cataracts.

In the cataractous lens, cross-linking of proteins and membrane damage caused by any means contribute to the increased light scattering and consequent lenticular opacity. Use of NAC to treat senile cataract can lead to diminishing of light scattering units in the lens, probably by prevention of the oxidative modification of crystallins and utilisation of lipid peroxides.[40,41]

Further evaluation of NAC is also warranted in the treatment of ocular inflammation, ocular manifestations of diabetes (including glycation reactions, microangiogenesis, vitreoproliferative disease), primary open-angle glaucoma and retinal disorders that involve pathological mechanisms associated with oxidative stress.[17,40-42]

Conclusion

In the USA, 300 000 to 400 000 new visually disabling cataracts occur annually, with complications of modern surgical techniques resulting in at least 7000 irreversibly blind eyes. Furthermore, senile cataract continues to be the main cause of visual impairment and blindness in the world. At least 5 to 10 million new visually disabling cataracts occur yearly, with modern surgical techniques resulting in 100 000 to 200 000 irreversibly blind eyes.

Most morbidity associated with senile cataracts occurs postoperatively. While the risk of dying as a result of cataract extraction is almost negligible,

studies have shown an increased risk of mortality in patients who underwent surgery. In a comparison of 167 patients aged 50 years or more who underwent cataract extraction at the New England Medical Center over a period of 1 year with 824 patients who elected one of six other surgical procedures, it was found that the former had almost twice the mortality of the latter. Further analysis showed no significant correlation between diabetes and increased mortality. In a similar 5-year mortality analysis, patients with cataracts who were younger than 75 years had significantly higher age-specific rates of mortality than would be expected from US life-tables.

We have evaluated an original pharmaceutical product, NAC, aimed at reversing cataracts in order to avoid the need for surgery. This ophthalmic drug shows potential for the non-surgical treatment of age-related cataracts and has been shown to have a high efficacy and good tolerability. NAC acts as a universal antioxidant both in the lipid phase of the cellular lens membranes and in the aqueous environment and protects the crystalline lens from oxidative stress-induced damage.

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