Medical Hypotheses

Medical Hypotheses (1994) 43, 307–311

A Hypothetical Mechanism for Toxic Cataract Due to Oxidative Damage to the Lens Epithelial Membrane

C. J. BENDER

Department of Molecular Pharmacology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA

Abstract — Lenticular opacities can be induced by numerous external agents that coincide with those that catalyze oxidative damage to lipids. One of the consequences of lipid peroxidation is that the affected membrane is rendered more permeable to protons. A proton leak in the tight epithelium of lens would uncouple the Na^+/K^+-ATPases that regulate the water and ionic content of the bounded tissue. Once regulatory control of the osmotic pressure is lost, the phase state of the cell's soluble proteins would change, leading to refractive changes or, in extreme cases, precipitation. The same does not occur in cornea because the stroma is an extracellular polymer blend rather than solution of soluble polymers. Polymeric phase transitions in the cornea require that divalent cations pass the epithelial membrane, which can occur only through the action of ionophores.

Toxic cataract and oxidative stress

The etiology of lenticular cataracts in the eye may be linked to several factors. One class of acquired opacities not associated with systemic disease are those cases in which the opacity is induced by an external agent, such as radiation or chemical. These agents have been extensively catalogued (1,2) and many of these can be described as oxidizing agents. As a result, the emphasis of proposed mechanisms of cataractogenesis lies with oxidative stress upon the tissues' constituent protein (3).

The vulnerability of proteins to oxidative stress follows from the reactive lability of exposed amino acid residues. For examples, thyils facilitate the exchange of hydrogen among organic radicals (4), and this suggests that exposed sulfur-bearing amino acid residues are susceptible to oxidative insult. Similarly, the proton on the peptide chain adjacent to the carbonyl may be prone to abstraction and therefore may be another site of protein crosslinking (5).

Oxidative damage to labile amino acids may cause crosslinkage or enzymatic inactivation of the lens protein (3). High molecular weight protein aggregates have been reported in lens homogenate obtained from senescence cataract (6), and cataractogenesis has been attributed to crystallin crosslinking and subsequent changes in the refractive index of the fiber cell milieu (3). Similarly, the Na^+/K^+-ATPase of the lens epithelial membrane may be inactivated by oxidizing any of several exposed thiol functionalities (7). Inhibition of this enzyme by oubain and vanadate have been shown to cause lenticular clouding (8), therefore oxidative insult to the ATPase of lens epithelia is another suggested mechanism of cataractogenesis (3,9).

In assessing the mechanism of toxic cataract and oxidative stress, only scant attention has been taken

Date received 16 February 1994
Date accepted 3 May 1994

of the prospect of damage to the epithelial and capsule membrane. Peroxidation of the membrane lipids is catalyzed by the same toxic agents noted for cataractogenesis, and the damage inflicted upon the membrane will initiate the same chain of events that lead to the observed trends described above. The thesis of this paper is that epithelial membrane lipids are an equally suitable target site of oxidative stress, and that oxidative damage to these lipids would alter the epithelial membrane's permeability and disrupt the osmotic balance that ensures the transparency of the lens. This alternative mechanism has merits in that it can account for many of the metabolic imbalances of low molecular weight solutes associated with cataract and acknowledges that the membrane is very likely to be the first ultrastructural feature encountered by the toxic agent.

Membrane damage and cataractogenesis

Membrane permeability changes occur as a result of a series of chain reactions that are collectively known as lipid peroxidation. One of the consequences of oxidative damage to lipid membranes is an increase in the permeability to protons (10). The chemistry of fatty acid oxidation is extensively reviewed (11,12), however the reactions that lead to a lipoidal conduit of protons are depicted in Scheme 1 (12). A hydroxy/epoxy tautomer is produced that can serve as a shuttle of protons in the otherwise hydrophobic region of the membrane.

The primary reaction is hydrogen abstraction from the highly susceptible divinyl group (-CH=CH-CH_2-CH=CH-) that is a common feature of the hydrophobic tails of biological lipids. The hydrogen abstraction is necessarily mediated by an initiator, many of which coincide with toxic agents of cataractogenesis. These include metals prone to single electron transfers, stable free radicals, ultraviolet and ionizing radiation (11,12). Of course, these oxidizing systems are non-specific, and the actual mechanism may entail a combination of both protein and lipid targets. Which of these is the principal target and mediator of cataractogenesis is a question of which entity is the more probable target and whose damage is consistent with the observed etiology.

The potential consequences of a proton leak in the lens epithelium are manifold as a result of functional disruption of the ion motive ATPases that are responsible for osmotic regulation and transport of metabolites. Na^+/K^+-ATPase is the most abundant (13), although Ca^{+2}-ATPase has been identified in rabbit and bovine lens (14). In both cases ion transport is tightly coupled to ATP hydrolysis, and therein lies a link to lipid peroxidation and the increased permeability to protons. A well known example from studies of oxidative phosphorylation, is the action of H^+-ATP synthase uncouplers (e.g. dinitrophenol) that serve as mediators of protons across the mitochondrial membrane (15).

The Ca^{+2}- and Na^+/K^+-ATPases would be directly affected because of their regulation by proton concentration. Other transport proteins would be affected in a secondary sense because they are coupled to ions whose intracellular concentrations are regulated by the ATPase. For example, glucose is the principal metabolic fuel of the lens cells and is typically co-transported with Na^+ in animal cells (16), and similar transport schemes are known for the import of amino acids across cell membranes. Lastly, it should be mentioned that a protein associated with the lens fiber cell membrane, MP26, demonstrates pH-sensitive ion channel properties when it is reconstituted into liposomes (17).

Formation of the opacity follows from either smotic shock or a cytoplasmic imbalance of specific cations, in particular Ca^{+2}. Decoupling the H^+/Ca^{+2}-ATPase (of indirectly via a Na^+ imbalance and the Ca^{+2}/Na^+ antiport), would disrupt the normally low intracellular calcium concentration. A connection between cataract and elevated Ca^{+2} levels has been documented (18,19), although there is no correlation between Ca^{+2} and the formation of high molecular weight aggregates (20).

The fiber cells of the lens contain a very high concentration of water-soluble globular proteins called crystallins. There is no long-range ordering of the dense protein solution, however, short-range order among protein neighbors has been identified as being the determinant factor of the cell's cytoplasmic refractive index (21). The short-range order among the crystallins and the resultant macromolecular network is due to the secondary structure of the protein. The exposed surface amino acid residues of crystallins tend to be conserved and it is believed that the associated forces derived from these lead to the characteristic compact conformational folding (22). The phase behavior of globular proteins in solution depend on the protein concentration and the presence of other small electolytes. The interactive forces between adjacent proteins are determined by the electrical double layer, that is, the surface charge and diffuse counterions (23). There is a dramatic effect upon double layer forces as divalent cations are admixed with monovalent ions in such a system. The surface potential can be lowered by divalent ions at concentrations 100 times lower than the concentration of monovalent ions needed to produce a commensurate effect. It is

also possible for some divalent cations, such as Ca^{+2}, to preferentially bind a surface despite a concentration excess of monovalent ions (23). If the uncoupling of H^+/Ca^{+2}-ATPase in the lens led to excess intracellular C^{+2}, electrostatic changes in the crystallin double layer would disrupt the protein's conformation and interactions.

The link between an uncoupling of ATPase activity from transmembrane proton gradients can be inferred from experimental and clinical studies. A lack of change in the activity of ATPase from senile cataract lens has been used to support a claim that cataract is not due to an enzymatic malfunction, but rather a change in membrane permeability (24,25). More direct evidence of a link between cataract and ATPase uncoupling follows from reports of cataract as a toxic side effect of therapeutic use of dinitrophenol as a weight loss aid (26–28). Dinitrophenol is the archetype of protonophores that uncouple oxidative phosphorylation from ATP synthesis (15).

The results of two experimental studies of dinitrophenol interaction with lens are consistent with a proposal that it is a proton carrier and uncouples the lens ATPase. Oxygen consumption of the lens capsule is altered following the addition of dinitrophenol (29), suggesting that the effect is analogous to the mitochondrial experiment. The consumption of oxygen by mitochondria following ATPase uncoupling by dinitrophenol is due to the uncontrolled reaction of the ATPase (O_2 is a reactant used by the ATPase) when the regulating proton gradient is disrupted. The permeability of the dinitrophenol-treated lens to a tracer dye was also examined (30), and data are reported that the lens is dye permeant when the bathing solution is not buffered, which suggests a connection to leak and pH gradient.

Comparison to cornea

Like the lens, cornea is prone to clouding under certain types of toxic stress, however the agents of toxic corneal opacity do not coincide with those associated with lenticular cataract. Both cornea and lens are bound by regulatory epithelia that are bathed by the aqueous humor. Although many of the electrolytes and small organic molecules in the aqueous humor are secreted and regulated by the ciliary body epithelia, some small organic molecules pass the blood/aqueous barrier via small capillaries. Presumably, toxic agents reach their target sites in the otherwise avascular lens and cornea via the aqueous humor. It follows that the corneal epithelium should be as prone to toxic oxidative stress as the lens, and the question of why cornea is less prone to cataract must be addressed.

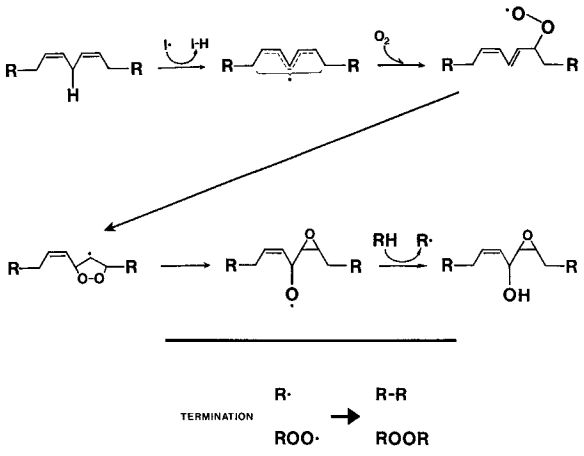

Fig.

Basic ultrastructural differences between lenticular and corneal tissue can account for the different response of the tissues to toxic agents. Although both are membrane bound, the corneal stroma is a polymeric matrix, whereas the lens cortex is cellular. Like the lenticular fiber cells, spatial organization of constituent fibers ensures corneal transparency, however, instead of a dense solution of globular proteins, the corneal stroma is a network of crosslinked extended chain polymers.

The corneal endothelium, which is bathed by the aqueous humor, shows NA^+/K^+-ATPase activity (31), although the net flux of ions across this membrane is limited to bicarbonate (31,32). The principal function of this epithelium is pumping out water that passively diffuses into the stroma. The corneal ATPases can be inhibited by oubain and vanadate (33,34), and uncoupled (35), although not by dinitrophenol. When these transport proteins of the corneal epithelium are poisoned the hypotonicity of the corneal stroma relative to the aqueous humor is no longer maintained, however the cornea swells without hazing. In contrast, treatment of lens with the same ATPase inhibitors leads to clouding of the lens (8).

Although the corneal stroma is structurally different from the polymeric solution of the lens, the disruptive action of surface modifying cations is analogous. Divalent cations are capable of unraveling the otherwise structured trifillar conformation of collagen (36), and the attractive interactions between collagen and proteoglycans can be overcome by low pH and exceeding a critical concentration of electrolyte (37,38). The difference between the propensity of cornea and lens towards toxic opacity is attributable

to the differing functions of their ion transport enzymes.

Clinical studies indicate that corneal opacities are caused by antibiotics and analgesics (39). The few exceptions are peroxide and embedded small metal clusters that cause localized hazing and might be attributable to oxidation via a Fenton-like mechanism. However, aside from this toxic effect of heavy metals and peroxide, there is no coincidence between agents of lenticular and corneal cataract. Antibiotics tend to be classified as carriers of ions or pore formers on the basis of their interaction with membranes (40), and this feature hints at the mechanism of corneal opacity formation. Disabling the pumps of the corneal epithelium does nothing to alter the surface properties of the stromal polymers since there are no transport mechanisms in place to actively load or remove divalent cations from the stroma. The cellular need for such ions is confined to the epithelia. In order to flood the stroma with divalent cations one needs ionophores of the type afforded by antibiotics and analgesics, and it is this mechanism that would lead to changes in the properties of the stromal polymeric network.

Conclusion and suggestion for experimentation

There is no question that cataract can be formed from an osmotic shock to the tissue and/or oxidative insult to the proteins of the lens fiber cell and epithelium. It is likely that non-specific oxidizing agents that leak into the aqueous humor damage both membrane and intracellular proteins, depending on kinetic factors. The kinetic factors that determine the rate and type of oxidative insult are: (1) the relative concentrations of toxic agent, dissolved oxygen, and antioxidants; (2) the relative reactivXve reactivities of protein versus lipid; and (3) the probability of the agent reaching a labile target, which is not independent of (1). These are all questions for further study. The intent of this paper has been to illustrate that the lipids of the epithelial membrane are also a likely target of insult that underlies cataractogenesis.

Cataractogenesis as a result of oxidative insult to the Na^+/K^+-ATPase and the lipids of the epithelial membrane are difficult to distinguish on the basis of experiments that entail the addition of some toxic agent because the resultant chain of events leading to cataract are identical. In order to conclusively prove that a proton leak in the membrane is an inducer of cataract, one needs to generate such a leak without the ambiguities of other potential forms of damage. Prospective experiments can be designed that have such characteristics.

The first of these experimental tests is suggested by known proton pores. An H^+-translocating protein of brown adipose tissue, which is responsible for uncoupling of ATPase synthase in the mitochondria of this tissue (41), can be isolated and reconstituted into liposomal membranes. Reconstitution of the protonophore into the lenticular epithelium would allow one to produce a proton leak without the ambiguity of side reactions of such proton uncouplers as dinitrophenol. If membrane damage is the causal basis of cataractogenesis, then modification of the epithelial membrane should ultimately lead to cataract and the many metabolic imbalances of molecular weight solutes. Furthermore, on the basis of the corneal ultrastructure and reasoning described above, the same reconstitution experiments applied to the corneal epithelium should alter the electrolyte content of the stroma, however it should do nothing more than cause the cornea to swell from the disabling of the fluid pumps.

The second line of proposed experimental testing is lipid-based and could possibly establish ties between diabetic insulin deficiency and cataract. Long chain fatty acids uncouple oxidative phosphorylation by acting as protonophores, in a manner analogous to the classical uncoupler dinitrophenol (42). One metabolic consequence of insulin deficiency is an elevated concentration of fatty acids in many tissues due to their release from adipose tissue. A test for toxic cataractogenesis by oleate and palmitate would determine whether uncoupling of the ion transport proteins of the lens epithelium is analogous to the mitochondrial process. If protonophores do indeed provide the mechanistic basis of cataractogenesis, then an assay for long chain fatty acids in diabetic cataract is warranted as a test for providing a systemic basis of cataract associated with diabetes.

References

1. Bellows JG ed. Cataract and Abnormalities of the Lens. New York: Grune and Stratton; 1975: 217–264.
2. Harding JJ, Crabbe JC. The Lens: Development, Proteins, Metabolism, and Cataract. In: H Davson, ed. The Eye, Vol 1B, 3rd edn. New York: Academic Press, 1984: 370–492.
3. Nugent J, Wheland J, eds. Human Cataract Formation. (CIBA Foundation Symposium 106) London: Pitman, 1984: 48–87.
4. Wang C-H, Cohen SG. Mercaptan catalysis in thermoneutral free radical exchange. J Amer Chem Soc 1957; 79: 1294–1299.
5. Pryor WA, Kneipp KG, Morkved EH, Stanley JP. Kinetic isotope effects in reactions of thiols with radicals formed by radiolysis. Radiat Res 1973; 53: 181–189.
6. Garner MH, Spector A. Selective oxidation of cysteine and methionine in normal and senile cataractous lenses. Proc Natl Acad Sci (USA) 1980; 77: 1274–1277.
7. Sweadner KJ, Goldin SM. Active transport of sodium and potassium ions: mechanism, function, and regulation. New England J Med 1980; 302: 777–783.
8. Korte I, Hockwin O, Cramer-Bartols G, Schmack W. Einwirkung von Oubain auf den Stoffwechsel von Rinder- und

Kalberlinsen. Arch Klin Ophthal 1968; 175: 242–245.
9. Garner WH, Garner MH, Spector A. H_2O_2-induced uncoupling of bovine lens Na^+,K^+-ATPase. Proc Natl Acad Sci (USA) 1983; 80: 2044–2048.
10. Vladmirov Yu A, Olenev VI, Suslova TB, Cheremisina ZP. Lipid peroxidation in mitochondrial membranes. Adv Lipid Res 1980; 17: 173–249.
11. Fraenkel EN, Lipid oxidation: mechanisms, products, and biological significance. J Am Oil Chem Soc 1984; 61: 1908–1917.
12. Wu G-S, Stein RA, Mead JF. Autoxidation of fatty acid monolayers adsorbed on silica gel II. Rates and products. Lipids 1977; 12: 971–978.
13. Bonting SL. Na-K activated adenosinetriphosphatase and active cation transport in the lens. Invest Ophthalmol 1965; 4: 723–738.
14. Borchman D, Delamore NA, Paterson CA. Ca-ATPase activity in rabbit and bovine lens. Invest Ophthalmol Vis Sci 1988; 29: 982–987.
15. Mitchell P. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol Rev 1966; 41: 445–502.
16. Wright JK, Seckler R, Overath P. Molecular aspects of sugar:ion cotransport. Ann Rev Biochem 1986; 55: 225–248.
17 Gooden MM, Takemoto LJ, Rintoul DA. Reconstitution of MIP26 from single human lenses into artificial membranes I. Difference in pH sensitivity of cataractous vs normal lens fiber protein. Curr Eye Res 1985; 4: 1107–1115.
18. Hightower KR, Reddy VN. Calcium content and distribution in human cataract. Exp Eye Res 1982; 34: 413–421.
19. Hightower KR. Cytotoxic effect of internal calcium on lens physiology: a review. Curr Eye Res 1985; 4: 453–459.
20. Bushell AR, Duncan G. The distribution of soluble, insoluble, and high molecular weight fractions of senile and normal cataractous human lenses as a function of internal calcium. Exp Eye Res 1978; 26: 223–226.
21. Delaye M, Tardieu A. Short-range order of crystallin protein accounts for eye lens transparency Nature (London) 1983; 302: 415–417
22. Slingsby C, Simpson A, Ferszt A, Bateman OA, Nalini V. Molecular Interactions in the eye lens. Biochem Soc Trans 1991; 19: 835–857.
23. Israelachvili JN. Intermolecular and Surface Forces. New York: Academic Press, 1985; 161–228.
24. Pasino M, Mariani G. Cation pump activity and membrane permeability in human senile cataractous lenses. Exp Eye Res 1982; 34: 887–893.
25. Gandolfi SA, Tomba MC, Mariani G. 86-Rb efflux in normal and cataractous human lenses. Curr Eye Res 1985; 4: 753–758.
26. Allen TD, Benson VM. Late development of cataract following use of dinitrophenol about one year before. J Amer Med Assoc 1935; 105: 795.
27. Boardman WW. Rapidly developing cataract after dinitrophenol. J Amer Med Assoc 1935; 105: 108.
28. Horner WD. Cataract following dinitrophenol treatment for obesity. Arch Ophthalmol 1936; 16: 447–457.
29. Field J, Tainter EG, Martin AW, Belding HS. Studies on the oxygen consumption of the rabbit lens and the effect of 2,4-dinitrophenol thereon. Amer J Ophthalmol 1937; 20: 779–794.
30. Borley WE, Tainter EF. Effects of dinitrophenol on the permeability of the capsule of the lens. Arch Opthalmol 1937; 18: 908–911.
31. Fischbarg J, Lim JJ. Fluid and electrolyte transports across the corneal endothelium. In: Zadunaisky JA, Davson H, eds. Current Topics in Eye Research, Vol 4. Orlando: Academic Press, 1984: 201–223.
32. Hodson S, Guggenheim J, Kaila D, Wigham C. Anion pumps in ocular tissues. Biochem Soc Trans 1991; 19: 849–852.
33. Green K. Stromal cation binding after inhibition of epithelial transport in cornea. Am J Physiol 1970; 218: 1642–1649.
34. Candia OA, Podos SM. Inhibition of active transport of chloride and sodium by vanadate in the cornea. Invest Ophthalmol Vis Sci 1981; 20: 733–737.
35. Dikstein S, Maurice DM. The metabolic basis to the fluid pump in the cornea. J Physiol 1972; 221: 29–41.
36. von Hippel PH, Wong K-Y. The collagen–gelatin phase transition I. Further studies of the effect of solvent environment and polypeptide chain composition. Biochemistry 1963; 2: 1387–1398.
37. Scott JE, Dorling J. Differential staining of acid glycosaminoglycans (mucopolysaccharides) by Alcian blue in salt solutions. Histochimie 1965; 5: 221–233.
38. Schodt, KP, Gelman RA, Blackwell JA. The effect of salt concentration and pH on the interaction between glycoaminoglycans and cationic polypeptides. Biopolymers 1976; 15: 1965–1977.
39. Grayson M. Diseases of the Cornea. 2nd edn. St Louis: C.V. Mosby, 1983; 573–598.
40. McLaughlin S, Eisenberg M. Antibiotics and membrane biology. Ann Rev Biophys Bioeng 1975; 4: 335–366.
41. Lin CS, Klingenberg M. Characteristics of the isolated purine nucleotide binding protein from brown fat mitochondria. Biochemistry 1982; 21: 2950–2956.
42. Schöfeld P, Schild L, Kunz, W. Long-chain fatty acids act as protonophoric uncouplers of oxidative phosphorylation in rat liver mitochondria. Biochim Biophys Acta 1989; 977: 266–272.