APPROACHES TO MODULATE APOPTOSIS

1. Pharmacological approaches: Involves the use of pharmacological agents to promote or inhibit apoptosis.

2. Genetic approaches: Involves the manipulation of genes involved in the apoptotic pathway.

3. Environmental approaches: Involves the manipulation of environmental factors that influence apoptosis.

4. Biological approaches: Involves the use of biological agents such as peptides, proteins, or RNA to modulate apoptosis.

5. Physical approaches: Involves the use of physical agents such as radiation or temperature to modulate apoptosis.

MODULATION OF APOPTOSIS

The modulation of apoptosis can have significant implications in various fields, including medicine and biology. It is important to understand the mechanisms and factors that control apoptosis to develop effective strategies for its modulation. This can lead to the development of new therapeutic interventions for various diseases and conditions where apoptosis plays a crucial role. The field of apoptosis research is rapidly expanding, and ongoing research is expected to contribute to the development of new and improved therapeutic strategies.
A number of pro- and anti-apoptotic factors are listed in Table I. The best characterized process for induction of apoptosis is through the Fas–Fas ligand system (Fig. 2). The Fas ligand is a soluble cytokine released by the cells which on binding to its receptors recruits other modulators (such as procaspase 8) into a death complex called apoptosome. Caspases are multiple cysteine-containing, aspartate-specific proteases which are produced as pro-enzymes that are activated by proteolytic cleavage. Caspase 8 and caspase 9 are called apical or initiator caspases which activate two prominent cascades leading to apoptosis. The formation of the Fas–Fas ligand complex activates caspase 8 and thus activates one of these pathways. The other pathway is activated by release of cytochrome C (from mitochondria) which activates caspase 9. Anti-apoptotic (bcl-2 and bcl-x) and proapoptotic (Bax) proteins are thought to control apoptosis by modulating cytochrome C release from the mitochondria. Caspase 8 and caspase 9 (apical caspases) further activate effector procaspases 3, 6 and 7. These downstream caspases have terminal effectors that lead to ultrastructural changes in the cell such as chromatin condensation, morphological changes of cell membrane and nuclear membrane breakage, which are characteristic of apoptosis. Many stimuli, inducers and effectors in this process are still unidentified. Studies have established that apoptotic pathways are not only stimulus-specific but also cell-specific.

Many approaches to control apoptosis are being developed from the understanding of its molecular mechanisms. These include:

1. pharmacological inhibition or overexpression of the involved genes;
2. gene therapy by viral transduction of apoptotic inhibitors;
3. inhibition of proteases;
4. inhibition of intracellular rise in calcium concentration; and
5. inhibition by scavenging mediators of apoptosis (e.g. reactive oxygen species).

**APOPTOSIS AND EMBRYOLOGY OF THE EYE**

Apoptotic cells have been identified in various layers of the developing mouse and human retina, and apoptotic bodies have been observed within phagosomes of neighbouring retinal cells. Abnormalities in the timing of apoptosis could thus be responsible for retinal dysplasias. Gene therapy or intrauterine pharmacological manipulation might help to avert such disastrous developments.

Apoptosis is required for the separation of the lens from the future corneal epithelium. It is also involved in removal of the tunica vasculosa lentis and the anterior pupillary membrane. A high frequency of persistent hyperplastic primary vitreous and cataracts has been found in p53-deficient mice. The number of apoptotic cells in the hyaloid artery, vasa hyaloidea propria and tunica vasculosa lentis was also reduced in these mice. Another study provides evidence of apoptosis in regression of the hyaloid artery in the rat eye. Thus, regression of these structures in the developing eye requires a p53-dependent apoptotic mechanism.

Apoptotic cells have been found in the lower nasal side of the developing optic cup of mice before the formation of the embryonic fissure whereas no such cells have been found in the corresponding area after closure of the embryonic fissure. Hence, apoptosis is also essential for the formation and persistence of the embryonic fissure.

In all these processes apoptosis is beneficial to the organism and its disruption would lead to congenital anomalies.

**APOPTOSIS AND THE CONJUNCTIVA**

At present, little is known about the aetiology of pterygium. Earlier, it was believed to be a degenerative condition but recent evidence suggests that it is a growth disorder. Apoptotic cells have been found to be restricted to a single basal layer in the epithelial layer of pterygia while in the normal conjunctiva they are seen throughout the epithelial layer. It is difficult to discern from this finding whether pterygia result from an aberration of the apoptotic pattern or another factor which leads to the disruption of apoptosis. However, if the final common pathway in the development of pterygia is a decrease in apoptosis, then the development of apoptotic stimulators might allow the condition to be treated medically.

**APOPTOSIS AND THE CORNEA**

Some amount of apoptosis is normally found in the healthy cornea. This is limited to the superficial epithelium with very little in the basal epithelium and none in the keratoctyes and endothelium. This is thought to be important for normal epithelial turnover. Apoptosis has also been identified in certain pathological conditions.
states in which keratocytes, endothelial cells and mononuclear cells invade the cornea. Keratocyte apoptosis is the first event in wound healing after epithelial debridement. It is thought to be initiated immediately by release of mediators such as interleukin-1 and Fas ligand from the damaged epithelium. Optimum apoptosis is present by about 4 hours after debridement and can be detected by TUNEL assay. It is hypothesized that this cell death induces hyperproliferative wound healing which might cause a corneal haze. Attempts have been made to inhibit this process using pharmacological agents such as zinc, caspase inhibitors and flunarizine. Modulation of this event may drastically alter wound healing.

Keratitis due to herpes simplex virus 1 induces apoptosis in the underlying keratocytes. Ishizaki et al. hypothesize that this is a physiological response to prevent further and deeper viral extension. Apoptosis has also been seen in corneal burns due to alkali. Fibroblastic cells in injured corneas were found to have the characteristics of myofibroblasts. Some of these cells were found to be TUNEL positive, suggesting that they were apoptotic. These myofibroblasts may contribute to wound contraction and scarring. Apoptosis may have a role in regulating the number of these cells and thus wound healing. Although its exact role is not known, staphylococcal alpha toxin has also been shown to induce apoptosis in the corneal epithelium.

Apoptosis may have a role in corneal allograft rejection as well as graft survival. Apoptotic keratocytes have been found in rejected corneal buttons. It has been postulated that the accompanying stromal inflammation consisting chiefly of T-lymphocytes and macrophages may be responsible for induction of apoptosis. Whether apoptosis induction or inhibition would enhance graft survival remains to be determined. Selective induction of apoptosis in T-lymphocytes vis-à-vis keratocytes might also tilt the balance in favour of graft survival.

Corneal endothelial cell apoptosis might be a determinant of the suitability of the donor button for transplantation. The number and distribution of apoptotic endothelial cells correlates well with the presence of corneal folds and the quality of the donor endothelium. It has been suggested that a mechanical induction due to stromal swelling might be responsible for apoptosis and its prevention might improve the quality of donor material.

Apoptosis might have a role in the pathogenesis of keratoconus. Fibroblasts from patients with keratoconus show 4-fold more interleukin-1 binding sites than normal fibroblasts. Interleukin-1 has been shown to have a major role in the induction of apoptosis. A study found that chronic epithelial injury leads to stromal thinning probably due to induction of apoptosis, suggesting a relation between excessive rubbing and development of keratoconus. Chronically wounded corneas become thin, with epithelial hyperplasia and a subepithelial acellular zone. Apoptosis has been detected in this acellular zone. Thus, apoptotic inhibitors might alter the course of this disease.

Another group of investigators have found endothelial cells to be TUNEL positive (apoptotic) in Fuchs' dystrophy but could not identify apoptosis using the transmission electron microscope. We do not know whether the primary pathology in Fuchs' dystrophy is in the Descemet's membrane or in the endothelial cells. However, if apoptosis in the latter turns out to be the primary event then its inhibitors would prove useful in the therapy of Fuchs' dystrophy.

Apoptosis has been studied in shed human corneal cells after contact lens use. It has been found that the number of shed cells increases and their size decreases on sequential removal of soft contact lenses. These cells stain positive by TUNEL and annexin V, but are morphologically different from classic apoptotic cells. It has been suggested that these could be cells in the last stages of differentiation which were shed due to friction-induced apoptosis. This fact could be used to assess trauma induced by contact lenses.

Amniotic membrane, when used as a graft, reduces inflammation. The amniotic membrane excludes polymorphonuclear cells (PMN). Many TUNEL-positive PMNs have been found adherent to the amniotic membrane matrix. As facilitation of PMN apoptosis is known to suppress inflammation, induction of apoptosis in PMNs by the amniotic membrane could probably explain its property of reducing inflammation.

Wilson and Hong have tried to explain the development and maintenance of Bowman's layer based on cytokine-mediated interactions between epithelial cells and keratocytes. As keratocytes move closer to the epithelium they are knocked off by apoptotic stimuli. This helps in maintaining an acellular layer below the epithelium. The same workers also hypothesize that the Bowman's layer may be dispensable and have no critical function.

The endothelial cell density of humans decreases with age. Oxidant species in the aqueous humour have been suggested to be one of the reasons for this cell loss. These oxygen species are generated from metabolites of normal cells, inflammatory cells and cells exposed to ultraviolet light from the sun. Reactive oxygen species can cause both apoptosis and necrosis of bovine corneal endothelial cells in vitro. Detection of apoptosis could be used for assessment of endothelial function and inhibition of apoptosis might prevent age-related endothelial cell loss. Dexamethasone has been shown to increase apoptosis of cultured human corneal keratocytes. Proliferation of cultured keratocytes was also induced as they expressed the glucocorticoid receptor. Whether in vivo the balance is towards cell death (apoptosis) or proliferation is yet to be determined.

APOPTOSIS IN REFRACTIVE SURGERY

Apoptosis in rabbit cornea after photorefractive keratectomy (PRK) was studied by Gao et al. in 1997. They found that after PRK the entire epithelial layer was apoptotic and keratocytes and endothelial cells also showed apoptosis. There was also increased bcl-2 induction in the basal keratocytes. This gene prevents apoptosis and its induction may be a part of the healing response. The presence of apoptosis in spite of bcl-2 induction suggests that there might be involvement of other inducers of apoptosis such as Fas, etc.

Even after LASIK, keratocytes undergo apoptosis. However, their number does not reach statistical significance. The apoptosis following LASIK is also qualitatively different, occurring in the deeper central keratocytes located anterior and posterior to the lamellar cut. It has been shown that laser scrape PRK has the lowest level of apoptosis, probably due to photodisruption of cytokines. Therefore, Helena et al. hypothesize that (i) epithelial injury is essential for keratocyte apoptosis, and (ii) diffusion of cytokines from the peripheral epithelium into the lamellar interface accounts for the pattern of apoptosis seen after LASIK.

Apoptosis after refractive surgery is probably mediated through the release of cytokines such as interleukin-1 and Fas ligand from the damaged epithelium. This cell loss due to apoptosis probably leads to hyperproliferative wound healing which is responsible for complications such as postoperative corneal haze and regression of correction following refractive surgery. Thus, the control of apoptosis has a potential in regulating wound healing after PRK or LASIK and in reducing the above complications.
Apoptosis of keratocytes can be inhibited by zinc, flunarizine, caspase inhibitor z-VAD-FMK and ubiquinone Q10 (free radical scavenger). Although apoptosis is inhibited by caspase inhibitors, an associated increase in the necrosis of keratocytes is also seen. Necrosis would release inflammatory mediators and again trigger wound healing. Thus, there would probably be no substantial clinical benefit in using the caspase inhibitor z-VAD-FMK.

APOPTOSIS AND GLAUCOMA

A common feature of glaucoma and indeed all optic neuropathies is progressive death of retinal ganglion cells. Thus, it is important to understand the exact mechanism of this process for therapeutic purposes. In animals, a high intraocular pressure has been shown to cause apoptosis of the ganglion cells. However, it is not clear how this is initiated. One postulation is that if the retrograde transport of brain-derived neurotrophic factor (BDNF) is reduced, the cells are deprived of the survival stimulus and die by apoptosis. This model could also explain the cell loss in optic nerve compression, transection or ischemia. In all these conditions the ganglion cells would be deprived of BDNF. Another model implicates glutamate-mediated toxicity as the cause of cell death. Ischemia leads to the release of glutamate which activates the N-methyl-D-aspartate (NMDA) receptor and opens the calcium channels. An increase in intracellular calcium leads to activation of nitric oxide synthase and formation of active nitric oxide. Nitric oxide, along with other reactive oxygen species, can directly cause DNA fragmentation or activate polyADP-ribose synthase which causes severe depletion of energy in the cells leading to cell death. Alternatively, it can activate p53 which can lead to apoptosis. Evidence is also accumulating that mild activation of the NMDA receptor causes apoptosis while intense activation causes necrosis. Based on the above theories, a number of apoptotic inhibitors which act at different steps in the apoptotic process are at present under trial. One such drug which is currently available commercially is an alpha-2 adrenergic agonist, brimonidine. This is said to have an effect by increasing expression of a survival factor (basic fibroblast growth factor) in the retina. Efforts are also being made to use gene therapy for glaucoma treatment. Experiments are being conducted to overexpress the bcl-x gene (apoptotic blocking gene) in retinal ganglion cells using viral vectors. However, just blocking apoptosis does not affect the primary stimulus in the disease process. Thus, apoptosis inhibition would be an adjunct to the control of intraocular pressure. Apoptosis of retinal ganglion cells has also been shown in cases of partial optic nerve injury and anterior ischemic optic neuropathy.

APOPTOSIS AND THE LENS

Many factors such as ultraviolet light and oxidative stress have been implicated in the formation of senile cataract. The lens is an avascular and non-nervated structure containing only a single layer of cells on its anterior surface. Most of these cells have a long half-life and are responsible for maintaining metabolic homeostasis and transparency of the lens. If the viability of these cells is disturbed, cataract may occur. The pattern of this cell loss in response to various stimuli has been studied. It has been found that in the anterior capsule of the human cataractogenic lens 4.4%–41.8% of epithelial cells are apoptotic and treatment of rat lens epithelial cells with hydrogen peroxide induces cell death by the apoptotic pathway. These apoptotic cells were identified both by TUNEL staining and transmission electron microscopy (TEM). To further confirm these findings, the authors showed that hydrogen peroxide activates endonuclease (causes DNA breakdown and apoptosis) and the c-fos gene (induction associated with apoptosis). They also found that almost all epithelial cells were apoptotic by 24 hours and complete cortical opacification followed at 36 hours.

Ultraviolet (UV) radiation generates reactive oxygen species such as hydrogen peroxide and superoxide. Rat lenses irradiated by UV light showed a large number of epithelial cells and opacification of a major part of the cortex. UVB also upregulated expression of the c-fos gene.

Another study on rats has shown that a brief, photochemically-induced, oxidative stress can cause apoptosis of the lens epithelial cells leading to cataract. The time required to develop cataract increased with a decrease in the period of insult and even 12 days after a 7-hour long insult the nucleus of the lens remained relatively transparent.

Calcium also plays an important role in the development of cataract as such lenses contain a higher concentration of calcium than normal lenses. It has been demonstrated that calcimycin, a calcium ionophore, triggers apoptotic death of rat lens epithelial cells in vitro. This is also associated with upregulation of the c-fos gene. The detection of apoptosis in calcimycin-treated lenses was subsequently followed by development of complete cortical opacification.

All these studies implicate apoptosis of lens epithelial cells as the final common pathway for the development of non-congenital cataract. This opens new avenues in the research for medical prevention of cataract. On the other hand, another study on capsulotomy specimens of patients undergoing cataract surgery and epithelium from cataractous lenses of eye bank eyes refutes these findings. The authors of this study did not find any significant apoptotic cells in the capsulotomy specimens. They also failed to find any correlation between epithelial cell density and cataract severity and age. The authors of this study comment that it is not rational to be able to detect a high rate of apoptosis (4%–40%) in lens epithelia as shown by previous studies. They have calculated that even if 5% of cells were to undergo apoptosis at a given time and the process took 24 hours, the lens would be devoid of epithelial cells in 4 months. Further studies are required to elucidate the role of apoptosis in age-related cataract formation.

APOPTOSIS AND POSTERIOR CAPSULAR OPAQUE FORMATION

Clinically, there are two morphological types of posterior capsular opacification—wrinkling and haze from fibrosis on the capsule, and epithelial pearls from regeneration of lens fibres. The cellular mechanisms that lead to these changes are not well understood. Cytokines such as transforming growth factor-beta (TGF-β), basic fibroblast growth factor and hepatocyte growth factor play an important role in this process. Rat lens epithelial cells cultured in the presence of TGF-β undergo apoptosis. It is also known that immediately after cataract surgery the concentration of TGF-β falls and rises later at around 2 weeks. A hypothesis has thus been put forward that initially due to low TGF-β there is lens epithelial cell proliferation (due to a fall in apoptotic stimulus) and later when TGF-β increases, cell proliferation decreases but other changes such as myofibroblast differentiation and extracellular matrix formation increase.

Caballero et al. have shown spontaneous disappearance of Elschnig pearls after YAG laser capsulotomy in 6 eyes of 5
patients. Of the possible causes they include one as being cell death by induction of apoptosis. Thus, modulation of apoptosis could help decrease one of the commonest complications of cataract surgery.

APOPTOSIS AND THE RETINA

Progressive photoreceptor cell degeneration after retinal detachment leads to irreversible loss of vision even after re-attachment. Apoptosis has been seen in some specimens of globe rupture with traumatic retinal detachment. Apoptosis in detached retinas was seen as early as 8 hours after trauma and the amount of apoptosis detected decreased as the interval between trauma and detachment increased. The authors of this study believe that as some of the globes were severely damaged they could not detect apoptosis in more than 25% of cases. The final pathway of visual loss could be apoptotic cell death and its early prevention might slow the progression of visual loss.

While the vascular changes in diabetic retinopathy have been studied extensively, the pathological changes in the retinal neurons that cause progressive visual loss have not. Apoptotic ganglion cells and other neuronal cells have been detected in retinal specimens of streptozotocin-induced diabetic rats. Insulin has been found to decrease the amount of apoptosis in the rats. It needs to be determined whether lack of insulin or hyperglycaemia is the stimulus for apoptosis. Apoptotic cells are also seen in human retinal specimens of diabetic as well as non-diabetic patients. However, the number of apoptotic cells in diabetic retinas is much more. Even retinal capillary pericytes have been shown to be apoptotic in the retinas of diabetic patients. Apoptosis is also seen in the endothelial cells of these retinas; however, the number of pericytes undergoing apoptosis is higher. Thus, apoptotic inhibitors could, at least theoretically, change the course of diabetic retinopathy.

Apoptosis has also been shown in other degenerative diseases such as anterior ischaemic optic neuropathy, retinitis pigmentosa, and inherited retinal dystrophy in rats. Chloroidal neovascular membranes (CNVMs) in age-related macular degeneration (ARMD) show progressive histological changes from active highly cellular membranes to inactive paucicellular scars. Many TUNEL-positive (apoptotic) cells have been detected in the region of neovascularization, whereas few such cells are seen in fibrotic areas of surgically excised CNVMs. The apoptotic cells were stromal pigment epithelial cells, endothelial cells and occasional macrophages. Though apoptosis seems to be an appropriate method of cell reduction in the subretinal space where necrosis-induced inflammatory reaction can cause havoc, the formation of a fibrous scar prevents diffusion of nutrients to the outer retina and further retinal damage. Thus, further understanding of the mechanisms of scar formation is required if pharmacological intervention is to be appropriately timed to give the best visual result.

Oxidative stress is implicated as one of the causes of retinal pigment epithelium (RPE) cell loss in ARMD. The preferred form of cell death in most ageing processes is apoptosis. Flupirtine, a non-opioid analgesic drug which is probably an NMDA antagonist, has been shown to inhibit apoptosis of neurons and lymphocytes. It has also been found to reduce the amount of reactive oxygen species and apoptosis of cultured RPE cells. Other NMDA antagonists have not been found to have the same effect. Thus it is possible that flupirtine inhibits apoptosis but by an NMDA-independent pathway and hence has potential for therapeutic use in ARMD.

A dysfunction of the RPE and/or choroid secondary to elevated levels of epinephrine and steroids has been associated with the pathogenesis of central serous retinopathy (CSR). It has been found that elevated levels of epinephrine, but not dexamethasone, induced apoptosis in cultured porcine retinal pigment epithelial cells. This could be a possible pathophysiological mechanism for the development of CSR.

Iron has been shown to induce apoptosis during photoreceptor cell death at an early phase of iron-induced retinopathy in rats. This apoptosis was mainly limited to the outer nuclear layer. An answer to when siderosis becomes irreversible might be obtained by determining when the apoptosis becomes independent of the iron stimulus.

APOPTOSIS AND UVEITIS

Most cases of anterior uveitis regress spontaneously. The mechanism of this spontaneous regression is not well understood. CD4+ lymphocytes have been found to be the most predominant cells in all stages of experimental uveitis. Apoptosis occurs in these cells and increases during the phase of resolution. The expression of Fas ligand derived from both the infiltrating cells and eye tissue has also been found to be increased in uveitis. A therapeutic approach to induce selective apoptosis in autoreactive cells could be helpful in the treatment of chronic uveitis.

The expression of Fas ligand has also been shown to be increased in patients with posterior uveitis. Inhibition of neutrophil apoptosis follows elective cataract surgery. As this could be the cause for postoperative inflammation, its modulation could alter the healing process. The degree of apoptosis does not vary significantly with the different types of anaesthesia used during cataract surgery.

APOPTOSIS AND INTRAOCULAR MALIGNANCY

Morphological changes closely resembling apoptosis have been seen in retinoblastoma specimens. The number of apoptotic cells in areas showing spontaneous regression are higher. The apoptotic index obtained by counting the number of apoptotic cells might be useful for pathological classification of the tumour, selection for treatment and prognostic evaluation.

Vincristine and cisplatin have been shown to induce apoptosis in retinoblastoma cell lines. The addition of sodium butyrate, a differentiating agent, increased the amount of apoptosis but zinc sulphate, an endonuclease inhibitor, does not. This augmentation effect may have implications for retinoblastoma therapy.

CONCLUSION

Apoptosis is a mechanism by which the body maintains cellular balance. In physiological processes this is delicately maintained and any disturbance in its control results in disease. Apoptosis could be the initiator or the final common pathway of disease manifestation. An in-depth understanding of this process will enable the development of apoptosis modulators which, when applied to a specific tissue in a properly timed and titrated dose, may alter the pathogenesis of disease.

In the eye, inhibition of apoptosis could play a role in altering the natural progression of cataract, glaucoma, retinal detachment, diabetic retinopathy and keratoconus. It has the potential to prevent corneal haze and regression following keratorefractive surgery. The induction of apoptosis may also be useful in the treatment of retinoblastoma, recurrent uveitis and graft rejection. Thus, future research may make available a new class of drugs for therapeutic use.
REFERENCES


