European Journal of Ophthalmology / Vol. 13 / Suppl. 3, 2003 / pp. S5-S10

Introduction to apoptosis in ophthalmology

G. CARELLA

Department of Ophthalmology and Visual Sciences, University Hospital San Raffaele, National Study Group on Apoptosis in Ophthalmology, Milano - Italy

ABSTRACT. *Apoptosis represents a mode of cellular death genetically programmed to maintain homeostasis of tissues. In specific pathologic circumstances, the death program may be activated by various environmental factors such as exposure to toxic substances or bacteria or deprivation of nutrients. From this point of view, apoptosis is considered the final event in several pathologies. In ophthalmology, experimental evidence has confirmed that apoptosis is a type of cellular death involved in various pathologic processes including glaucoma, retinitis pigmentosa, ischemic retinopathy, corneal reparative processes, cataract, and retinoblastoma. The aim of this article is to review the most recent results published in this field and to describe some of the molecular mechanisms responsible for the activation of the apoptotic program in some important ocular disorders. The understanding of such mechanisms could outline new therapeutic strategies for the prevention of cellular death in ophthalmology.* Eur J Ophthalmol 2003; 13 (Suppl. 3): S5-S10

KEY WORDS. *Apoptosis, Eye diseases, Cell death*

Accepted: December 18, 2002

Apoptosis as a type of cellular extinction was first described by Kerr et al in 1972 (1). In contrast to necrosis, apoptotic death is an active process that requires energy and in which the cell participates actively in self-elimination by activating the death program (2, 3). This process is initiated by expression of specific genes, the protein products of which activate a cascade of molecular events that in turn result in elimination of the cell. Typically, apoptosis affects singular cells in the tissue, which disappear in few dozens per minute without inducing inflammatory or immune responses. Following cell detachment from its matrix, its cytoplasm undergoes a rapid reduction in size (cell shrinkage) followed by blebbing and breaking up in variously sized fragments (apoptotic bodies). These quickly disappear, mostly ingested by sister cells or by circulating nearby phagocytes. In contrast to necrosis, the earliest degenerative changes affect the nucleus: adhesion of semilunar plaques of condensed chromatin onto nuclear membrane is accompanied by internucleosomal cleavage of DNA in distinct fragments. Paradoxically, cytoplasm and organelles appear intact even inside apoptotic bodies. Figures 1 through 6 illustrate the peculiar morphology of apoptotic cells belonging to a corneal human retinal pigmented cell line (RPE), examined by scansion electron microscopy (SEM) or transmission electron microscopy (TEM). Figures 7 and 8 show the microscopic appearance of RPE cells stained by TdT-mediated dUTP nick end-labeling (TUNEL) technique, which allows identification of apoptotic cells, which contain dark brown spots of fragmented DNA inside green cytoplasm, in contrast to viable cells, which appear uniformly green or unstained.

Apoptosis may commence spontaneously at a determined moment of cellular life, to allow maintenance of homeostasis of the tissues, or may be triggered in advance by pathologic stimuli such as exposure to

© Wichtig Editore, 2003 1120-6721/S5-06$03.00/0

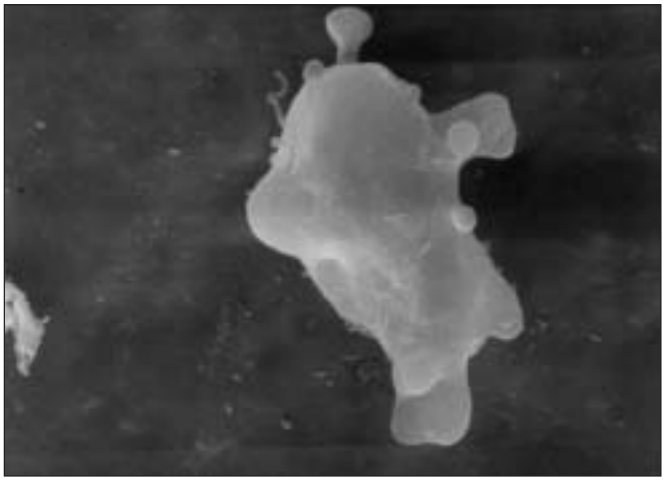

Fig. 1 - *Scanning electron microscopy analysis of a rat-1 fibroblast treated with antimycin A 200 µM. The image shows the cellular blebbing typical of programmed cell death. Magnification x2000.*

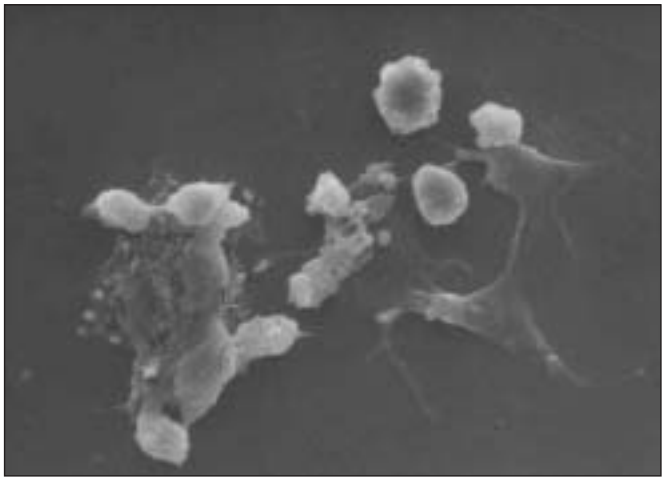

Fig. 2 - *Scanning electron microscopy analysis of rat-1 fibroblasts treated with antimycin A 200 µM. Several features typical of apoptosis are visible: cellular shrinkage (on the left) and apoptotic bodies (on the right). Magnification x2000.*

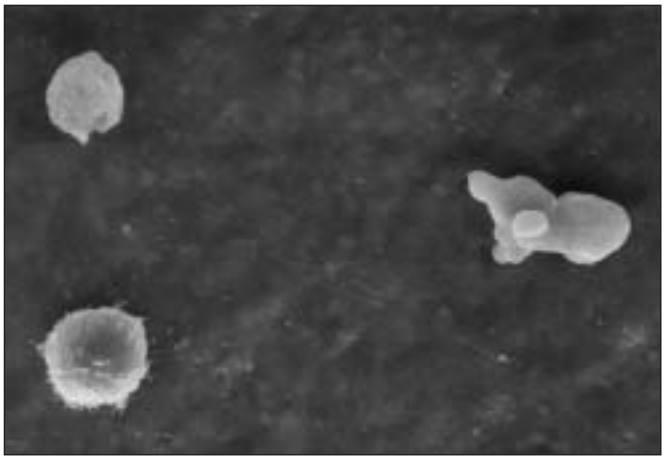

Fig. 3 - *Scanning electron microscopy analysis of rat-1 fibroblasts treated with antimycin A 200 µM. The image shows two healthy cells (on the left) and one apoptotic cell (on the right). Magnification x1000.*

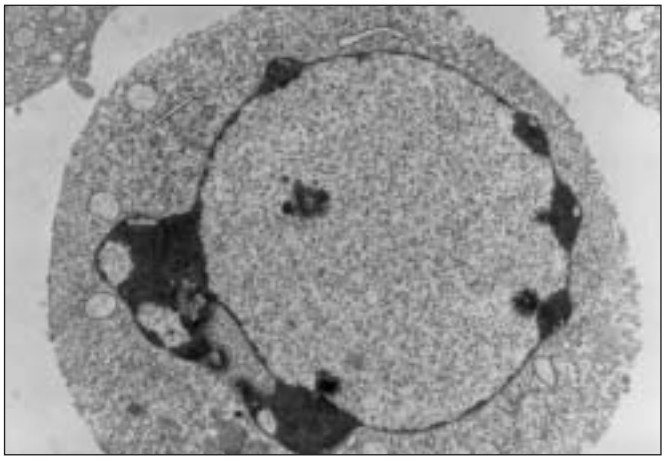

Fig. 4 - *Transmission electron microscopy analysis of rat-1 fibroblast treated with antimycin A 300 µM. The nucleus shows chromatin condensation and margination typical of programmed cell death. Magnification x5000.*

toxic substances or micro-organisms or deprivation of cellular nutritional factors (2, 3). Therefore, apoptotic death represents a final event of numerous pathologies, not only in ophthalmology (4-6) but also in practically all biomedical disciplines (3). Moreover, apoptosis, being a process consisting of a succession of molecular events, could be blocked or triggered by genetic or pharmacologic manipulation (2, 3). The discovery of this possibility has provoked considerable clinical interest, predicting new potentials for therapeutic intervention. This review aims to outline the most recent developments in the study of the role of apoptosis in ocular pathology, and to underline new clinical perspectives that these new discoveries have opened.

Wilson and others have demonstrated that various types of epithelial insults activate the process of cel-

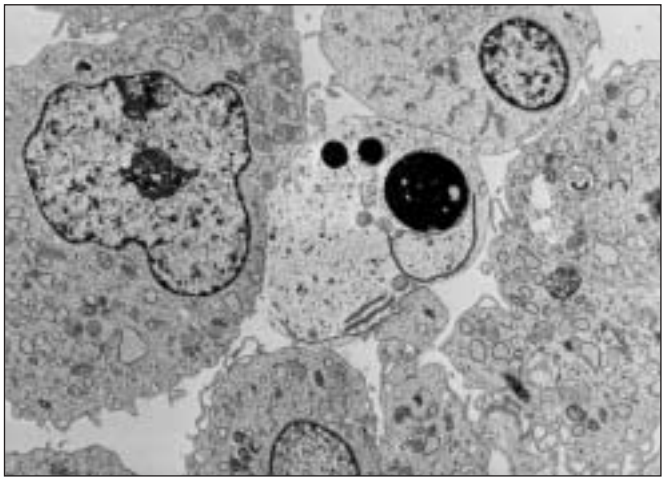

Fig. 5 - *Transmission electron microscopy analysis of rat-1 fibroblasts treated with 200 µM antimycin A. Several features typical of apoptosis are visible. Magnification x3000.*

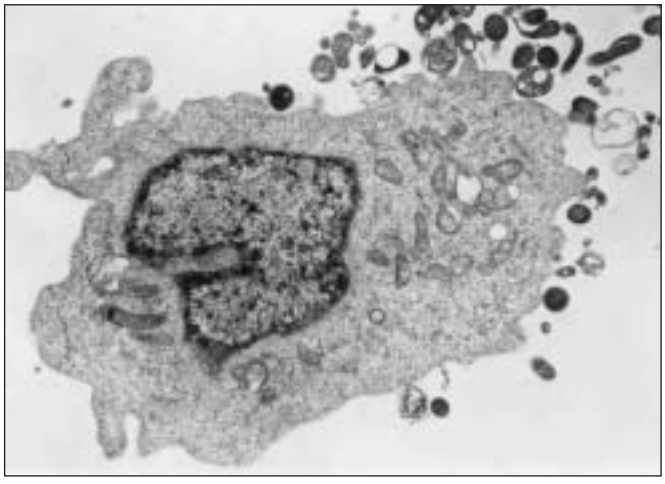

Fig. 6 - *Typical aspect of an apoptotic cell: nuclear chromatin condensation and margination, apoptotic bodies formation in a rat-1 fibroblast exposed to 200 µM antimycin A. Magnification x3000. (Figures 1-6, courtesy of Sandra Zecchi-Orlandini and Lucia Formigli.)*

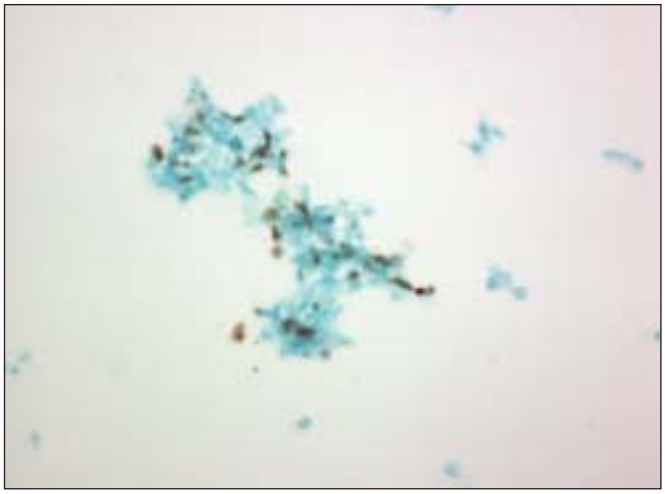

Fig. 7 - *TdT-mediated dUTP nick end-labeling analysis of cultured retinal pigment epithelial cells treated with antimycin A 150 µM. Apoptotic cells are brown, living cells are green. Magnification x40.*

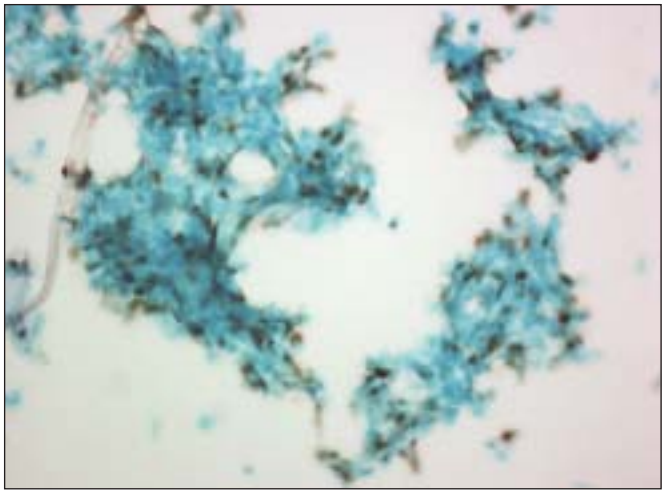

Fig. 8 - *TdT-mediated dUTP nick end-labeling analysis of retinal pigment epithelial cells treated with 150 µM antimycin A. Magnification x40. (Figures 7-8, courtesy of Laura Papucci, Ewa Witort, and Sergio Capaccioli.)*

lular death by apoptosis of stromal keratocytes (7-9). The apoptosis was observed as a result of lesions following treatment with excimer laser, microkeratectomy by laser *in situ* keratomileusis, or other manipulations required during corneal surgical procedures. Moreover, death by apoptosis of these cells was also documented in the case of epithelial infection by herpes virus. However, the physiopathologic significance of apoptotic response is not clear. In the case of viral infection, it was hypothesized that induction of the death of keratocytes may represent a defense system against propagation of the virus in the deeper layers of the tissues. The interpretation of apoptotic events following surgical manipulation in the epithelium is more complicated. Progressive repopulation of the stroma by new keratocytes deriving from

replication of the residual stromal cells was noted 3 days after the triggering of the apoptotic process (7-9). These new cells, similar to the fibroblasts, show morphologic characteristics typical of activated cells and, in addition, develop endoplasmic reticulum, and their processes of cellular synthesis are enhanced. It was hypothesized that apoptosis of the keratocytes represents the primary event of the corneal reparative process and, in the case of refractive surgical procedures, it is responsible for the haze and the phenomenon of regression of the functional result.

The possibility of intervention in the initial stage offers the most efficient way to control the reparative response. Therefore, numerous studies were directed at determining the modality of the initiation of apoptosis in order to prevent the death of keratocytes, rendering these cells insensitive (resistant) to the traumatic insult (10, 11). Recently, Brancato et al (11) have reported that pretreatment of keratocytes with coenzyme Q10 (ubiquinone) prevents apoptotic death following excimer laser irradiation *in vitro*. This molecule can reduce production of free radicals by the cell and possibly can interfere with the opening of the megapores of the mitochondrial membrane by modulation of the permeability transition pore (PTP), both events related to activation of the apoptotic program. These results, if confirmed by experimental models *in vivo*, could open the way to new strategies for prevention of postsurgical complications in refractive surgery.

The results of studies performed in experimental animal models (12) and in human studies (13) have shown that glaucoma is associated with activation of the apoptotic program of cellular death of retinal ganglion cells and, as a result, leads to progressive impoverishment of residual cells.

These observations opened the way for studies aiming to individuate mechanisms responsible for priming apoptosis in glaucoma and to design improved therapeutic procedures (5, 6, 14, 15).

It was hypothesized that increased intraocular pressure, causing reduction of axonal flow, may limit intake of neurotrophic factors directed from lateral geniculate body to the retina and trigger apoptotic process. The role of glutamate involvement in the excytotoxic pathway has also been identified in parallel. This hypothesis is based on the well-documented observation that non-excessive but chronic increase of glutamate concentration inside the vitreous body is selectively toxic for ganglion cells of the retina. Also, in patients with glaucoma and in animals in which this disease was experimentally induced, the levels of glutamate in the vitreous body are markedly elevated compared to controls (16).

The neurotoxicity of glutamate, demonstrated in other experimental models, can be attributed to activation of type NMDA and non-NMDA receptors (5, 6, 14, 15). These, in turn, promote calcium entry inside the cell, increased levels of which determine activation of the series of molecular cascades leading to cellular death. Calcium is the important secondary messenger that can stimulate release of neurotransmitters, induce gene expression, and activate increased number of enzymes that are Ca^{2+}-dependent, such as nitric oxide synthase, proteases, protein kinases, phospholipases, and endonucleases.

Moreover, activation of the NMDA-type glutamate receptors and the consequent increase of intracellular concentrations of calcium, determine production of the free radicals, highly reactive molecules involved in activation of apoptosis (5, 6, 14, 15).

Based on these results, new projects are under study to treat the multiform clinical aspects of glaucoma. The aim is to protect the retinal ganglion cells by interfering with the apoptotic process on its various compartmental levels.

Recently, based on experimental models, hereditary degeneration of the retinal membrane has been reported to result from apoptotic death of photo receptors (17). These data have aroused noteworthy interest. Retinitis pigmentosa can originate from a number of genetic mutations; consequently, human genetic therapy has to overcome extreme genotypic complexity to eliminate the specific mutation responsible for this pathology. In contrast, pharmacologic or genetic therapy that interferes with a common event such as apoptosis may represent a more feasible type of treatment (18). The degenerative modifications observed in the hereditary disorders of the retina are extremely slow developing; hence the diagnosis may take decades to establish. Therefore the theory prevails that even a modest reduction in the rate of cell apoptosis may preserve vision for long periods (18).

Ischemia is the final result of numerous pathologic processes in the optic nerve and the retina, including ischemic opticopathy, diabetic and hypertensive retinopathy, and occlusions of veins and arteries. For

this reason, understanding the molecular mechanisms involved in retinal damage resulting from oxygen deprivation attract interest worldwide (5, 6, 19). The rat model of reperfusion injury established that cellular death of retinal cells is related to apoptosis (20). Levine and Louhab have reported the occurrence of cellular death by apoptosis in the ganglion cell layer of the retina in a 70-year-old patient that arose 20 days after an episode of anterior ischemic optic neuropathy (21).

The mechanism responsible for the apoptotic cellular death in ischemic events of the retina cannot be completely explained. The excytotoxic pathway involving glutamate has been identified in pathologic cellular death of ischemic lesions in the central nervous system (22). Glutamate administered subcutaneously to newborn mice resulted in degeneration of the internal layer of the retina, with major damage occurring in the ganglion cell layer (23). In the experimental mouse model of ischemia, it was noted that during the phase of hypoxia the glutamate concentrations measured in the intracellular space by microdialysis were twice as high as normal levels. However, the concentrations of glutamate in the reperfusion phase (in which the anatomical damage is most evident) were approximately six to seven times greater. The new hypothesis emerged that, analogically to the ischemic processes occurring in central nervous system, hypoxia in the retina induces massive release of glutamate and this is the starting point for injury to the retina. Several studies have been undertaken to follow-up and penetrate the molecular mechanisms that form the base of these alterations.

Glutamate interacts with receptors of the NMDA type and this determines opening of the calcium ion channels. This has been confirmed by use of MK801. This antagonist, noncompetitive with the NMDA receptors, protected ganglial cells against cytotoxicity of glutamate even when administered 1 to 4 hours after the insult (24).

Increase of the calcium concentrations resulting from activation of NMDA receptors can in turn stimulate release of the neurotransmitters, induce expression of genes, and activate elevated numbers of calcium-dependent enzymes such as proteases, protein kinases, phospholipases, nitric oxide synthase, and endonucleases. These events are considered to be involved in the activation of the cellular death program (19). Thus it is acknowledged that molecules able to prevent increase of the intracellular calcium concentration, including calcium antagonists, may reduce glutamate-associated neurotoxicity to retinal ganglion cells *in vitro* and *in vivo* (19, 25).

Activation of the type NMDA glutamate receptors and consequent increase in intracellular calcium concentrations is associated with the formation of free radicals (26-28). It is well established that in the reperfusion phase that follows ischemic insult, a massive quantity of reactive oxygen species (ROS) is produced. ROS are involved in the process of neurodegeneration via peroxidation of the fatty acids and nucleic acids, and also from the breakdown of the protein bonds. These data allowed clarification of the precise role of the antioxidants, scavengers of the free radicals, in the treatment of ischemic diseases of the retina, pathologic occurrences which are grow exponentially worldwide.

Cellular death by apoptosis has been well documented for epithelial cells of the lens in patients with senile opacity and in animals exposed to ultraviolet radiation, leading to the hypothesis that it can also have a role in mechanisms responsible for the onset of cataract (29). Apoptosis is also responsible for cellular death during detachment of the retina (30), in posterior uveitis (31), in retinoblastoma (4), and in rat experimental model of amblyopia (32).

In ophthalmology, apoptosis represents the final event involved in many pathologic disorders. Improved understanding of the molecular mechanisms responsible for activation of the apoptotic program and the cascade of events leading to the cellular death in ophthalmology is necessary. New therapeutic strategies need to be outlined for disorders that strike on the morphologic and functional level.

Reprint requests to:
Prof. Giuseppe Carella
Viale Pubblico Passeggio, 16
29100 Piacenza, Italy
g.carella@agonet.it

REFERENCES

1. Kerr JFR, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972; 26: 239-57.
2. Bosman FT, Visser BC, van Oeveren J. Apoptosis: pathophysiology of programmed cell death. Pathol Res Pract 1996; 192: 676-83.
3. Samali A, Gorman AM, Cotter TG. Apoptosis - the story so far. Experientia 1996; 52: 933-41.
4. Nickells RW, Zack DJ. Apoptosis in ocular disease: a molecular overview. Ophthalmic Genet 1996; 17: 145-65.
5. Capaccioli S, Nucci C, Schiavone N, Quattrone A, Carella G. L'apoptosi nelle angioneurotticopatie. In: Bisantis C, Carella G, eds. I sistemi vascolari del nervo ottico e dell'area periottica. Relazione ufficiale al 78° Congresso della Società Oftalmologica Italiana. Roma: I.N.C., 1998: 81-130.
6. Capaccioli S, Nucci C, Quattrone A, Carella E.G. L'apoptosi in oftalmologia. Monografie della Società Oftalmologica Italiana. Roma: I.N.C., 1998; 2: 30-76.
7. Wilson SE. Molecular cell biology for the refractive corneal surgeon: programmed cell death and wound healing. J Refract Surg 1997; 13: 171-75.
8. Wilson SE, Kim WJ. Keratocyte apoptosis: implications on corneal wound healing, tissue organization, and disease. Invest Ophthalmol Vis Sci 1998; 39: 220-6.
9. Helena MC, Bearveldt F, Kim WJ, Wilson SE. Keratocyte apoptosis after corneal surgery. Invest Ophthalmol Vis Sci 1998; 39: 276-83.
10. Kuo IC, Seitz B, LaBree L, McDonnell PJ. Can zinc prevent apoptosis of anterior keratocytes after superficial keratectomy? Cornea 1997; 16: 550-5.
11. Brancato R, Schiavone N, Siano S, et al. Prevention of corneal keratocyte apoptosis after argon fluoride excimer laser irradiation with the free radical scavenger ubiquinone Q10. Eur J Ophthalmol 2000; 10: 32-8.
12. Quigley HA, Nickells RW, Kerrigan LA, Pease ME, Thibault DJ, Zack DJ. Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. Invest Ophthalmol Vis Sci 1995; 36: 774-86.
13. Kerrigan LA, Zack DJ, Qugley HA, Smith SC, Pease ME. TUNEL-positive ganglion cells in human primary open-angle glaucoma. Arch Ophthalmol 1997; 115: 1031-5.
14. Nickells RW. Retinal ganglion cell death in glaucoma: the how, the why, and the maybe. J Glaucoma 1996; 5: 345-56.
15. Caprioli J. Neuroprotection of the optic nerve in glaucoma. Acta Ophthalmol 1997; 75: 364-7.
16. Dreyer EB, Zurakowsky D, Schumer RA, Podos SM, Lipton SA. Elevated glutamate in the vitreous of humans and monkeys with glaucoma. Arch Ophthalmol 1996; 114: 299-305.
17. Portera-Cailliau C, Sung CH, Nathans J, Adler R. Apoptosis photoreceptor cell death in mouse models of retinitis pigmentosa. Proc Natl Acad Sci U S A 1994; 91: 974-8.
18. Adler R. Mechanisms of photoreceptor death in retinal degenerations. Arch Ophthalmol 1996; 114: 79-83.
19. Sucher NJ, Lipton SA, Dreyer EB. Molecular basis of glutamate toxicity in retinal ganglion cells. Vision Res 1997; 37: 3483-93.
20. Kuroiwa S, Katai N, Shibuki H, et al. Expression of cell cycle-related genes in dying cells in retinal ischemic injury. Invest Ophthalmol Vis Sci 1998; 39: 610-7.
21. Levin LA, Louhab A. Apoptosis of retinal ganglion cells in anterior ischemic optic neuropathy. Arch Ophthalmol 1996; 114: 488-91.
22. Lipton SA, Rosenberg PA. Excitatory aminoacids as a final common pathway for neurologic disorder. N Engl J Med 1994; 330: 613-22.
23. Lucas DR, Newhouse JP. The toxic effect of sodium L-glutamate on the inner layers of the retina. Arch Ophthalmol 1957; 58: 193-201.
24. Levy DI, Lipton SA. Comparison of delayed administration of competitive and uncompetitive antagonists in preventing NMDA receptor-mediated neuronal death. Neurology 1990; 40: 852-5.
25. Sucher NJ, Lei SZ, Lipton SA. Calcium channel antagonists attenuate NMDA receptor-mediated neurotoxicity of retinal ganglion cells in culture. Brain Res 1991; 559: 179-81.
26. Takahasi K, Lam TT, Edward DP, Buchi ER, Tso OM. Protective effects of flunarizine on ischemic injury in the rat retina. Arch Ophthalmol 1992; 110: 862-70.
27. Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Science 1993; 262: 689-95.
28. Pellegrini-Giampiero D, Cherici G, Alesiani M, Moroni F. Excitatory amino acid release and free radical formation may cooperate in the genesis of ischemia-induced neuronal damage. J Neurosci 1990; 10: 1035-41.
29. Li WC, Kuszak JR, Dunn K, et al. Lens epithelial apoptosis appears to be a common cellular basis for non-congenital cataract development in humans and animals. J Cell Biol 1995; 130: 169-81.
30. Berglin L, Algvere PV, Seregard S. Photoreceptor decay over time and apoptosis in experimental retinal detachment. Greafes Arch Clin Exp Ophthalmol 1997; 235: 306-12.
31. Chan CC, Matteson DM, Li Q, Whitcup SM, Nussenblatt RB. Apoptosis in patients with posterior uveitis. Arch Ophthalmol 1997; 115: 1559-67.
32. Nucci C, Piccirilli S, Rodinò P, et al. Apoptosis in the dorsal lateral geniculate nucleus after monocular deprivation involves glutamate signalling, NO production and PARP activation. Biochem Biophys Res Commun 2000; 278: 360-7.