N-[Frontiers in Bioscience 15, 934-956, June 1, 2010]

Telomere attrition in lens epithelial cells - a target for N-acetylcarnosine therapy

Mark A. Babizhayev [1,2], Yegor E. Yegorov[3]

[1]Innovation Vision Products, Inc., 3511 Silverside Road, Suite 105, County of New Castle, Delaware, USA 19810, [2]Moscow Helmholtz Research Institute of Eye Diseases, Str. Sadovaya-Chernogryazskaya 14/19, Moscow 103064, Russian Federation, [3]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 32 Vavilov street, Moscow, 119991, Russian Federation

TABLE OF CONTENTS

1. ABSTRACT

The lens epithelium is especially vulnerable to oxidative stress. The erosion and shortening of telomeres in human lens epithelial cells in the lack of telomerase activity has been recognized as a primary cause of premature lens senescence phenotype that trigger human cataractogenesis. Carnosine, released ophthalmically from N-acetylcarnosine prodrug lubricant eye drops , at physiological concentration might remarkably reduce the rate of telomere shortening in the lens cells subjected to oxidative stress in the lack of efficient antioxidant lens protection. The data of visual functions (visual acuity, glare sensitivity) in older adult subjects and older subjects with cataract treated with 1% N-acetylcarnosine lubricant eye drops showed significant improvement as compared, by contrast with the control group which showed generally no improvement in visual functions, with no difference from baseline in visual acuity and glare sensitivity readings. Prevention of cellular senescence with ophthalmic prodrug N-acetylcarnosine may be a novel therapeutic target in a management of cataract, basic preventive health care and in arresting of after-cataract following extracapsular cataract extraction.

2. INTRODUCTION

Originality is simply a pair of fresh eyes....Thomas W. Higginson

Numerous reports indicate that oxidative stress is involved in cataractogenesis (1-4). Many epidemiological studies (5-10), although controversial (11), have shown that sunlight exposure, which generates reactive oxygen, could represent a major risk factor for senile cataract. Experimental data have also established that oxygen-derived free radicals induce lens opacification in animals: (12-18), and that antioxidant therapy can delay the onset of cataract in animal models (19-22). Moreover, many analytical investigations have,demonstrated that cataractous lenses contain products of oxidative processes (23-28) and are deficient in antioxidant defences (29-32).

3. N-ACETYLCARNOSINE AND TELOMERE SHORTENING IN HUMAN LENS CELLS

3.1. Lens cellular structures

The lens contains a single layer of epithelial cells that, in the equatorial region, terminally differentiate into

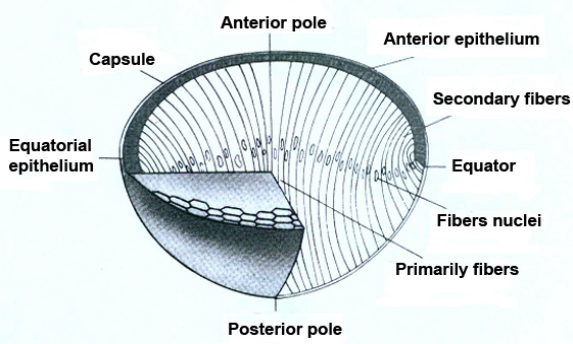


Figure 1. Scheme of the human lens structure. Reproduced with permission from (99).

fiber cells (33,34). There is a growth during this process, with the cell elongating toward both anterior and posterior poles. The newly formed fiber cell develops over the older fibers, increasing the volume of the lens and simultaneously displacing the older fibers in toward the center of the tissue. Thus, the inner region represents the embryonic lens, the oldest part of the tissue designated as the nucleus, and the periphery contains the youngest section, the cortex, which is the metabolically active region (Figure 1).

The nucleus, mitochondria, and other cellular organelles are degraded during the terminal differentiation process so that the fully differentiated lens fiber, which represents a large proportion of the mature lens, has lost its ability to synthesize protein and maintain metabolic processes. A consequence of this process is that the fraction of the lens that is metabolically active is constantly diminishing. It is generally believed, but not fully established, that only the epithelial cells can carry on all of the typical cellular functions.

The lens epithelium is especially vulnerable to oxidative stress (35-37). Damage to this single layer of cuboidal epithelial cells on the anterior surface of the lens can precede and contribute to lens opacification. For example, generation of reactive oxygen species (ROS) in a cultured rat lens system led to irreversible damage in the epithelial cell layer (35). Cellular and mitochondrial swelling, DNA fragmentation, and loss of cell viability were observed before cataract formation. These data emphasize the fact that the lens epithelium plays a crucial role in lens transparency.

3.2. Peroxide threat and cellular signaling

Generation of ROS can cause deleterious peroxidation of lipids, modification of proteins, and cleavage of DNA (38). However, at low concentrations, ROS, which are generated as by-products of normal mitochondrial electron transport and typically, by NAD (P)H oxidase isoforms, can serve a valuable function in cell signaling (39-42). For example, an increase in intracellular ROS via the increased expression of the GTP-binding protein racl leads to activation of the transcription factor nuclear factor-κB (41). In prokaryotes, H_2O_2 acts as a second messenger activating OxyR protein, a transcriptional activator of many antioxidant genes, including hydroperoxidase I and glutathione reductase (43).

3.3. Telomeres structures

Telomeres are special structures at the end of chromosomes. They shorten during each round of replication and this has been characterized as a mitotic counting mechanism. Telomeres are composed of repetitive nucleotide sequences and associated proteins that protect chromosomes from degradation and recombination. Vertebrate telomeric DNA consists of a conserved hexameric sequence (5´-TTAGGG-3´) arranged in tandem repeats (44-48). Telomere length is maintained by a balance between processes that lengthen and those that shorten telomeres. Evidence from various organisms suggests that several factors influence telomere length regulation, such as oxidative stress, telomere binding proteins, telomerase, and DNA replication enzymes. Telomerase is a ribonucleoprotein polymerase that specifically elongates telomeres. In most human cells telomere length is not maintained and telomerase is not active. The catalytic core of human telomerase is composed of an RNA subunit known as hTER (human telomerase RNA) and a protein subunit named as hTERT (human telomerase reverse transcriptase) (49-52). Expression of hTERT is a key step of regulation of telomerase in human cells and plays a crucial role in cellular immortalization and cancer development (53-57).

Normal human somatic cells shorten their telomeres during their lifespan leading eventually to dysfunctional telomeres, growth arrest and replicative senescence (58). Overexpression of ***TERT***, the catalytic subunit of telomerase, counteracts telomere shortening, extends the replicative potential and prevents replicative senescence (Figure 2a-d) (59). It may also exert an antiapoptotic action at an early stage of the cell death process prior to mitochondrial dysfunction and caspase activation (60). Telomerase activity in mammals (dog, rabbit, rodents) , but not humans, is likely important in the germinative epithelium to maintain its proliferative potential and prevent cell senescence (61-63). Telomerase in lens epithelial cells in mammals may function in the quiescent, central lens to maintain telomeres damaged by oxidative stress and ultraviolet light exposure, thereby preventing accelerated loss of these elements which triggers cell senescence. It remains to be determined if the increase in telomerase activity in lens epithelial cells from cataractous lenses (61) is a primary dysregulation that may have a role in the development of the cataract, or is secondary to cataract formation (Figure 3).

In addition, there is evidence for telomere length-independent functions of telomerase, which appear to promote cell survival and stress resistance (64-68). For example, telomerase expression conferred increased resistance to specific DNA damaging agents (66,69,70) and decreased apoptosis (60,68,71,72).

3.4. Oxidative insult and cataract

Oxidative stress associated with the formation of lipid peroxides is suggested to contribute to pathological

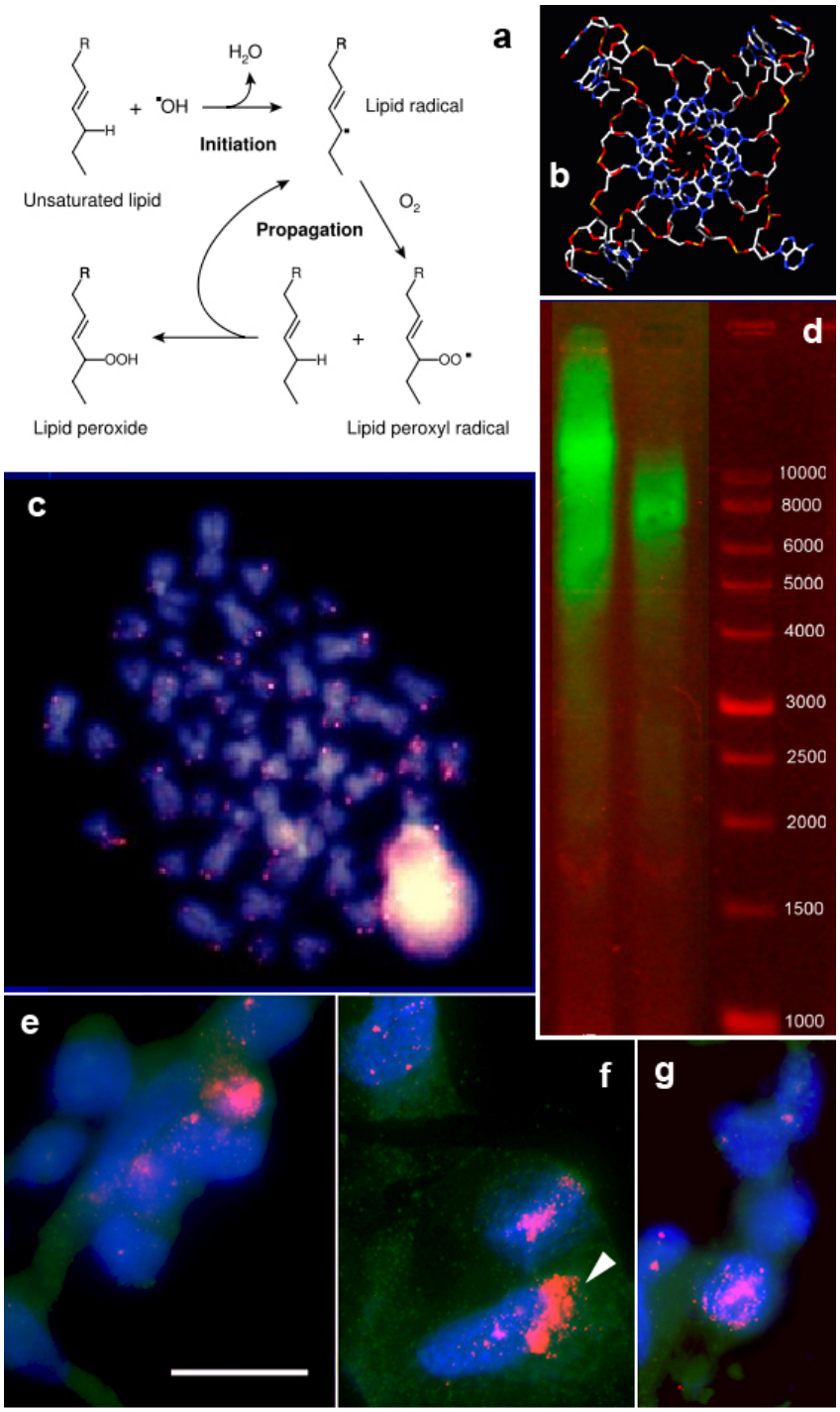


Figure 2. (A): Lipid hydroperoxide mediated damaging mechanism shortening telomers (B) in the human lens epithelial cells and inducing cellular senescence phenotype during aging and human cataract formation. (C,D). Increase of telomere length after administration of the hTERT gene in several cell types including human neural stem cells (presented case studies) , fibroblasts or epithelial cells (Provided from Professor Yegor E. Yegorov's personal studies). (E,F,G). Detection of hTERT protein . The arrow shows on the presence of hTERT in the cytoplasm of the cells. Bar- 25 µm. (The data are provided from Professor Yegor E. Yegorov's personal studies).

processes in ageing and systemic diseases, such as diabetes, atherosclerosis, chronic renal failure, inflammation and retinal degenerative diseases known as statistically significant risk factors for cataract (15, 34, 73-76). Glutathione-dependent enzymes in the lens have been suggested to play an important role in the detoxification of

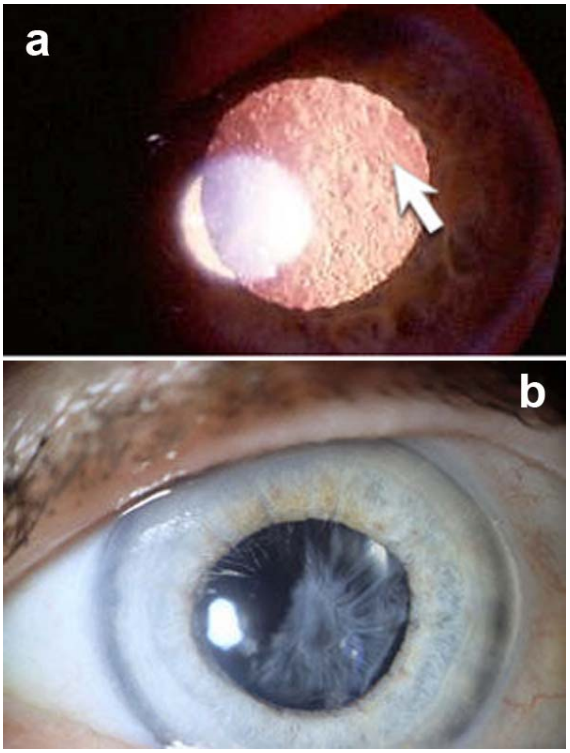


Figure 3. The senescent phenotype of lens epithelial cells and formation of secondary cataract can be attenuated with N-acetylcarnosine therapeutic platform. A: This lens epithelium proliferation is shown that has developed a rough and uneven surface from secondary cataract (The small, bright circle is the camera light); B: The white discoloration is secondary cataract located behind the lens implant

endogeneous toxicants that reach the lens from different sources (macular, retinal and other age-related ocular changes), such as hydroperoxides, 4-hydroxyalkenals, dialdehydes (including malondialdehyde), fatty acid epoxides generated during lipid peroxidation (3). The observation that lipid peroxides are elevated in the lens membranes of some patients with cataract has drawn attention to these toxic oxidant species (3,74-82). Lipid peroxides can cause cataract, producing damages to both cell membrane and cytosol regions (77-84). At the membrane, lipid hydroperoxides induce changes in permeability (85-88), refashion the micro-viscosity (order) of its lipid-protein environment (89-93), cause an uncoupling of the membrane-bound enzyme Na,K-ATPase and oxidative inhibition of Ca^{2+}-ATPase in several tissues including the lens (94,95). Within the cell, lipid peroxides can damage DNA (96), induce a drop in total glutathione and dramatic change in the redox ratio of glutathione, lead to the appearance of new fluorophores and large protein aggregates with low solubility (clouding matrix) in the lens matter (97-102). Some authors advanced the hypothesis that H_2O_2, free radical ($O_2^{-\cdot}$, $OH^{\cdot}$) and singlet ($^1\Delta_gO_2$) forms of oxygen are toxic, and may be generated in excessive amounts in several experimental cataracts and in human senile cataract as a consequence of lens photooxidation (103,104), radical generation by xenobiotics (105), or impaired enzymatic and non-enzymatic defenses which exist in the eye lens (106-110). The lens is well equipped with enzymatic antioxidant protective systems (i.e., superoxide dismutase , catalase, glutathione peroxidase) and endogenous free radical scavengers (e.g. glutathione, ascorbic acid), which both lose their efficiency with age and in cataracts (105,106, 111-118). The high levels of reduced glutathione (GSH) in the normal human lens (in excess of 10 mM) (118) and ascorbate in the lens and the aqueous humour (1 mM) (112-114) have been proposed to be a natural protection against photooxidation (116,119). The paradox of lens oxidative alterations in the eye, despite the high concentrations of antioxidants can be manifested since the above-mentioned reductants may undergo pro-oxidant activation in the presence of transition metal ions or endogenous photosensitizers (120). Both copper and iron promote H_2O_2 and free radical production from ascorbate or the cysteine residue of glutathione and the extent of this catalysis correlates with the cell toxicity (120,121). Transition metal ions are important components of non-enzymatic tissue peroxidation and they may be present in the eye tissues and fluids in catalytically active concentrations (122,123). Besides, aqueous and plasma Cu^{2+} levels have been reported to increase with age, in ocular inflammation and in diabetes, the etiologies which are considered to be the risk factors of cataract (124,125). An endogeneous photosensitizer, riboflavin, can catalyze the generation of active oxygen forms and the photo-oxidation of ascorbate and GSH and this is similarly associated with phototoxicity to lens epithelial cells (104,112,113, 120). Thus, the crystalline lens, a reservoir of reductants such as GSH and ascorbic acid, in addition to transition metal ions or photosensitizers, may become an extremely effective redox-coupled free radical generating system (126-130).

3.5. Oxidative stress, the replicative lifespan of cells, telomere integrity and cellular senescence

The replicative lifespan of primary human cells is telomere dependent ; however, its heterogeneity is not understood (Figure 2c,d). Concerning human ageing, many questions about molecular mechanisms have been addressed using *in vitro* senescence models derived from normal diploid human cells. The proliferative potential of human primary cells in culture is limited, and extended passaging of such cells leads to a state of terminal growth arrest, referred to as replicative senescence (Figure 4). While the erosion of telomeres, due to the lack of telomerase activity (for recent review, see (131)), has been recognized as a primary cause of replicative cellular senescence, a variety of other events have been identified that trigger premature senescence. Most notably, oxidative stress was found to induce premature senescence in human fibroblasts (132,133), endothelial cells (134,135), and a variety of other cell types (reviewed in ref. (136)).

The mitochondrial theory of ageing (137) suggests a critical role for mitochondrial dysfunction and subsequently increased ROS production as an inducer of ageing and premature senescence. Accordingly, replicative senescence of human diploid fibroblasts (HDF) has been associated with mitochondrial dysfunction and

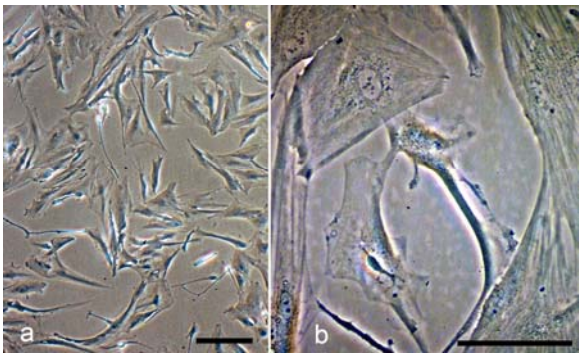


Figure 4. Senescent culture of human neural stem cells (NSC). Bars: a – 250 μm, b – 100 μm**.** (The data are provided from Professor Yegor E. Yegorov's personal studies).

mitochondrial ROS were identified as important players in the senescence response of HDF (138-140). However, mitochondrial dysfunction does not seem to be uniformly responsible for senescence in all cell types. This indicates mitochondrial production of ROS as one of the causes of replicative senescence. By sorting early senescent (SES) cells from young proliferating fibroblast cultures, it has been shown that SES cells have higher ROS levels, dysfunctional mitochondria, shorter telomeres, and telomeric gamma-H2A.X foci (140). The authors propose that mitochondrial ROS is a major determinant of telomere-dependent senescence at the single-cell level that is responsible for cell-to-cell variation in replicative lifespan (140). The intracellular ROS function as signalling molecules and that oxidants play a central role as mediators of cellular senescence. The fact that telomere length correlates with the final inhibition of proliferation under conditions of varied oxidative stress, while the population doubling level does not, suggests that telomere shortening provides the signal for cell cycle exit in senescence. In postmitotic cells, no further telomere shortening occurs (133). However, the sensitivity of terminal restriction fragments to S1 nuclease increases, indicating the accumulation of single-strand breaks in telomeres of nondividing fibroblasts. This effect is found both under normoxic and hyperoxic culture, although it is more pronounced under conditions of higher oxidative stress. It might be speculated that accumulation of single-strand breaks and the resultant loss of distal single-stranded fragments during replication could be a major cause of telomere shortening, possibly more important than incomplete replication per se (133). To determine if increased oxidative stress may contribute to the senescent phenotype, cells were treated with tert-butyl hydroperoxide (tBHP), which is known to increase oxidative stress by decreasing the intracellular glutathione levels. It has been documented that mild tBHP stress induces a phenotype of premature senescence in a subpopulation of the treated cells, which closely resembles the phenotype of naturally senescent human umbilical vein endothelial cells (HUVEC), including growth arrest, senescence-associated beta-gal activity, and apoptotic cell death. These results establish a model of premature senescence for human endothelial cells, suitable to analyze mechanisms of age-associated cell death (134). Furthermore, telomere-shortening rate and cell replicative lifespans can be greatly modified by DNA-damaging oxidative stress (141-142) via a telomere-specific repair deficiency, which causes stress-dependent accumulation of single-strand breaks (145) and accelerates telomere shortening during DNA replication (146). This had led to the suggestion that telomere reduction is not strictly programmed, with telomere length acting as a mere cell-division counting device, but instead that telomeres act as sentinels for cumulative oxidative and/or environmental stress triggering division arrest when the damage burden (detected through telomere length) becomes too great (141). It was hypothesized that accelerated telomere shortening is due to preferential accumulation of oxidative damage in telomeres (145). The data suggest that metabolic time-dependent single-strand degradation is a major cause of telomere shortening. This is supporting the idea that telomere shortening plays an important role in triggering cellular senescence (146). Telomeres in most human cells shorten with each round of DNA replication, because they lack the enzyme telomerase. This is not, however, the only determinant of the rate of loss of telomeric DNA. Oxidative damage is repaired less well in telomeric DNA than elsewhere in the chromosome, and oxidative stress accelerates telomere loss, whereas antioxidants decelerate it. The oxidative stress is an important modulator of telomere loss and telomere-driven replicative senescence is primarily a stress cell response (141) inherent to lens epithelial cells survival and human cataract formation as well.

3.6. Telomeres and Telomerase Activity in Lens Epithelial Cells of Normal and Cataractous Lenses

The adult crystalline lens is lined on its anterior surface by a monolayer of lens epithelial cells (LEC) with diverse replicative potential, including the anterior or central zone, the germinative zone and the equatorial zone. The central LEC are rarely mitotic and generally considered to be quiescent, the germinative LEC have mitotic activity and some of these cells have the potential to divide throughout life and the equatorial LEC terminally differentiate into the lens fiber cells (147-150).

Telomerase activity was found in LEC in all three mammalian species (in canine, feline and murine groups) investigated in the study (61) (but not in human species), confirming that the presence of telomerase activity in these cells is not a canine-specific phenomenon. Telomerase activity was detected in all three LEC regions of the canine lenses (central, germinative, and equatorial) with no significant differences in the level of activity between the different regions . Telomerase activity was absent in canine lens fiber cells, corneal endothelium, corneal epithelium from both the limbal and central regions, and rabbit nonpigmented ciliary epithelium. Surprisingly, telomerase activity was significantly lower in all three regions analysed in canine LEC from dogs less than 1 year of age than in adult dogs (61). Telomerase activity in 23 central capsulotomy specimens collected from naturally occurring canine cataracts were compared to levels in the central lens capsule from normal canine eyes, and surprisingly, significantly higher telomerase activity was found in the cataractous specimens.

Cells that have telomerase activity generally have longer telomeres than cells lacking this activity (151). Since cataractous canine LEC had a higher level of telomerase activity than normal LEC in canine group, it was important to determine if this was reflected in altered telomere lengths in LEC from cataractous canine lenses. In the normal lens capsule samples, there was some heterogeneity in telomere length within each sample as indicated by the number of bands in each lane. However, all normal lens capsules studied and all regions of the LEC had similar banding patterns ranging 4-20 kb, indicating telomere lengths did not differ between the three regions (61). The longest telomere in all normal canine samples measured was consistently 20 kb. Telomeres from cataractous canine capsules also had the same lower range but the upper limits were higher than the normal samples varying 20-24 kb (61). Similar to telomerase activity, pretreatment of normal eyes with topical medications used prior to cataract surgery did not affect telomere lengths. It was not surprising to find telomerase activity in the germinative LEC, as this cell population has the potential to undergo mitosis throughout the life of the canine host (148,149). Cells in the equatorial region of the lens, though not highly mitotic, may retain telomerase activity as the germinative cells migrate into this region. However, once the cells terminally differentiate into fiber cells, they lose telomerase activity, as no activity was found in the fiber cells. Although some post-mitotic cells have telomerase activity (152), many terminally differentiated normal somatic cells do not (153). As previous studies have indicated that central LEC are quiescent and rarely undergo mitosis (148,149) , the presence of telomerase activity in this cell population of mammals was surprising and more difficult to explain. Telomerase negative cells can divide many times in culture, population doublings of 56 and 88 in retinal pigment epithelial cells and foreskin fibroblasts, respectively are possible (154) before telomeres become critically short and cell senescence is triggered. The crystalline lens is exposed to constant nonionizing radiation in the form of ultraviolet light which causes chromosomal damage by inducing DNA strand breaks (155). Damage to genomic DNA is more effectively repaired than damage to telomeric DNA and oxidative damage to DNA in cells without telomerase activity can result in critical shortening of telomeres and cell senescence after just one cell division (145). When cells become senescent they are unable to re-enter the cell cycle and their phenotype dramatically changes (156), they begin to elaborate senescence-associated beta-galactosidase, proteases, extracellular matrix components and inflammatory cytokines. Conversion of LEC to the senescent phenotype is likely to be deleterious to normal lens physiology and transparency. Therefore, the presence of telomerase activity in the central epithelium might be necessary to prevent ultraviolet light induced DNA damage and critical telomere shortening. The loss of telomerase activity upon cell passage and telomere shortening may lead to conversion to the senescent phenotype as evidenced by increasing numbers of cells stained with the senescence marker, beta-galactosidase.

The ability of ultraviolet light to up regulate telomerase activity could have some effects on an age-related increase in activity of this enzyme. Dogs less than 1 year of age had significantly less telomerase activity than adult dogs (61). This suggests that the LEC of young dogs possess a basal level of telomerase activity that increases as the animal matures. It is possible that other local environmental factors, such as H_2O_2 and other peroxide compounds , could contribute to DNA damage in LEC leading to activation of telomerase activity.

The telomere shortening in rodents may play some role at least as a marker of cell aging in rats although perhaps less of a causal one than in humans (157). In order to examine telomere shortening directly in lens epithelial cells (LECs) *in situ*, the authors utilized fluorescence *in situ* hybridization (FISH) of a peptide nuclei acid (PNA) probe to the telomere repeat sequence (TELO-FISH). Although it is not clear whether such moderate telomeric erosion can limit cell division in rodent LECs, the telomeric shortening correlated well with previous studies demonstrating reduced clonal replicative potential, and reduced rates of *in vivo* DNA replication in LECs from old rodents and a delay in this attenuation in animals on chronic caloric restriction (157). Examination of LECs in each of nine young and nine old ad lib fed (AL), and nine old calorically restricted (CR) rat lenses, demonstrated that rat LEC telomeres were shortened by 21% in old AL fed rats relative to young controls ($P< 0.01$) and that CR reduced this loss to 12% ($P< 0.05$) (157). This suggests a possible relationship between telomeric shortening, loss of replicative potential in LECs, and cataract appearance in rodents. Oxidative or other damage to the telomeric DNA appears to act synergistically increasing the probability of capping failure and cell senescence even with only moderate average shortening (144,158,159). Numerous genes regulate telomerase activity within the cell (160) but the process is quite complex and currently not well understood. Perhaps telomerase is up regulated in cataractogenesis in dogs as a protective response to DNA damage, as it has been shown that there is significant damage to DNA in LEC from human cataractous lenses compared to normal lenses (161).

There has been some time ago a renewed interest in the association between DNA damage to the human lens epithelium and the development of lens opacities in human patients (161) . In approximately 50% of the cataractous samples analyzed, the proportion of cells containing DNA single strand breaks was significantly higher than in control lenses. It is important that no relationship between age and DNA damage was noted. These findings are consistent with the hypothesis that in some human patients with cataract, DNA damage in the lens epithelial cell population may be related to the development of lens fiber cell opacity (161). The possible relationships and effect for these phenomena are suggestive for oxidative damage, telomere shortening and loss of replicative capacity and corresponding to senescent phenotype alteration of gene expression patterns contributing to human age-related cataract formation, in that order.

In the recent study (162) the authors reported that human telomerase reverse transcriptase (hTERT) displays additional functions beyond telomere synthesis. They have demonstrated that hTERT introduced into Bovine lens

epithelial cells (LECs) can suppress differentiation. Furthermore, the authors provided the evidence (162) that hTERT can regulate the RAS/RAF/MEK/ERK pathway to mediate the suppression of bovine lens epithelial cell differentiation. This study provides evidence that hTERT regulates both proliferation and differentiation in eukaryotes. The results of another study suggest that hTERT, when overexpressed in human lens epithelial cells, accelerates cell growth rate through regulation of RB/E2F pathway and possibly other genes (163).

Among the human, bovine, and rabbit lenses examined, only the central epithelium from the 6-month rabbit lens displayed telomerase activity (164). In both transparent and cataractous human lenses, hTERT activity and expression were not detected. However, the template RNA was present in both types of human lenses (164). The telomeres found in transparent human lenses were approximately 1 kb longer than those in cataractous human lenses. These results suggest the possibility that telomere shortening is associated with human cataractogenesis. The primary cultures and later passages of HLECs also displayed no detectable telomerase activity. Introduction of hTERT cDNA into HLECs followed by G418 selection yielded a stable line of HLECs expressing hTERT. In this line, hTERT has supported normal growth after 48 population doublings and also enhanced antiapoptotic activity against oxidative stress (164). hTERT introduced into HLECs prevents replicative senescence through telomere synthesis. The hTERT-transfected cells with normal growth have a maximum telomere length of approximately 13 kb. Shortening of this telomere length to a certain degree signals cellular senescence, as observed in vector-transfected HLECs, which have a maximum telomere length of approximately 10 kb. Thus, HLECs use a telomerase-dependent mechanism to maintain their telomere stability (164).

3.7. N-acetylcarnosine as a therapeutic tool to manage age-related cataracts in human eyes or visually disabling eye diseases as well as systemic health conditions

During the adult years, the increased visual demands of our technological society bring about the need for regular optometric care (Figures 5a,b) (165,166). While the incidence of ocular disease is low for young adults, vocational and recreational visual demands are significant. To maintain visual efficiency, productivity, and optimum eye health, periodic examinations are recommended.

Adults, beginning in their early to mid-forties, can experience changes in their ability to see clearly at close distances. This normal aging change in the eye's focusing ability will continue during the forties and fifties. In addition, increases in the incidence of eye health problems occur during these years. Individuals age 61 or older have an increasing risk for the development of cataracts, glaucoma and macular degeneration and other sight threatening or visually disabling eye conditions as well as systemic health conditions. Therefore, annual eye examinations are recommended. At risk are individuals diagnosed with diabetes or hypertension, or who have a family history of glaucoma or cataracts, and those taking systemic medications with ocular side effects or those with other health concerns or conditions.

Carnosine (beta-alanyl-L-histidine) and related compounds are natural constituents of excitable tissues possessing diverse biological activities (167,168). The level of carnosine in tissues is controlled by a number of enzymes transforming carnosine into other carnosine related compounds, such as carcinine, N-acetylcarnosine, anserine or ophidine (by decarboxylation, acetylation or methylation, respectively) or its cleavage into the amino acids, histidine and β-alanine. Hydrolysis is mainly due to tissue carnosinase (EC 3.4.13.3) which is widely distributed among different tissues (169,170) or serum carnosinase (EC 3.4.13.20), found in brain and blood plasma of primates and humans (171,172). Both carnosine and N-acetylcarnosine compounds are found primarily in the heart and skeletal muscles and in the brain. We have found appreciable levels of L-carnosine in transparent human lenses which are markedly depleted in mature cataracts (173). The concentration of carnosine in transparent crystalline lenses detected was about 25 μM. At different stages of cataract development, the level of carnosine fell, reaching about 5 μM (173). Carnosine has been proven to scavenge reactive oxygen species (ROS) as well as alpha-beta unsaturated aldehydes formed from peroxidation of cell membrane fatty acids during oxidative stress (174-176). It can oppose glycation (177,178) and it can chelate divalent metal ions . The important studies have produced clinical and experimental evidence of beneficial effects of N-acetylcarnosine in treating cataracts of the eyes, these and other ophthamological benefits have been proven (2,179-186). Research with N-acetylcarnosine (NAC) demonstrates that it is effective not only in preventing cataracts but also in treating them. NAC has been shown to improve vision by partially reversing the development of the cataract, thus increasing the transmissivity of the lens to light (181).

One of the obscure aspects of the carnosine physiological role is the biological significance of the enzymatic metabolism of carnosine or its derivatives in tissues. We found that , in order to change the antioxidant status, tissue enzymes can modify the NAC prodrug molecule and that deacetylation increases *in vivo* the resistance of lens tissues and its cells to oxidative stress. 1% N-acetylcarnosine (Figure 5a) is a universal bioactivating antioxidant for vision in the developed and patented drug delivery system lubricant eye drop formulations containing mucoadhesive cellulose-based compound combined with corneal absorption promoters. Its topical administration delivers pure L-carnosine and allows its increased intraocular absorption into the aqueous humor surrounding the lens, enabling significant improvements in anti-cataract drug efficacy and the minimization of side-effect from either local or systemic drug absorption/bioavailability to the eye, and also creates optimization effects in the number of ocular degenerative age-dependent disorders (182). The formulation was also found to be non-irritant and well tolerable. The developed system can be a viable alternative to conventional eye

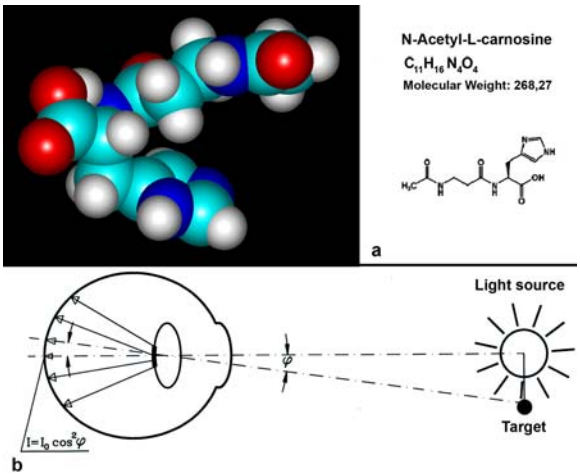


Figure 5. (a). The sight threatening age-related eye diseases are managed with imidazole-containing peptide based compounds, such as patented N-acetylcarnosine (3D space-filling model of the N-acetylcarnosine molecule is presented) lubricant eye drops (128-132). N-Acetylcarnosine (NAC) eye drops (Can-C ^{TM}) contained a 1% solution of NAC (185,190,191) 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. (**b).** Periodic optometric examinations are an important part of routine preventive health care. Many eye and vision conditions present no obvious symptoms. Therefore, individuals are often unaware that a problem exists. Early diagnosis of lens opacities with disability glare Halometer DG ® and treatment of cataract with N-acetylcarnosine lubricant eye drops are important for maintaining good vision and when possible preventing permanent vision loss.

drops for the treatment of various ocular diseases and is suitable for clinical application. N-acetylcarnosine prodrug and codrug ophthalmic formulations applied topically to the eye and moreover, its controlled time released ophthalmic ingredient L-carnosine exerts anti-glycation, bioactivating antioxidant properties in the lens and cornea as a scavenger of lipid peroxides, singlet oxygen and OH· radicals and provides the spatial aspects of intracellular pH regulation (126,181,183).

3.8. Intraocular transcorneal and systemic absorption of L-carnosine from N-acetylcarnosine ocular drug delivery system: Suitable molecular therapeutic interventions and visual sensory signaling responses

The provided studies with N-acetylcarnosine ophthalmic prodrug confirmed the increased intraocular uptake of L-carnosine active ingredient in the aqueous humor (186-189).

Histidyl compounds of aqueous humor have been examined by reverse phase analytical HPLC (Figure 6) as used in separation of imidazole-containing amino acids and dipeptides described earlier (187-189). Amino acids can be detected by the absorbance of carboxylate (~ 200 nm) and peptides by absorbance of carboxylate and the peptide bond (200-220 nm). Chromatograms of solutions of L-carnosine and its putative N-acetyl derivative are well separated. The elution order of the compounds was compared to a predicted order based upon their relative hydrophobicities as outlined by Rekker (190). Peaks were unequivocally identified by comparison of their retention times to those of the authentic standard compounds or of putative acetylated compound run singly . Tests for specific chemical reactivity (191) provided additional evidence for the identification of L-carnosine and N-acetylcarnosine.

Due to its relative hydrophobicity compared to L-carnosine, NAC might cross the cornea of the treated eye gradually and maintain longer the concentration of the active principle (L-carnosine) reaching the aqueous humor. The HPLC pattern of an extract of the aqueous humor obtained 30 min after instillation to the rabbit eye of ophthalmic formulation containing 1% NAC, lubricants carboxymethylcellulose, glycerine and preservative benzyl alcohol in the borate buffer confirms that the peak characteristic of L-carnosine has a concentration and a retention time (3.225 min) clearly distinct from N-acetylcarnosine (6.0 min) and basically different from the dead time of the column (3.0 min) (Figure 6). This identified peak of L-carnosine quantified and integrated by the data processor showed that virtually all N-acetylcarnosine after the overall extraction efficiency is converted into the L-carnosine compound with a retention time of 3.225 min (Figure 6). The data on the L-carnosine-related product were blanked with the control placebo data applied to the paired eyes of the animals. The mean ratio of L-carnosine (C) / (NAC) relevant to the L-carnosine release in the aqueous humor 30 min after instillation of Formulation A (Can-C ^{TM}) with 1% N-acetylcarnosine into the rabbit eye corresponded to **C/NAC**=6.64±0.06 (n =8, where n =number of the examined treated rabbit eyes; only right eyes were treated). In the control placebo formulation-treated eyes the same indices could not be quantified at statistically significant rate. Concentrations of imidazole products in the aqueous humor corresponded to those of intact rabbit eyes and refer to baseline values of L-carnosine 0.19± 0.10 μg/ml related products variously detected in extracts from normal animals.

Our data demonstrate that topical administration of pure L-carnosine (1% solution) to the rabbit eye (instillation, subconjunctival injection) does not lead to accumulation of this natural compound in the aqueous humor over 30 min in concentration exceeding that in the placebo-treated matched eyes, and its effective concentration is exhausted more rapidly (189,192). In another aspect, the data demonstrate the prospects of applications of an ophthalmic composition comprising NAC, or its pharmacologically acceptable salt in combination with a cellulose compound to treat the eye complex of symptoms. This complex of symptoms may have an oxidative component in their genesis, such as senile cataract, glaucoma, inflammation or diabetic ocular complications. The topical administration of N-

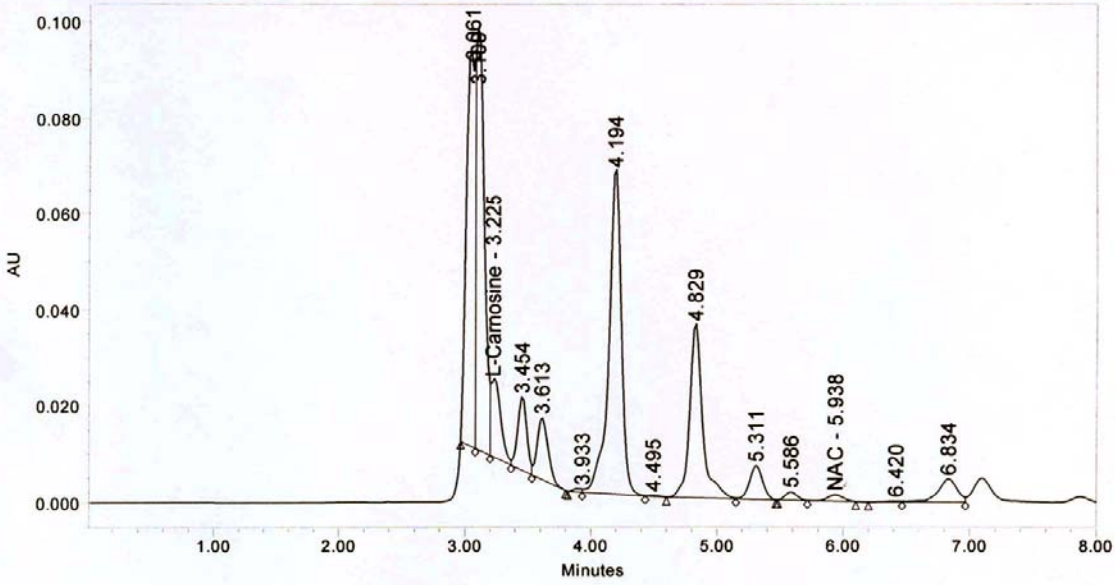
MMA

Project Name: Peptides
Reported by User: Makarov

Breeze

SAMPLE INFORMATION

Sample Name:	No.2 OD	Acquired By:	Makarov
Sample Type:	Narrow Unknown	Date Acquired:	7/25/02 12:21:47 PM
Vial:	1	Acq. Method:	Rabbit
Injection #:	4	Date Processed:	7/25/02 12:32:01 PM
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	8.00 Minutes	Sample Set Name	
Column Type:			



	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height	Amount	Units
1		3.061	402670	21.41	83190	24.86		
2		3.100	389886	20.73	87965	26.28		
3	L-Carnosine	3.225	89479	4.76	16173	4.83	3.392	mkg/ml
4		3.454	76173	4.05	15081	4.51		
5		3.613	76279	4.06	12690	3.79		
6		3.933	2892	0.15	625	0.19		
7		4.194	463529	24.65	67930	20.30		
8		4.495	1150	0.06	174	0.05		
9		4.829	257552	13.69	36153	10.80		

Figure 6. HPLC of extract of aqueous humor aspirated 30 min after the instillation of ophthalmic formulation with 1% NAC and lubricants into the rabbit eye. The integrated concentration of the carnosine related product (3.392 µg/ml, 3.225 min) is attributed to accumulation of carnosine in the ophthalmic formulation-treated eye. Chromatograms of solutions of L-carnosine and its putative N-acetyl derivative show that these compounds are well separated. Tests for specific chemical reactivity provided additional evidence for the identification of L-carnosine and N-acetyl-carnosine (189). Extractions of imidazole-containing compounds from the aqueous humor aliquots were performed according to Babizhayev *et al.*, 1996 (189). The published data showed that all the desired imidazole-containing compounds in the aqueous humor thus obtained could be of good purity and recovery (189).

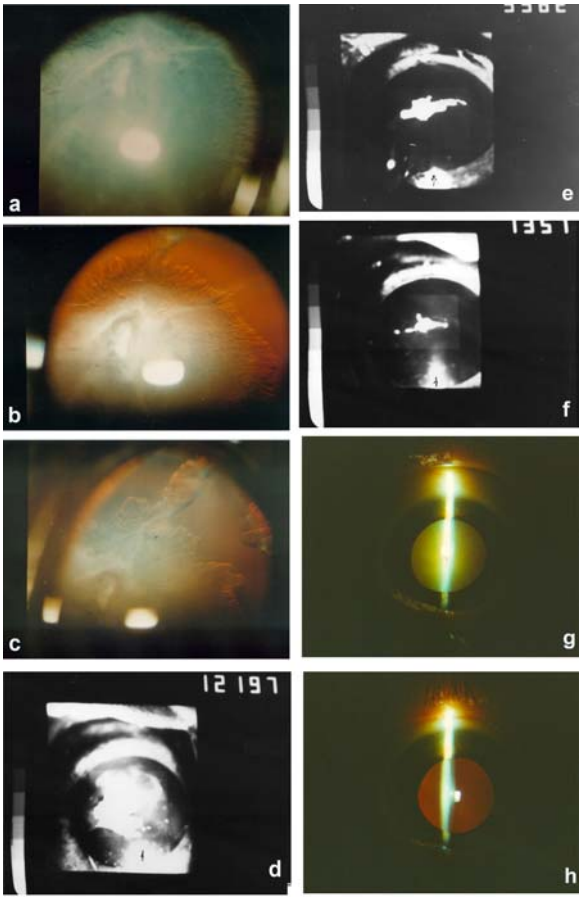
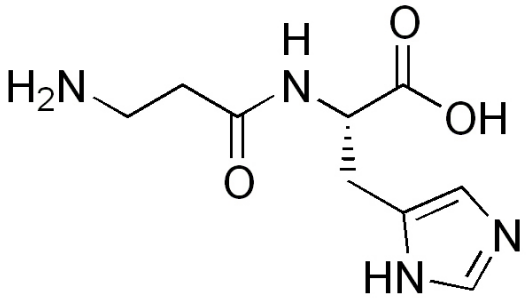


Figure 7. Therapeutic treatment with 1% N-acetylcarnosine lubricant eye drops (patented formulation by Dr. Mark A. Babizhayev/Innovative Vision Products, Inc.) of age-related cataract in canine eye (A (1-3)). Pictures A,B,C showing the reduction of cataract in canine eye. Top picture: this shows the canine cataract before treatment with Can-C^{TM} including 1% n-acetylcarnosine eye drops. B picture: this shows 2 weeks of the treatment of the canine cataract with topical administration of Can-C^{TM} eye drops including 1% N-acetylcarnosine. C: Results of the treatment after 1 month (bottom image). Already one begins to see the break-up of the impaired proteins - an effect that for obvious reasons has been described as "melting snow". The eyes of animals in each group were examined at regular intervals using a Zeiss SL-10 slit lamp. The appearance and progression of opacity was different in animals so the staging varied. Posterior subcapsular opacity (PSC) and cortical opacities occurred during the early stages of cataract formation. Treatment of the 6-month rabbit eye with traumatic cataract for 2 weeks (D,E,F)) Estimation of opacity of the lens by quantitative morphometric analysis; The figures in the upper right segment of the images demonstrate that the area of the lens opacity is significantly diminished during the treatment with 1% N-acetylcarnosine lubricant eye drops. Treatment of ripe age-related cataract in human patient's eye for 6 months (G,H). The appearance of rose reflex in the area of the lens (H) demonstrates that the cataractous human lens (G) becomes clearer upon this interval of treatment with 1% N-acetylcarnosine lubricant eye drops.

acetylcarnosine in the developed and patented lubricant eye drop formulation delivers pure L-carnosine and allows its increased intraocular absorption into the aqueous humor surrounding the lens, thus enabling significant improvements in anti-cataract efficacy Figs. 6,7 (126-130).

The important pharmacokinetic observation activities of the tested ophthalmic formulation Can-C ^{TM} including 1% N-acetylcarnosine and lubricant carboxymethylcellulose relate to the accumulation of L-carnosine in the aqueous humor of the contralateral rabbit eyes after 30 min of instillation of the medication to the tested rabbit eyes. The detected measure of L-carnosine in the aqueous humor of the contralateral untreated eyes corresponded to 1.45 ± 0.08 μg/ml (n=9). The data indicate that intraocular route for the administered medication includes (at least partially) a systemic drug absorption in the preferred intraocular site of L-carnosine released from 1% N-acetylcarnosine ophthalmic prodrug enhanced with the addition into the ophthalmic formulation of a cellulose derivative, i.e. carboxymethylcellulose (bioadhesive and absorption enhancer) that is also used as a mucoadhesive carrier for the patented ocular drug, due to its ability to coat the cornea and remain on the eye for a longer time. This route of systemic absorption of biologically active compound avoids the first-pass effect normally observed after oral presentation of a compound, and the pharmacological sequelae resemble those seen after an intravenous administration. This pharmacokinetic phenomenon have been given in clinical situations with topical administration of anti-glaucoma medicines to the eye , since the systemic effects of drugs such as timolol can be quite pronounced (193).

The systemically absorbed L-carnosine released from the topically administered to the eyes 1% N-acetylcarnosine ophthalmic prodrug Can-C ^{TM} as lubricant eye drops, is acting not only as a radical scavenger but also represents a possible neurotransmitter-like molecule that regulates neuronal functions such as hypothalamic control of the autonomic nervous system. CN2 (CNDP2) is a cytosolic enzyme that can hydrolyze carnosine to yield l-histidine and beta-alanine. CN2-immunoreactivity is highly concentrated in neuronal cells in the dorsal part of the tuberomammillary nucleus of the posterior hypothalamus (194). Since the tuberomammillary nucleus is the exclusive origin of histaminergic neurons, further investigations were focused, whether CN2 is present in the histaminergic neurons. It was found that CN2-immunoreactivity was colocalized with that of histidine decarboxylase, which is the key enzyme for histamine biosynthesis specifically expressed in the histaminergic neurons of the tuberomammillary nucleus (194). These results clearly indicate that CN2 is highly expressed in the histaminergic neurons in the tuberomammillary nucleus, implying that systemically absorbed L-carnosine released from the Can-C ^{TM} ophthalmic formulation topically administered to the eyes serves as a metabolic source to supply histidine and activates histaminergic neurons for histamine synthesis (Figure 8). Axons of the tuberomammillary nucleus project primarily to the cerebral cortex, thalamus, basal ganglia, basal forebrain, and hypothalamus. Tuberomammillary



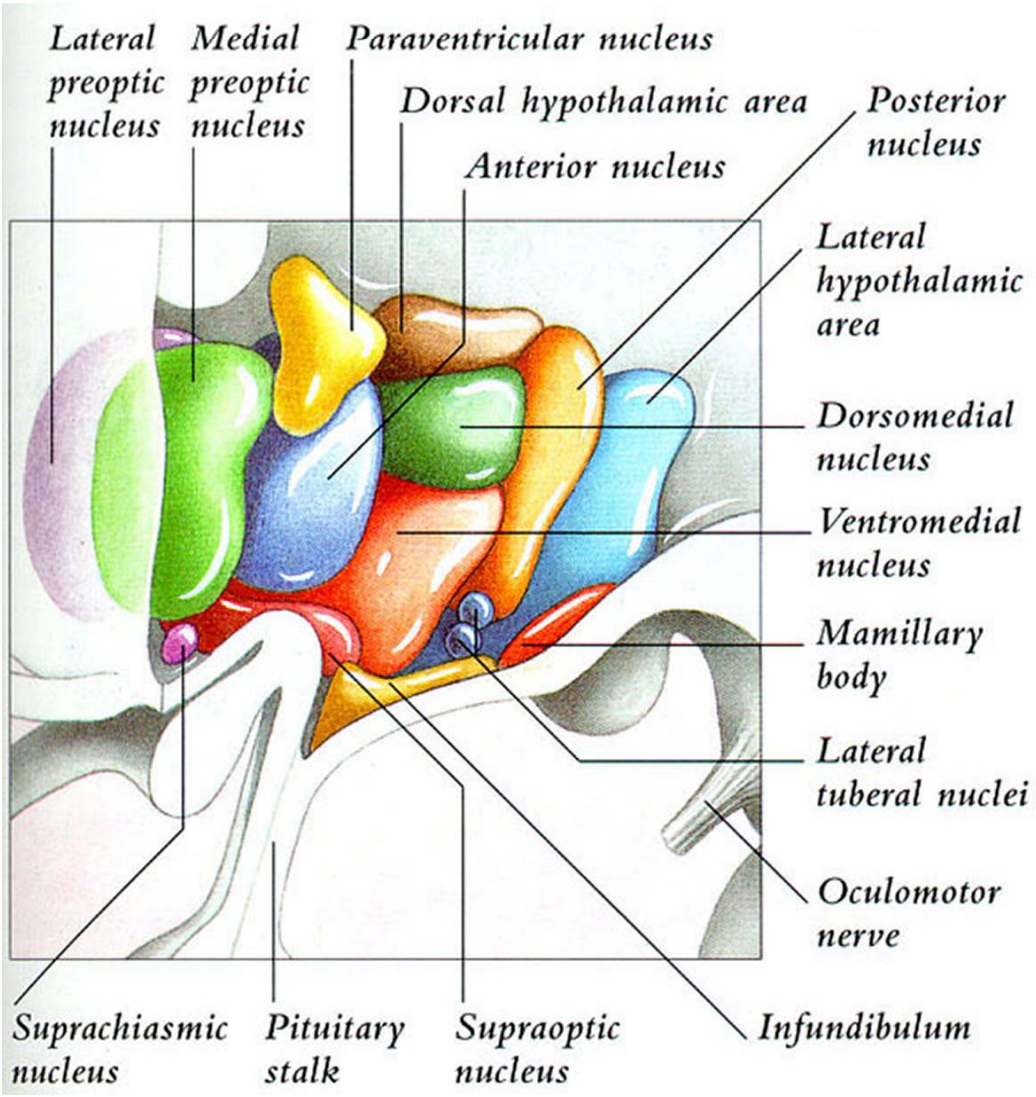


Figure 8. The signaling role of L-carnosine released from the ophthalmic prodrug N-acetylcarnosine lubricant eye drops in hypothalamus after the systemic absorption. The hypothalamus is a portion of the brain that contains a number of small nuclei with a variety of functions. One of the most important functions of the hypothalamus is to link the nervous system to the endocrine system responsible for prevention of ageing via the (hypophysis). Tuberomammillary activation causes a small phase lag in the visual response that is similar at all spatial frequencies, consistent with the induced shift from burst to tonic firing mode. These results indicate a significant histaminergic effect of L-carnosine on LGN thalamocortical cells, with no clear effect on thalamic inhibitory neurons . The data are supported by the Personal Dr. Mark A. Babizhayev's studies (Reviewed in Ref. 129).

activation usually results in a rapid and significant increase in the amplitude of baseline activity and visual responses in lateral geniculate nucleus (LGN) neurons (195). Tuberomammillary activation causes a small phase lag in the visual response that is similar at all spatial frequencies, consistent with the induced shift from burst to tonic firing mode. These results indicate a significant histaminergic effect of L-carnosine on LGN thalamocortical cells, with no clear effect on thalamic inhibitory neurons.

3.9. Human Lens Telomeres as a Prospective Biological Target for N-acetylcarnosine Ophthalmic Indications

In this work, we originally propose that the existence of shortened telomeres in the human lens epithelial cells is the molecular clock that triggers cellular senescence phenotype during aging and cataract progression and is the result of oxidative attack to the lens by phospholipid hydroperoxides present in the aqueous humor and lens cellular membrane structures in the lack of efficacy of the antioxidant protection in the lens towards these damaging oxidant promoters (Figure 2a) (196). The cumulative results from the literature demonstrated that carnosine at physiological concentration might remarkably reduce the rate of telomere shortening in the continuously surviving lens cells subjected to oxidative stress induced by phospholipid hydroperoxides in the lack of efficient antioxidant lens protection (196-200). Carnosine and related dipeptides have been shown to prevent peroxidation of model membrane systems leading to the suggestion that they represent water-soluble counterparts to lipid-soluble antioxidants such as alpha-tocopherol in protecting cell membranes from oxidative damage (189).

Other roles ascribed to these dipeptides in ophthalmics include actions as neurotransmitters, modulation of enzymic activities, chelation of heavy metals and transition metal ions , the inhibition of nonenzymic glycosylation of proteins (transglycating activities) (126-130). We proposed a deglycation system involving removal, by transglycation of sugar or aldehyde moieties from the Schiff bases by ophthalmic aldehyde scavenger L-carnosine derived from its ocular bioactivating sustained release prodrug 1% N-acetylcarnosine (NAC) lubricant eyedrops containing a mucoadhesive cellulose compound combined with corneal absorption promoters in drug delivery system (126).

Carnosine attenuates the development of senile features when used as a supplement to a standard diet of senescence accelerated mice (SAM) devoid of serum carnosinase activity (201). Its effect is apparent on physical and behavioral parameters and on average life span. A striking anti-senescence effect of carnosine was demonstrated by McFarland and Holliday (197-199). They showed that human diploid fibroblasts grown in 20 mM carnosine had an extended lifespan, both in population doublings (PDs) and chronological time. The dipeptide L-carnosine had beneficial effects on cultured human fibroblasts. Physiological concentrations in standard media prolong their *in vitro* lifespan and strongly reduce the normal features of senescence. Late passage cells in normal medium were rejuvenated when transferred to medium containing carnosine, and became senescent when carnosine was removed (197). Neither D-carnosine, (beta-alanyl-D-histidine), homocarnosine, anserine, nor beta-alanine had the same effects as carnosine on human fibroblasts.

In the recent work (200), the authors studied the effect of carnosine on a human fetal lung fibroblast strain (HPF), which was either kept in a continuously proliferating or proliferation-inhibited state. The results indicate that carnosine can reduce telomere shortening rate possibly by protecting telomere from damage. Cells continuously grown in 20 mM carnosine exhibited a slower telomere shortening rate and extended lifespan in population doublings. When kept in a long-term nonproliferating state, they accumulated much less damages in the telomeric DNA when cultured in the presence of carnosine. The authors suggest that the reduction in telomere shortening rate and damages in telomeric DNA made an important contribution to the life-extension effect of carnosine (200).

In our review we propose the effects of carnosine on telomeres of the human lens epithelial cells during aging and cataract formation based on the established clinical efficacy of L-carnosine ophthalmic prodrug N-acetylcarnosine to partially reverse and prevent human cataracts in clinics (Figure 7) (126-130;179-185).

3.10. The clinical effects of N-acetylcarnosine ophthalmic prodrug through protections from telomere attrition and shortening: prevention and reversal of cataracts, basic preventive health care

The data of visual functions (visual acuity, glare sensitivity) in older adult subjects and older subjects with cataract treated with 1% NAC showed significant improvement as compared, by contrast with the control group which showed generally no improvement in visual functions, with no difference from baseline in visual acuity and glare sensitivity readings (126-130;179-185). In most of the patients treated, study treatment was well tolerated and no ocular or systemic adverse events were reported. Use of NAC to treat cataract and aging lens can lead to diminishing of light scattering units in the lens which raise glare effect, probably by prevention of the oxidative modification of crystallins and utilization of lipid peroxides (126-130;179-185).The present investigation indicates that carnosine derived from N-acetylcarnosine ophthalmic prodrug can preserve telomere structures in the lens epithelial cells, mitochondrial function, cell viability, and ATP levels in human lens cells during oxidative stress. Although the precise mechanism responsible for protection by the carnosine against oxidative stress remains to be determined, the ability of non-hydrolized carnosine, to protect against peroxide toxicity indicates that this type of biological activity is likely to be mediated through scavenging effect of carnosine towards toxic aldehydes and phospholipid hydroperoxide compounds (lipid peroxidation products) inducing shortening of telomeres in the lens epithelial cells. Carnosine promotes the protection of normal cells from acquiring phenotypic characteristics of

cellular senescence switching human lens into the process of ageing and cataractogenesis.

Aging is postulated to be a telomere-shortening process, and may be a phenomenon in which some telomeres have already undergone partial shortening in early individual development. The latter situation would result in these shortened telomeres undergoing further shortening during the individual's lifetime, leading to abnormally accelerated imbalances in functioning of neuroendocrine and neurotrophic cells and provoking neurodegenerative events. If aging is an intrinsically programmed process and if it is engendered by a growing deficit of telomeric activity, then identification of telomere-associated processes, and their artificial reproduction and therapeutic usage targeting telomers and their products would open new perspectives in overcoming age-related diseases that currently appear to be incurable (202).The systemic absorption of carnosine released from the ophthalmic prodrug N-acetylcarnosine can have an appropriate therapeutic influence on the lifelong clock which is regulated by the shortening of telomere DNA during ageing and age-related disorders in postmitotic neurons of the hypothalamus. Shortening of these DNA sequences occurs in humans on a monthly basis and is controlled by release of growth hormone discharged from the anterior pituitary directly into the hypothalamus via local blood vessels (202).

3.11. Prevention of complications of cataract surgery with N-acetylcarnosine ophthalmic prodrug: targeting secondary cataracts through modulation of cell senescent behavior

Once a clouded lens develops, surgical removal is still the usual remedy. Each year about 1.5 million cataract operations are performed, making it the most common operation in the country in people over 65 (Reviewed in Refs. 127,130).

Problems after cataract surgery are rare, but they can occur. These problems can include infection, bleeding, inflammation (pain, redness, swelling), loss of vision, double vision and high or low eye pressure . The following side effects are documented : retinal detachment, cystoid macula edema, posteriorly dislocated lens material, endophthalmitus, choroidal hemorrhage, secondary cataracts (Reviewed in Refs. 3,130, 181).Often the eye tissue that endoses the intraocular lens (IOL) becomes cloudy and may blur the vision of the eye subjected to cataract surgery. This condition is called after-cataract (secondary cataract). An after-cataract can develop months or years after cataract surgery. Residual lens epithelial cells (LECs) undergo fibrous proliferation after cataract surgery, resulting in capsular fibrosis (Reviewed in Ref. 203).

3.11.1. Secondary cataracts (Posterior Capsular Opacification) and their treatments

About 30% of patients who undergo extracapsular cataract surgery develop a secondary "after-cataract" called posterior capsular opacification. Posterior capsular opacification generally occurs because of the following events:- After surgery, there are still some natural lens cells left behind that proliferate on the back of the capsule. The capsule gradually becomes cloudy and interferes with clear vision the same way the original cataract did.

According to a 2001 study, the probability of developing a secondary cataract was 6% at one year, 15% at two years, 23% at three years, and 38% at nine years (Reviewed in Refs. 127,130). The risk is lower with phacoemulsification. Secondary cataracts are more likely to occur in younger patients, in those with diabetes, or when cataract surgery is combined vitrectomy (clearance of debris from the fluid in the eye). The appearance of a secondary cataract constitutes the most common complication of cataract surgery since the advent of phacoemulsification. Prevention is today one of the crucial challenges of research in ophthalmology.

3.11.2. Treatment for Posterior Capsular Opacification

The standard treatment is laser surgery known as a *YAG capsulotomy*. (Capsulotomy means cutting into the capsule, and YAG is an abbreviation of yttrium aluminum garnet, the laser most often used for this procedure). However, laser surgery carries its own risks and possible complications, similar to those of cataract surgery itself, and can also lead to poorer vision or blindness. About 1% of laser surgery patients develop a detached retina, which is much higher than the risk from the original cataract surgery.

The present benefits of N-acetylcarnosine ophthalmic therapeutic modality relates to a method for treatment or prevention of secondary cataract, comprising contacting the surface of the eye with an effective amount of a therapeutically active and physiologically acceptable N-acetylcarnosine combined with the cellulose compound releasing carnosine in the aqueous humor that prevents the senescent phenotype of residual germinative lens epithelial cells in in the equatorial regions of the lens capsule after cataract surgery.

We have developed a drug-sustained N-acetylcarnosine delivery system releasing carnosine in anterior chamber of the eye. This system targets the goal of combining advantageous pharmacological properties of carnosine with the ability to interfere on the epithelial cellular processes of secondary cataract. Clinical evaluation based on biomicroscopy and histology showed a lesser reaction of secondary cataract treated in eyes with the N-acetylcarnosine ophthalmic prodrug. The decrease in secondary cataract reaction in cases of N-acetylcarnosine treatment of after-cataract demonstrated the advantages of its pharmacological effect (Figure 3). The pharmacological and theoretical concept of a drug-sustained delivery system seems attractive and thus calls for further evaluation of cellular telomeres modulators and of cell behavior.

4. SUMMARY AND PERSPECTIVE

Although human cataractogenesis is associated with aging, direct evidence of lens epithelial cellular senescence and the mechanism of senescence associated

with chronic oxidative stress and accompanying ocular and systemic abnormalities or diseases during aging and cataractogenesis is lacking. This work demonstrates that oxidants induced premature senescence in human lens epithelial cells, with accelerated telomere shortening and reduced telomerase activity (although many human lenses lack detectable telomerase activity **(**164)**)**. We conclude that human cataractogenesis is characterized by senescence of lens cells, accelerated by oxidative stress-induced DNA damage, inhibition of telomerase and marked telomere shortening. Prevention of cellular senescence with ophthalmic prodrug N-acetylcarnosine may be a novel therapeutic target in a management of cataract, basic preventive health care and in arresting of after-cataract following extracapsular cataract extraction.

5. ACKNOWLEDGEMENTS

This work was planned, organized, and supported by Innovative Vision Products, Inc. (County of New Castle, DE, USA), by Russian Foundation for Basic Research (grant 09-04-01071a and Federal Agency for Education (State contract P1293). Innovative Vision Products, Inc. is a holder of the worldwide patent (including PCT International Publication Number WO 2004/028536 A1) for the application of *N*-acetylcarnosine for the Treatment of ophthalmic disorders, including cataracts as well as (PCT International Publication Number WO 2004/064866 PCT/JP2004/000351) protecting the described in the article therapeutic applications. Innovative Vision Products Inc. 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 last decade IVP has developed a track record in developing these technologies to effectively address the unmet needs of specific diseased populations. The authors report the interest in the Intellectual Property and marketing of the described modalities protected with the patents. The authors bear primary responsibility for accuracy of statements made in this document.

7. REFERENCES

1. Abraham Spector: Oxidative stress and disease. *J Ocul Pharmacol Ther*16, 193-201 (2000)

2. Bojana Kisić, Dijana Mirić, Lepsa Zorić, Ilija Dragojević and Aleksandra Stolić: Role of lipid peroxidation in pathogenesis of senile cataract (Article in Serbian). *Vojnosanit Pregl* 66, 371-375 (2009)

3. Mark A. Babizhayev, Anatoly I. Deyev, Valentina N. Yermakova, Igor V. Brikman, Johan Bours: Lipid peroxidation and cataracts: N-acetylcarnosine as a therapeutic tool to manage age-related cataracts in human and in canine eyes. *Drugs R D* 5, 125-139 (2004)

4. Douglas Borchman and Marta CeciliaYappert: Age-related lipid oxidation in human lenses. *Invest Ophthalmol Vis Sci* 39, 1053-1058 (1998)

5. Hideko Sawada, Takeo Fukuchi and Haruki Abe: Oxidative stress markers in aqueous humor of patients with senile cataracts. *Curr Eye Res* 34, 36-41 (2009)

6. O. P. S. Maurya, Lipa Mohanty, Gautam Bhaduri and Abhishek Chandra: Role of anti-oxidant enzymes superoxide dismutase and catalase in the development of cataract: study of serum levels in patients with senile and diabetic cataracts. *J Indian Med Assoc* 104, 396-397 (2006)

7. Mostafa Saadat, Majid Farvardin-Jahromi and Hooshang Saadat: Null genotype of glutathione S-transferase M1 is associated with senile cataract susceptibility in non-smoker females. *Biochem Biophys Res Commun* 319, 1287-1291 (2004)

8. Mostafa Saadat and Majid Farvardin-Jahromi: Occupational sunlight exposure, polymorphism of glutathione S-transferase M1, and senile cataract risk. *Occup Environ Med* 63, 503-504 (2006)

9. Vishnubhatla Sreenivas, Ashok K. Prabhakar, Sengamedu S. Badrinath, Thomas Fernandez, Indra Sekhar Roy, Tarun Sharma and Binal Shah: A rural population based case-control study of senile cataract in India. *J Epidemiol* 9, 327-336 (1999)

10. G. Tissié, S. Flangakis, L. Missotten, F. D'Hermies, J.J. de Laey, H. Bourgeois, C. Zenatti, J.R. Hermet, M.C. Rigeade and C. Bonne: Antioxidant activity of plasma from subjects with and without senile cataract. *Doc Ophthalmol* 83, 357-361 (1993)

11. Jonathan C Javitt and Hugh R. Taylor: Cataract and latitude. *Doc Ophthalmol* 88, 307-325 (1994-1995)

12. Durga K. Bhuyan, X. Huang, G. Kuriakose, W.H. Garner and Kailash C. Bhuyan: Menadione-induced oxidative stress accelerates onset of Emory mouse cataract *in vivo*. *Curr Eye Res* 16, 519-526 (1997)

13. Durga K. Bhuyan and Kailash C. Bhuyan: Assessment of oxidative stress to eye in animal model for cataract. *Methods Enzymol* 233, 630-639 (1994)

14. Kailash C. Bhuyan, Durga K. Bhuyan, W. Chiu, S. Malik and Irwin Fridovich: Desferal-Mn (III) in the therapy of diquat-induced cataract in rabbit. *Arch Biochem Biophys* 288, 525-532 (1991)

15. Mark A. Babizhayev and Anatoly I. Deyev: Lens opacity induced by lipid peroxidation products as a model of cataract associated with retinal disease. *Biochim Biophys Acta* 1004, 124-133 (1989)

16. R. Manikandan, R. Thiagarajan, S. Beulaja, S. Chindhu, K. Mariammal, G. Sudhandiran and M. Arumugam: Anti-cataractogenic effect of curcumin and aminoguanidine against selenium-induced oxidative stress in the eye lens of Wistar rat pups: An *in vitro* study using isolated lens. *Chem Biol Interact* 181, 202-209 (2009)

17. Eva M. Olofsson, Stefan L. Marklund and Anders Behndig: Enhanced diabetes-induced cataract in copper-zinc superoxide dismutase-null mice. *Invest Ophthalmol Vis Sci* 50, 2913-2918 (2009)

18. B.N. Rooban, Y. Lija, P.G. Biju, V. Sasikala, V. Sahasranamam and A. Abraham: Vitex negundo attenuates calpain activation and cataractogenesis in selenite models. *Exp Eye Res* 88, 575-582 (2009)

19. Kavita R. Hegde, Svitlana Kovtun and Shambhu D. Varma: Induction of ultraviolet cataracts *in vitro*: prevention by pyruvate. *J Ocul Pharmacol* Ther 23, 492-502 (2007)

20. T.N. Raju, C.Sanat Kumar, V. Rajani Kanth, B.Venkata Ramana, P. Uma Maheswara Reddy, P. Suryanarayana and G. Bhanuprakash Reddy: Cumulative antioxidant defense against oxidative challenge in galactose-induced cataractogenesis in Wistar rats. *Indian J Exp Biol* 44, 733-739 (2006)

21. P. Geraldine, B.B. Sneha, R. Elanchezhian, E. Ramesh, C.M. Kalavathy, J. Kaliamurthy and P.A. Thomas: Prevention of selenite-induced cataractogenesis by acetyl-L-carnitine: an experimental study. *Exp Eye Res* 83, 1340-1349 (2006)

22. Ibrahim Kocer, Seyithan Taysi, Mustafa Vecdi Ertekin, Ihsan Karslioglu, Akcahan Gepdiremen, Orhan Sezen and Korkmaz Serifoglu: The effect of L-carnitine in the prevention of ionizing radiation-induced cataracts: a rat model. *Graefes Arch Clin Exp Ophthalmol* 245, 588-594 (2007)

23. Mark A. Babizhayev: Accumulation of lipid peroxidation products in human cataracts. *Acta Ophthalmol (Copenh)* 67, 281-287 (1989)

24. Xingjun Fan, Jianye Zhang, Mathilde Theves, Christopher Strauch, Ina Nemet, Xiaoqin Liu, Juan Qian, Frank J Giblin and Vincent M Monnier: Mechanism of lysine oxidation in human lens crystallins during aging and in diabetes. *J Biol Chem* 284, 34618-34627 (2009)

25. Zuhal Yildirim, Filiz Yildirim, N Irem Ucgun and Nedret Kilic: The evaluation of the oxidative stress parameters in nondiabetic and diabetic senile cataract patients. *Biol Trace Elem Res* 128, 135-143 (2009)

26. Mikhail Linetsky, Ekaterina Shipova, Rongzhu Cheng and Beryl J Ortwerth: Glycation by ascorbic acid oxidation products leads to the aggregation of lens proteins. *Biochim Biophys Acta* 1782, 22-34 (2008)

27. Li Huang, Marta C. Yappert, James J. Miller and Douglas Borchman: Thyroxine ameliorates oxidative stress by inducing lipid compositional changes in human lens epithelial cells. *Invest Ophthalmol Vis Sci* 48, 3698-704 (2007)

28. Xingjun Fan, LixingW. Reneker, Mark E. Obrenovich, Christopher Strauch, Rongzhu Cheng, Simon M. Jarvis, Beryl J. Ortwerth and Vincent M. Monnier: Vitamin C mediates chemical aging of lens crystallins by the Maillard reaction in a humanized mouse model. *Proc Natl Acad Sci USA* 103, 16912-16917 (2006)

29. Paulo S. M. Barros, Angelica M. V. Safatle, Lelio Queiroz, Vanessa V. Silva and Silvia B. M. Barros: Blood and aqueous humour antioxidants in cataractous poodles. *Can J Ophthalmol* 39, 19-24 (2004)

30. Orkide Donma, Eda Yorulmaz, Hamiyet Pekel and Nezir Suyugül: Blood and lens lipid peroxidation and antioxidant status in normal individuals, senile and diabetic cataractous patients. *Curr Eye Res* 25, 9-16 (2002)

31. Zehra Hashim and Shamshad Zarina: Antioxidant markers in human senile and diabetic cataractous lenses. J Coll Physicians Surg Pak 16, 637-640 (2006)

32. Wanchao Ma, Irene Nunes, C. S. Hamish Young and Abraham Spector: Catalase enrichment using recombinant adenovirus protects alphaTN4-1 cells from H2O2. *Free Radic Biol Med* 40, 335-340 (2006)

33. J. Horwitz and N. Jaffe: Anatomy and embryology. In: Textbook of Ophthalmology 3, Lens and Cataract. Eds: Podos S. M. and Yanoff, M., Cower Medical Publishing, New York (1992)

34. John J. Harding and M.J. Crabbe: The lens: development, proteins, metabolism and cataract. In: The Eye. Eds. Davson H., Vol. lB. Academic Press, Orlando, Florida (1984).

35. Abraham Spector, Guo-Ming Wang, Ren-Rong Wang, Wan-Cheng Li, Jer R. Kuszak: A brief photochemically-induced oxidative insult causes irreversible lens damage and cataract I: transparency and epithelial cell layer. *Exp Eye Res* 60, 471-481 (1995)

36. Venkat N. Reddy, L.R. Lin, Y.S. Ho, J.L. Magnenat, N. Ibaraki, F.J. Giblin and L. Dang: Peroxide-induced damage in lenses of transgenic mice with deficient and elevated levels of glutathione peroxidase. *Ophthalmologica* 211, 192-200 (1997)

37. Abraham Spector, Yinqing Yang, Ye-Shih Ho, Jean-Luc Magnenat, Ren-Rong Wang, Wanchao Ma and Wan-Cheng Li: Variation in cellular glutathione peroxidase activity in lens epithelial cells, transgenics and knockouts does not significantly change the response to H2O2 stress. *Exp Eye Res* 62, 521-540 (1996)

38. Dana R. Crawford, Yanhong Wang, Gary P. Schools, John Kochheiser and Kelvin J. Davies: Down-regulation of mammalian mitochondrial RNAs during oxidative stress. *Free Radic Biol Med* 22, 551-559 (1997)

39. Ming Zheng, Fredrik Aslund and Gisela Storz: Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science* 279, 1718-1721 (1998)

40. Maitrayee Sundaresan, Zu-Xi Yu, Victor J. Ferrans, Kaikobad Irani and Toren Finkel: Requirement for

generation of H_2O_2 for platelet-derived growth factor signal transduction. *Science* 270, 296-299 (1995)

41. David J. Sulciner, Kaikobad Irani, Zu-Xi Yu, Victor J. Ferrans, Pascal Goldschmidt-Clermont and Toren Finkel: racl regulates a cytokine-stimulated, redox-dependent pathway necessary for NFκB activation. *Mol Cell Biol* 16, 7115-7121 (1996)

42. Matthias Meyer, Rhona Schreck and Patrick A. Baeuerle: H_2O_2 and antioxidants have opposite effects on activation of NF-κB and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO J* 12, 2005-2015 (1993)

43. G. Storz and B.S. Polla: Transcriptional regulators of oxidative stress-inducible genes in prokaryotes and eukaryotes. In: Stress-Inducible Cellular Responses. Eds. U. Feige, R.I. Morimoto, I. Yahara, B. Polla, Basel, Switzerland: Birkhauser Verlag, 239-254 (1996)

44. Elizabeth H. Blackburn: Structure and function of telomeres. *Nature* 350, 569-573 (1991)

45. Tracy M. Bryan, Anna Englezou, Jay Gupta, Silvia Bacchetti and Roger R. Reddel: Telomere elongation in immortal human cells without detectable telomerase activity. *EMBO J* 14, 4240-4248 (1995)

46. Peter T. Rowley: Telomerase: Putting an end to DNA. *Cancer Invest* 16, 170-174 (1998)

47. Catherine A. Egan, Isabelle Savre-Train, Jerry W. Shay, Steven E. Wilson and William M. Bourne: Analysis of telomere lengths in human corneal endothelial cells from donors of different ages. *Invest Ophthalmol Vis Sci* 39, 648-653 (1998)

48. Bas van Steensel, Agata Smogorzewska, Titia de Lange: TRF2 protects human telomeres from end to end fusions. *Cell* 92, 401-413 (1998)

49. Carol W. Greider: Telomere length regulation. *Annu Rev Biochem* 65, 337-365 (1996)

50. Kathleen Collins: Mammalian telomeres and telomerase. *Curr Opin Cell Biol* 12, 378-383 (2000)

51. Jiannan Feng, Walter D. Funk, Sophia S. Wang, Scott L. Weinrich, Ariel A. Avilion, Chihwei P. Chiu, Richard R. Adams, Ellen Chang, Rebecca C. Allsopp, Jinshan Yu, Scheherazade Le, Michael D. West, Calvin B. Harley, Wallace H. Andrews, C.W. Greiolen and Bryant Villeponteau: The RNA component of human telomerase. *Science* 269, 1236-1241 (1995)

52. Toru M. Nakamura, Gregg B. Morin, Karen B. Chapman, Scott L. Weinrich, William H. Andrews, Joachim Lingner, Calvin B. Harley and Thomas R. Cech: Telomerase catalytic subunit homologs from fission yeast and human. *Science* 277, 955-959 (1997)

53. Nancy Axelrod: Of telomeres and tumors. *Nat Med* 2, 158-159 (1996)

54. Matthew Meyerson, Christopher M. Counter, Elinor N. Eaton, Leif W. Ellisen, Philipp Steiner, S.D. Caddle, Liuda Ziaugra, Roderick L. Beijersbergen, Michael J. Davidoff, Qiang Liu, Silvia Bacchetti, Daniel A. Haber and Robert A. Weinberg: hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. *Cell* 90, 785-795 (1997)

55. Aloysius J. Klingelhutz: The roles of telomeres and telomerase in cellular immortalization and the development of cancer. *Anticancer Res* 19, 4823-4830 (1999)

56. Jerry W. Shay and Woodring E. Wright: Hallmarks of telomeres in ageing research. *J Pathol* 211, 114-123 (2007)

57. Matthew Meyerson: Role of telomerase in normal and cancer cells. *J Clin Oncol* 18, 2626-2634 (2000)

58. Shaheda Ahmed, Joao F. Passos, Matthew J. Birket, Tina Beckmann, Sebastian Brings, Heiko Peters, Mark A. Birch-Machin, Thomas von Zglinicki and Gabriele Saretzki: Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress. J Cell Sci 121, 1046-1053 (2008)

59. Andrea G. Bodnar, Michel Ouellette, Maria Frolkis, Shawn E. Holt, Choy-Pik Chiu, Gregg B. Morin, Calvin B. Harley, Jerry W. Shay, Serge Lichtsteiner and Woodring E. Wright: Extension of life-span by introduction of telomerase into normal human cells. Science 279, 349-352 (1998)

60. Weiming Fu, Michael Killen, Carsten Culmsee, Sonu Dhar, Tej K. Pandita and Mark P. Mattson: The catalytic subunit of telomerase is expressed in developing brain neurons and serves a cell survival-promoting function. J Mol Neurosci 14, 3-15 (2000)

61. Carmen M. Colitz, Michael G. Davidson and M.Christine McGanan: Telomerase activity in lens epithelial cells of normal and cataractous lenses. *Exp Eye Res* 69, 641-649 (1999)

62. William R. Pendergrass, Patricia E. Penn, Jinbo Li and Norman S. Wolf: Age-related telomere shortening occurs in lens epithelium from old rats and is slowed by caloric restriction. *Exp Eye Res* 73, 221-228 (2001)

63. Xiao-Qin Huang, Juan Wang, Jin-Ping Liu, Hao Feng, Wen-Bin Liu, Qin Yan, Yan Liu, Shu-Ming Sun, Mi Deng, Lili Gong, Yun Liu and David W. Li: hTERT extends proliferative lifespan and prevents oxidative stress-induced apoptosis in human lens epithelial cells. *Invest Ophthalmol Vis Sci* 46, 2503-2513 (2005)

64. Donatella del Bufalo, Antonella Rizzo, Daniela Trisciuoglio, Gianluigi Cardinali, Maria Rosaria Torrisi, Uwe Zangemeister-Wittke and Anna Biroccio: Involvement of hTERT in apoptosis induced by

interference with Bcl-2 expression and function. *Cell Death Differ* 12, 1429-1438 (2005)

65. Hyo Jung Kang, Yoon Sik Choi, Seung-Beom Hong, Kee-Won Kim, Ran-Sook Woo, Seok Joon Won, Eun Ju Kim, Hee Kyung Jeon, So-Young Jo, Tae Kook Kim, Robert Bachoo, Ian J. Reynolds, Byoung Joo Gwag and Han-Woong Lee: Ectopic expression of the catalytic subunit of telomerase protects against brain injury resulting from ischemia and NMDA-induced neurotoxicity. J Neurosci 24, 1280-1287 (2004)

66. Antoinette Ludwig, Gabriele Saretzki, Per Sonne Holm, Frank Tiemann, Michael Lorenz, Thomas Emrich, Calvin B. Harley and Thomas von Zglinicki: Ribozyme cleavage of telomerase mRNA sensitizes breast epithelial cells to inhibitors of topoisomerase. *Cancer Res* 61, 3053-3061 (2001)

67. Hidemasa Oh, George E. Taffet, Keith A. Youker, Mark L. Entman, Paul A. Overbeek, Lloyd H. Michael and Michael D. Schneider:Telomerase reverse transcriptase promotes cardiac muscle cell proliferation, hypertrophy, and survival. *Proc Natl Acad Sci USA* 98, 10308-10313 (2001)

68. Peisu Zhang, Sic L. Chan, Weiming Fu, Marty Mendoza and Mark P. Mattson: TERT suppresses apoptotis at a premitochondrial step by a mechanism requiring reverse transcriptase activity and 14-3-3 protein-binding ability. FASEB J 17, 767-769 (2003)

69. Yasuko Kondo, Seiji Kondo, Yoshikazu Tanaka, Talat Haqqi, Barbara P. Barna and John K. Cowell: Inhibition of telomerase increases the susceptibility of human malignant glioblastoma cells to cisplatin-induced apoptosis. *Oncogene* 16, 2243-2248 (1998)

70. Girdhar G. Sharma, Arun Gupta, Huichen Wang, Harry Scherthan, Sonu Dhar, Varsha Gandhi, Georg Iliakis, Jerry W. Shay, Charles S. Young and Tej K. Pandita: hTERT associates with human telomeres and enhances genomic stability and DNA repair. *Oncogene* 22, 131-146 (2003)

71. Christophe Massard, Yael Zermati, Anne-Laure Pauleau, Nathanael Larochette, Didier Métivier, Laure Sabatier, Guido Kroemer and Jean-Charles Soria: hTERT: a novel endogenous inhibitor of the mitochondrial cell death pathway. Oncogene 25, 4505-4514 (2006)

72. Huanzhang Zhu, Wei Fu and Mark P. Mattson: The catalytic subunit of telomerase protects neurons against amyloid beta-peptide-induced apoptosis. *J Neurochem* 75, 117-124 (2000)

73. R.M. Clayton, J. Cuthbert, J. Duffy, J. Seth, C.I. Phillips, R.S. Bartholomew and J.M. Reid: Some risk factors associated with cataract in S.E. Scotland: a pilot study. *Trans Ophthalmol Soc UK* 102, 331-336 (1982)

74. Ciro Costagliola, Giovanni Iuliano, Massimo Menzione, Anna Nesti, Francesca Simonelli and Ernesto Rinaldi: Systemic human diseases as oxidative risk factors in cataractogenesis. I. Diabetes. *Ophthalmic Res* 20, 308-316 (1988)

75. Ciro Costagliola, Giovanni Iuliano, Massimo Menzione, Francesca Simonelli, Achille Tortori, Bruno Masturzi, Attilio di Benedetto and Ernesto Rinaldi: Systemic human diseases as oxidative risk factors in cataractogenesis. II. Chronic renal failure. *Exp Eye Res* 51, 631-635 (1990)

76. Francesca Simonelli, Anna Nesti, Manuela Pensa, L Romano, S Savastano, Ernesto Rinaldi and G Auricchio: Lipid peroxidation and human cataractogenesis in diabetes and severe myopia. *Exp Eye Res* 49, 181-187 (1989)

77. Kailash C. Bhuyan, Ravidatt W. Master, Robert S. Coles and Durga K. Bhuyan: Molecular mechanisms of cataractogenesis: IV. Evidence of phospholipid . malondialdehyde adduct in human senile cataract. *Mech Ageing Dev* 34, 289-296 (1986)

78. Douglas Borchman, Marta Cecilia Yappert, R.Q. Rubini and Christopher A. Paterson: Distribution of phospholipid-malondialdehyde-adduct in the human lens. *Curr Eye Res* 8, 939-946 (1989)

79. Kailash C. Bhuyan, Durga K. Bhuyan and Steven M. Podos: Lipid peroxidation in cataract of the human. *Life Sci* 38, 1463-1471 (1986)

80. Kailash C. Bhuyan, Durga K. Bhuyan and Steven M. Podos: Free radical enhancer xenobiotic is an inducer of cataract in rabbit. *Free Radic Res Commun* 12-13, 609-620 (1991)

81. Hiroyuki Mibu, Masanobu Nagata and Mitsushi Hikida: A study on lipid peroxide-induced lens damage *in vitro*. *Exp Eye Res* 58, 85-90 (1994)

82. Sanjay K. Srivastava, Sanjay Awasthi, Lifei Wang, Aruni Bhatnagar, Yogesh C. Awasthi and Aseem H. Ansari: Attenuation of 4-hydroxynonenal-induced cataractogenesis in rat lens by butylated hydroxytoluene. *Curr Eye Res* 15, 749-754 (1996)

83. Hideo Nishigori, Jung W. Lee, Yasuhisa Yamauchi and Motoharu Iwatsuru: The alteration of lipid peroxide in glucocorticoid-induced cataract of developing chick embryos and the effect of ascorbic acid. *Curr Eye Res* 5, 37-40 (1986)

84. K. Yagi, S. Komura, N. Ihara, H. Abe, H. Konishi and S. Arichi: Serum lipid peroxide levels in rats with inherited cataracts. *J Appl Biochem* 7, 202-206 (1985)

85. J. Samuel Zigler Jr., Richard S. Bodaness, Igal Gery and Jin H. Kinoshita: Effects of lipid peroxidation products on the rat lens in organ culture: a possible mechanism of cataract initiation in retinal degenerative disease. *Arch Biochem Biophys* 225, 149-156 (1983)

86. J. Samuel Zigler Jr., Igal Gery, D. Kessler and Jin H. Kinoshita: Macrophage mediated damage to rat lenses in culture: a possible model for uveitis-associated cataract. *Invest Ophthalmol Vis Sci* 24, 651-654 (1983)

87. John D. Goosey, W.M. Tuan and C.A. Garcia: A lipid peroxidative mechanism for posterior subcapsular cataract formation in the rabbit: a possible model for cataract formation in tapetoretinal diseases. *Invest Ophthalmol Vis Sci* 25, 608-612 (1984)

88. J. Samuel Zigler Jr. and H.H. Hess: Cataracts in the Royal College of Surgeons rat: evidence for initiation by lipid peroxidation products. *Exp Eye Res* 41, 67-76 (1985)

89. Mark A. Babizhayev, Boris A. Dainyak and Alexandra H. Maxina: ESR spin label and ultrastructural monitoring of protein-lipid interactions in the lens fiber-cell plasma membranes in relation to human ageing and cataractogenesis. *Mech Ageing Dev* 64, 133-147 (1992)

90. Douglas Borchman, Om P. Lamba, Samira Salmassi, Marjorie Lou and M. Cecilia Yappert: The dual effect of oxidation on lipid bilayer structure. Lipids 27, 261-265 (1992)

91. Douglas Borchman, Om P. Lamba and M. Cecilia Yappert: Structural characterization of lipid membranes from clear and cataractous human lenses. Exp Eye Res 57, 199-208 (1993)

92. Li Huang L, Rosendo Estrada, Marta C. Yappert and Douglas Borchman: Oxidation-induced changes in human lens epithelial cells. 1. Phospholipids. *Free Radic Biol Med* 41, 1425-1432 (2006)

93. Li Huang, Daxin Tang, Marta C. Yappert and Douglas Borchman: Oxidation-induced changes in human lens epithelial cells 2. Mitochondria and the generation of reactive oxygen species. *Free Radic Biol Med* 41, 926-936 (2006)

94. Douglas Borchman, Christopher A. Paterson and Nicholas A. Delamere: Oxidative inhibition of Ca2+-ATPase in the rabbit lens. *Invest Ophthalmol Vis Sci* 30, 1633-1637 (1989)

95. V.E. Kagan: Lipid Peroxidation in Biomembranes, p.181. CRC Press, Boca Raton, FL (1988).

96. Jeen-Woo Park and Robert A. Floyd: Lipid peroxidation products mediate the formation of 8-hydroxydeoxyguanosine in DNA. *Free Radic Biol Med* 12, 245-250 (1992)

97. Mark A. Babizhayev, Anatoly I. Deyev and Leonid F. Linberg: Lipid peroxidation as a possible cause of cataract. *Mech Ageing Dev* 44, 69-89 (1988)

98. Mark A. Babizhayev: Lipid fluorophores of the human crystalline lens with cataract. *Graefes Arch Clin Exp Ophthalmol* 227, 384-391 (1989)

99. Mark A. Babizhayev: Antioxidant activity of L-carnosine, a natural histidine-containing dipeptide in crystalline lens. *Biochim Biophys Acta* 1004, 363-371 (1989)

100. Kiyomi Kikugawa, Tetsuta Kato, Masatoshi Beppu and Akira Hayasaka: Development of fluorescence and cross-links in eye lens crystallin by interaction with lipid peroxy radicals. *Biochim Biophys Acta* 1096, 108-114 (1991)

101. Jasmine V. Ferrer, Eduardo Gascó, Juan Sastre, Federico V. Pallardó, Miguel Angel Asensi and Jose Viña: Age-related changes in glutathione synthesis in the eye lens. *Biochem J* 269, 531-534 (1990)

102. Ai Hyang Shin, Chang Joo Oh and Jeen-Woo Park: Glycation-induced inactivation of antioxidant enzymes and modulation of cellular redox status in lens cells. *Arch Pharm Res* 29, 577-581 (2006)

103. John D. Goosey, J. Samuel Zigler Jr. and Jin H. Kinoshita: Cross-linking of lens crystallins in a photodynamic system: a process mediated by singlet oxygen. *Science* 208, 1278-1280 (1980)

104. Sergei Iu. Egorov, Mark A. Babizhaev, Alexander A. Krasnovskiĭ Jr. and Anna A. Shvedova. Photosensitized generation of singlet molecular oxygen by endogenous substances of the eye lens. Biofizika 32, 169-171 (1987)

105. Kailash C. Bhuyan and Durga K. Bhuyan: Regulation of hydrogen peroxide in eye humors. Effect of 3-amino-1H-1,2,4-triazole on catalase and glutathione peroxidase of rabbit eye. *Biochim Biophys Acta* 497, 641-651 (1977)

106. Kailash C. Bhuyan and Durga K. Bhuyan: Superoxide dismutase of the eye: relative functions of superoxide dismutase and catalase in protecting the ocular lens from oxidative damage. *Biochim Biophys Acta* 542, 28-38 (1978)

107. Kailash C. Bhuyan and Durga K. Bhuyan: Molecular mechanism of cataractogenesis: III. Toxic metabolites of oxygen as initiators of lipid peroxidation and cataract. *Curr Eye Res* 3, 67-81 (1984)

108. Mark A. Babizhaev, Anatoly I. Deev, Yuri A. Vladimirov and Irina B. Deeva: Decomposition of H_2O_2 by human cataractous lenses. Biull Eksp Biol Med 102, 158-160 (1986)

109. John V. Fecondo and Robert C. Augusteyn: Superoxide dismutase, catalase and glutathione peroxidase in the human cataractous lens. *Exp Eye Res* 36, 15-23 (1983)

110. G.N. Rao, B. Sadasivudu and E. Cotlier: Studies on glutathione S-transferase, glutathione peroxidase and glutathione reductase in human normal and cataractous lenses. *Ophthalmic Res* 15, 173-179 (1983)

111. Abraham Spector and William H. Garner: Hydrogen peroxide and human cataract. *Exp Eye Res* 33, 673-681 (1981)

112. Alex Pirie: Glutatione peroxidase in lens and a source of hydrogen peroxide in aqueous humour. *Biochem J* 96, 244-253 (1965)

113. Alex Pirie: A light-catalysed reaction in the aqueous humor of the eye. *Nature* 205, 500-501 (1965)

114. Rama S. Dwivedi and V.B. Pratap: Alteration in glutathione metabolism during cataract progression. *Ophthalmic Res* 19, 41-44 (1987)

115. J. Scharf, A. Dovrat and David Gershon: Defective superoxide-dismutase molecules accumulate with age in human lenses. *Graefes Arch Clin Exp Ophthalmol* 225, 133-136 (1987)

116. Venkat N. Reddy: Glutathione and its function in the lens--an overview. *Exp Eye Res* 50, 771-778 (1990)

117. W.B. Rathbun: In Glutathione: Chemical, Biochemical, and Medical Aspects, Part B. Eds: Dolphin D, Poulson R and Avramovic O. 467-510, Wiley, New York (1989)

118. John J. Harding: Free and protein-bound glutathione in normal and cataractous human lenses. *Biochem J* 117, 957-960 (1970)

119. Shambhu D.Varma, Sushil Kumar and R.D. Richards: Light-induced damage to ocular lens cation pump: prevention by vitamin C. *Proc Natl Acad Sci USA* 76, 3504-3506 (1979)

120. Simon P. Wolff, Guo-Ming Wang and Abraham Spector: Pro-oxidant activation of ocular reductants. 1. Copper and riboflavin stimulate ascorbate oxidation causing lens epithelial cytotoxicity *in vitro*. *Exp Eye Res* 45, 777-789 (1987)

121. Guillermo Saez, Paul J. Thornalley, Holly A. Hill, Reginald Hems and Joe V. Bannister: The production of free radicals during the autoxidation of cysteine and their effect on isolated rat hepatocytes. *Biochim Biophys Acta* 719, 24-31 (1982)

122. Reema Nath, Sanjay K. Srivastava and K. Singh: Accumulation of copper and inhibition of lactate dehydrogenase activity in human senile cataractous lens. *Indian J Exp Biol* 7, 25-26 (1969)

123. J.P. Gerhard: Study of copper in the aqueous humor. *Doc Ophthalmol* 20, 104-110 (1966)

124. Denham Harman: The free radical theory of aging: effect of age on serum copper levels. *J Gerontol* 20, 151-153 (1965)

125. Riccardo Noto, Rosaria Alicata, Luciano Sfogliano, Sergio Neri and Maurizio Bifarella: A study of cupremia in a group of elderly diabetics. *Acta Diabetol Lat* 20, 81-85 (1983)

126. Mark A. Babizhayev, Andrea Guiotto and Anne Kasus-Jacobi: N-Acetylcarnosine and histidyl-hydrazide are potent agents for multitargeted ophthalmic therapy of senile cataracts and diabetic ocular complications. *J Drug Target* 17, 36-63 (2009)

127. Mark A. Babizhayev and Anne Kasus-Jacobi: State of the art clinical efficacy and safety evaluation of N-acetylcarnosine dipeptide ophthalmic prodrug. Principles for the delivery, self-bioactivation, molecular targets and interaction with a highly evolved histidyl-hydrazide structure in the treatment and therapeutic management of a group of sight-threatening eye diseases. *Curr Clin Pharmacol* 4, 4-37 (2009)

128. Mark A. Babizhayev, Philip Micans, Andrea Guiotto and Anne Kasus-Jacobi: 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. *Am J Ther* 16, 517-533 (2009)

129. Mark A. Babizhayev, Leslie Burke, Philip Micans and Stuart P. Richer: N-Acetylcarnosine sustained drug delivery eye drops to control the signs of ageless vision: Glare sensitivity, cataract amelioration and quality of vision currently available treatment for the challenging 50,000-patient population. *Clin Interv Aging* 4, 31-50 (2009)

130. Mark A. Babizhayev: Current ocular drug delivery challenges for N-acetylcarnosine: novel patented routes and modes of delivery, design for enhancement of therapeutic activity and drug delivery relationships. *Recent Pat Drug Deliv Formul* 3, 229-265 (2009)

131. May Shawi and Chantal Autexier: Telomerase, senescence and ageing. *Mech Ageing Dev* 129, 3-10 (2008)

132. Qin Chen and Bruce N. Ames: Senescence-like growth arrest induced by hydrogen peroxide in human diploid fibroblast F65 cells. *Proc Natl Acad Sci USA* 91, 4130-4134 (1994)

133. Thomas von Zglinicki, Gabriele Saretzki, Wolf Döcke and Christian Lotze: Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence? *Exp Cell Res* 220, 186-193 (1995)

134.Hermann Unterluggauer, Barbara Hampel, Werner Zwerschke and Pidder Jansen-Dürr: Senescence-associated cell death of human endothelial cells: the role of oxidative stress. *Exp Gerontol* 38, 1149-1160 (2003)

135. David J. Kurz, Stephanie Decary, Ying Hong, Elisabeth Trivier, Alexander Akhmedov and Jorge D. Erusalimsky: Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. *J Cell Sci* 117, 2417-2426 (2004)

136. Renata Colavitti and Toren Finkel: Reactive oxygen species as mediators of cellular senescence. *IUBMB Life* 57, 277-281 (2005)

137. Jaime Miquel: An integrated theory of aging as the result of mitochondrial-DNA mutation in differentiated cells. *Arch Gerontol Geriatr* 12, 99-117 (1991)

138. Eveline Hutter, Kathrin Renner, Gerard Pfister, Petra Stöckl, Pidder Jansen-Dürr and Erich Gnaiger: Senescence-associated changes in respiration and oxidative phosphorylation in primary human fibroblasts. *Biochem J* 380, 919-28 (2004)

139. Petra Stöckl, Christina Zankl, Eveline Hütter, Hermann Unterluggauer, Peter Laun, Gino Heeren, Edith Bogengruber, Dietmar Herndler-Brandstetter, Michael Breitenbach and Pidder Jansen-Dürr: Partial uncoupling of oxidative phosphorylation induces premature senescence in human fibroblasts and yeast mother cells. *Free Radic Biol Med* 43, 947-958 (2007)

140. Joao F. Passos, Gabriele Saretzki, Shaheda Ahmed, Glyn Nelson, Torsten Richter, Heiko Peters, Ilka Wappler, Matthew J. Birket, Graham Harold, Karin Schaeuble, Mark A. Birch-Machin, Thomas B. Kirkwood and Thomas von Zglinicki: Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence. *PLoS Biol* 5, e110 (2007)

141.Thomas von Zglinicki: Oxidative stress shortens telomeres. *Trends Biochem Sci* 27, 339-344 (2002)

142. Violeta Serra, Thomas von Zglinicki, Mario Lorenz and Gabriele Saretzki: Extracellular superoxide dismutase is a major antioxidant in human fibroblasts and slows telomere shortening. *J Biol Chem* 278, 6824-6830 (2003)

143.Gabriele Saretzki and Thomas von Zglinicki: Replicative aging, telomeres, and oxidative stress. *Ann N Y Acad Sci* 959, 24-29 (2002)

144. Thomas von Zglinicki: Role of oxidative stress in telomere length regulation and replicative senescence. *Ann N Y Acad Sci* 908, 99-110 (2000)

145. Simone Petersen, Gabriele Saretzki and Thomas von Zglinicki: Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. *Exp Cell Res* 239, 152-160 (1998)

146. Nicolle Sitte, Gabriele Saretzki and Thomas von Zglinicki: Accelerated telomere shortening in fibroblasts after extended periods of confluency. Free Radic Biol Med 24, 885-893 (1998)

147. Christopher A. Paterson and Nicholas A. Delamere: The Lens. In: Adler's Physiology of the Eye. Eds: Hart W. M. Mosby Year Book, St. Louis, pp. 348-90 (1992)

148. Jerome R. Kuszak: A re-examination of primate lens epithelial cell size, density and structure as a function of development, growth and age. *Nova Acta Leopoldina* 75, 45-66 (1997)

149. Clifford V. Harding, John R. Reddan, Nalin J. Unakar and Mihir Bagchi: The control of cell division in the ocular lens. *Int Rev Cytol* 31, 215-300 (1971)

150. John W. McAvoy and Joan McDonald: Proliferation of lens epithelial explants in culture increases with age of donor rat. *Curr Eye Res* 3, 1151-1153 (1984)

151. Andrea G. Bodnar, Nam W. Kim, Rita B. Effros and Choy-Pik Chiu: Mechanism of telomerase induction during T cell activation. *Exp Cell Res* 228, 58-64 (1996)

152. Isabella Russo, Andrew R. Silver, Andrew P. Cuthbert, Darren K. Griffin, Deborah A. Trott and Robert F. Newbold: A telomere-independent senescence mechanism is the sole barrier to Syrian hamster cell immortalization. *Oncogene* 17, 3417-3426 (1998)

153. Futoshi Ishikawa: Regulation mechanisms of mammalian telomerase. *Biochemistry (Mosc)* 62, 1332-1337 (1997)

154. Andrea G. Bodnar, Michel Ouellette, Maria Frolkis, Shawn E. Holt, Choy-Pik Chiu, Gregg B. Morin, Calvin B. Harley, Jerry W. Shay, Serge Lichtsteiner and Woodring Wright: Extension of life-span by introduction of telomerase into normal human cells. *Science* 279, 349-352 (1998)

155. Norman J. Kleiman, Ren-Rong Wang and Abraham Spector: Ultraviolet light induced DNA damage and repair in bovine lens epithelial cells. *Curr Eye Res* 9, 1185-1193 (1990)

156. Vincent J. Cristofalo, Christoph Volker, Mary K. Francis and Maria Tresini: Age-dependent modifications of gene expression in human fibroblasts. *Crit Rev Eukaryot Gene Expr* 8, 43-80 (1998)

157. William R. Pendergrass, Patricia E. Penn, Jing Li and Norman S. Wolf: Age-related telomere shortening occurs in lens epithelium from old rats and is slowed by caloric restriction. *Exp Eye Res* 73, 221-228 (2001)

158. Elizabeth H. Blackburn: Telomere states and cell fates. *Nature* 408, 53-56 (2000)

159. Thomas von Zglinicki, Rita Pilger and Nicolle Sitte: Accumulation of single-strand breaks is the major cause of telomere shortening in human fibroblasts. *Free Radic Biol Med* 28, 64-74 (2000)

160. Constance I. Nugent and Victoria Lundblad: The telomerase reverse transcriptase: components and regulation. *Genes Dev* 12, 1073-1085 (1998)

161. Norman J. Kleiman and Abraham Spector: DNA single strand breaks in human lens epithelial cells from patients with cataract. *Curr Eye Res* 12, 423-431 (1993)

162. Juan Wang, Hao Feng, Xiao-Qin Huang, Hua Xiang, Ying-Wei Mao, Jin-Ping Liu, Qin Yan, Wen-Bin Liu, Yan Liu, Mi Deng, Lili Gong, Shuming Sun, Chen Luo, Shao-Yun Liu, Xuan-Jie Zhang, Yun Liu and David Wan-Cheng Li: Human telomerase reverse transcriptase immortalizes bovine lens epithelial cells and suppresses differentiation through regulation of the ERK signaling pathway. *J Biol Chem* 280, 22776-22787 (2005)

163. Hua Xiang, Juan Wang, Yingwei Mao, Mingyao Liu, Venkan N. Reddy and David Wan-Cheng Li: Human telomerase accelerates growth of lens epithelial cells through regulation of the genes mediating RB/E2F pathway. *Oncogene* 21, 3784-3791 (2002)

164. Xiao-Qin Huang, Juan Wang, Jin-Ping Liu, Hao Feng, Wen-Bin Liu, Qin Yan, Yan Liu, Shu-Ming Sun, Mi Deng, Lili Gong, Yun Liu and David Wan-Cheng Li: hTERT extends proliferative lifespan and prevents oxidative stress-induced apoptosis in human lens epithelial cells. *Invest Ophthalmol Vis Sci* 46, 2503-2513 (2005)

165. Mark A. Babizhayev, Anatoly I. Deyev, Valentina N. Yermakova, Nina G. Davydova, Natalya I. Kurysheva, Valerii S. Doroshenko and Alexander V. Zhukotskii: Image analysis and glare sensitivity in human age-related cataracts. *Clin Exp Optom* 86, 157-172 (2003)

166. Mark A. Babizhayev and Marie-Christine Seguin: Process of assessment of ocular disfunctions and implementation devices of this process. US Patent No. 6,007,203. Date of patent: December 28, 1999.

167. Sergei E. Severin: Problems concerned with the biological activity of naturally occurring imidazole compounds. *Proceedings of Plen. Sess. Seventh International Biochemical Congress NY*, pp.45-61 (1964)

168. Alexander A. Boldyrev and Sergei E. Severin: The histidine-containing dipeptides, carnosine and anserine: distribution, properties and biological significance. *Adv Enzyme Regul* 30, 175-194 (1990)

169. James F. Lenney: Specificity and distribution of mammalian carnosinase. *Biochim Biophys Acta* 429, 214-219 (1976)

170. Mel C. Jackson, Christine M. Kucera and James F. Lenney: Purification and properties of human serum carnosinase. *Clin Chim Acta* 196, 193-205 (1991)

171. James F. Lenney: Separation and characterization of two carnosine-splitting cytosolic dipeptidases from hog kidney (carnosinase and non-specific dipeptidase). *Biol Chem Hoppe Seyler* 371, 433-440 (1990)

172. Norbert Kunze, Horst Kleinkauf and Karl Bauer: Characterization of two carnosine-degrading enzymes from rat brain. Partial purification and characterization of a carnosinase and a beta-alanyl-arginine hydrolase. *Eur J Biochem* 160, 605-613 (1986)

173. Alexander A. Boldyrev, Alexander M. Dupin, Aron Y. Bunin, Mark A. Babizhayev and Sergei E. Severin: The antioxidative properties of carnosine, a natural histidine containing dipeptide. *Biochem Int* 15, 1105-1113 (1987)

174. Giancarlo Aldini, Roberto Maffei Facino, Giangiacomo Beretta and Marina Carini: Carnosine and related dipeptides as quenchers of reactive carbonyl species: from structural studies to therapeutic perspectives. *Biofactors* 24, 77-87 (2005)

175. Giancarlo Aldini, Marica Orioli, Marina Carini and Roberto Maffei Facino: Profiling histidine-containing dipeptides in rat tissues by liquid chromatography/electrospray ionization tandem mass spectrometry. *J Mass Spectrom* 39, 1417-1428 (2004)

176. Marica Orioli, Giancarlo Aldini, Giangiacomo Beretta, Roberto Maffei Facino and Marina Carini: LC-ESI-MS/MS determination of 4-hydroxy-trans-2-nonenal Michael adducts with cysteine and histidine-containing peptides as early markers of oxidative stress in excitable tissues. *J Chromatogr B* 827, 109-118 (2005)

177. V. Prakash Reddy, Matthew R. Garrett, George Perry and Mark A. Smith: Carnosine: a versatile antioxidant and antiglycating agent. *Sci Aging Knowledge Environ* 2005, pe12 (2005).

178. Imran Rashid, David M. van Reyk and Michael J. Davies: Carnosine and its constituents inhibit glycation of low-density lipoproteins that promotes foam cell formation *in vitro*. *FEBS Lett* 581, 1067–1070 (2007)

179. Mark A. Babizhayev, Anatoly I. Deyev, Valentina N. Yermakova, Yuri A. Semiletov, Nina G. Davydova, Natalya I. Kurysheva, Alexander V. Zhukotskii and Ita M. Goldman: N-Acetylcarnosine, a natural histidine-containing dipeptide, as a potent ophthalmic drug in treatment of human cataracts. *Peptides* 22, 979-994 (2001)

180. Mark A. Babizhayev, Anatoly I. Deyev, Valentina N. Yermakova, Yuri A. Semiletov, Nina G. Davydova, Valerii S. Doroshenko, Alexander V. Zhukotskii and Ita M. Goldman: Efficacy of N-acetylcarnosine in the treatment of cataracts. *Drugs Res Dev* 3, 87-103 (2002)

181. Mark A. Babizhayev, Anatoly I. Deyev, Valentina N. Yermakova, Valerii V. Remenschikov and Johan Bours: Revival of the lens transparency with N-acetylcarnosine. *Current Drug Therapy* 1, 91-116 (2006)

182. Mark A. Babizhayev: Method for topical treatment of eye disease and composition and device for said treatment. PCT Patent Application. International Publication Number WO 2004/028536 A1. International Publication Date: 8 April 2004.

183. Mark A. Babizhayev: Ophthalmic pharmacology of N-acetylcarnosine lubricant eye drops. *J Pharmacol Toxicol* 1, 201-233 (2006)

184. Mark A. Babizhayev: Rejuvenation of visual functions in older adult drivers and drivers with cataract during a short-term administration of N-acetylcarnosine lubricant eye drops. Rejuvenation Res 7, 186-198 (2004)

185. Mark A. Babizhayev, Valentina N. Yermakova, Anatoly I. Deyev and Marie-Christine Seguin: Imidazole-containing peptidomimetic NACA as a potent drug for the medicinal treatment of age-related cataract in humans. *J. Anti-Aging Medicine* 3, 43-62 (2000)

186. Mark A. Babizhayev, Valentina N. Yermakova, Natalya L. Sakina, Rima P. Evstigneeva, Elena A. Rozhkova and Galina A. Zheltukhina: N^{α}-Acetylcarnosine is a prodrug of L-carnosine in ophthalmic application as antioxidant. *Clin Chim Acta* 254, 1–21 (1996)

187. Mark A. Babizhayev and Edoardo Bozzo Costa: Composizioni farmaceutiche contenenti *N*-acetilcarnosina per il trattamento della cataratta. Italian Patent A61K gruppo 37/00 20122 MI, Priority Oct. 15, 1993.

188. Mark A. Babizhayev and Edoardo Bozzo Costa E: Pharmaceutical compositions containing *N*-acetylcarnosine for the treatment of cataract. Patent PCT/EP 94/03340 SCB 238 PCT, Oct. 10, 1994.

189. Mark A. Babizhayev: Ocular drug metabolism of the bioactivating antioxidant N-acetylcarnosine for vision in ophthalmic prodrug and codrug design and delivery. *Drug Dev Ind Pharm* 34, 1071-1089 (2008)

190. R.F. Rekker. The hydrophobic fragmental constant. Elsevier, Amsterdam (1977)

191. John J. O'Dowd, David J. Robins and David J. Miller: Detection, characterisation, and quantification of carnosine and other histidyl derivatives in cardiac and skeletal muscle. *Biochim Biophys Acta* 967, 241-249 (1988)

192. Mark A. Babizhayev, Valentina N. Yermakova, Yuri A. Semiletov and Anatoly I. Deyev: The natural histidine-containing dipeptide N-acetylcarnosine as an antioxidant for ophthalmic use. *Biochemistry (Moscow)* 65, 588– 598 (2000)

193. Ibrahim F. Hepsen, Zeki Yildirim, Harun Yilmaz and Mahir Kotuk: Preventive effect of lacrimal occlusion on topical timolol-induced bronchoconstriction in asthmatics. *Clin Experiment Ophthalmol* 32, 597-602 (2004)

194. Hiroto Otani, Akiko Okumura, Katsuya Nagai and Nobuaki Okumura: Colocalization of a carnosine-splitting enzyme, tissue carnosinase (CN2)/cytosolic non-specific dipeptidase 2 (CNDP2), with histidine decarboxylase in the tuberomammillary nucleus of the hypothalamus. *Neurosci Lett* 445, 166-169 (2008)

195. Daniel J. Uhlrich, Karen A. Manning, and Jin-Tang Xue: Effects of activation of the histaminergic tuberomammillary nucleus on visual responses of neurons in the dorsal lateral geniculate nucleus. *J Neuroscience* 22, 1098–1107 (2002)

196. Mark A. Babizhayev: Failure to withstand oxidative stress induced by phospholipid hydroperoxides as a possible cause of the lens opacities in systemic diseases and ageing. *Biochim Biophys Acta* 1315, 87–99 (1996)

197. Robin Holliday and G.A. McFarland: A role for carnosine in cellular maintenance. *Biochemistry (Moscow)* 65, 843-848 (2000)

198. G.A. McFarland and Rjbin Holliday: Retardation of the senescence of cultured human diploid fibroblasts by carnosine. *Exp Cell Res* 212, 167-175 (1994)

199. Robin Holliday and G.A. McFarland: Further evidence for the rejuvenating effects of the dipeptide L-carnosine on cultured human diploid fibroblasts. Exp Gerontol 34, 35-45 (1999)

200. Lan Shao, Qing-huan Li and Zheng Tan: L-carnosine reduces telomere damage and shortening rate in cultured normal fibroblasts. *Biochem Biophys Res Commun* 324, 931-936 (2004)

201. Alexander A. Boldyrev, Steven C. Gallant and Gennady T. Sukhich: Carnosine, the protective, anti-aging peptide. *Biosci Rep* 19, 581-587 (1999)

202. Alexei M. Olovnikov: Hypothesis: lifespan is regulated by chronomere DNA of the hypothalamus. *J Alzheimers Dis* 11, 241-252 (2007)

203. Okihiro Nishi, Kayo Nishi, Tsutomu Fujiwara and Eiichi Shirasawa: Types of collagen synthesised by the lens epithelial cells of human cataracts. *Br J Ophthalmol* 79, 939–943 (1995)

Abbreviations: VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; ESAF:endothelial cell-stimulating
angiogenic growth factor; Ang: angiopoietin; TGF: transforming growth factor; PDGF: platelet derived growth factor; Ach:
acetylcholine; NO: nitric oxide; NP: nitroprusside

Key Words Telomere Shortening, Telomerase, Human Lens Cells, Aging And Cataract, Lifespan, Telomere-Dependent Senescent Phenotype, Biological Marker, Carnosine, N-Acetylcarnosine Ophthalmic Prodrug, Cataract Treatment, Basic Preventive Health Care, After-Cataract Prevention, Review

Send correspondence to: Mark A. Babizhayev, Innovative Vision Products, Inc., Moscow Division, Ivanovskaya 20, Suite 74 Moscow 127434 Russian Federation, Tel/Fax: 7-495-977-2387, Fax: 7-495-977-2387, E-mail: markbabizhayev@mail.ru

http://www.bioscience.org/current/vol15.htm