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Stress Response of the Trabecular Meshwork

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Abstract

The trabecular meshwork (TM) is known to be subjected to different types of stress such as mechanical, oxidative, and phagocytic stress. Although short-term exposure to these stresses is expected to elicit adaptive responses, long-term exposure may lead to permanent alterations in the tissue physiology and contribute to the pathologic increase in aqueous humor outflow resistance frequently associated with glaucoma. A fuller understanding of the cell-specific and tissue-specific responses to stress in the TM, including changes in gene and protein expression, signal transduction, and potential pathogenic effects, could lead to novel prevention and therapeutic strategies for glaucoma. This review summarizes the current information available about how the TM responds to mechanical, oxidative, and phagocytic stress, as well as the evidence supporting the role that such responses may have in the alterations of the TM in aging and glaucoma.

Keywords

trabecular meshwork; mechanical stress; oxidative stress; phagocytosis; glaucoma

Living tissues are exposed to different stress conditions that can have a considerable impact in tissue physiology. Short-term stress generally elicits adaptive responses that have protective effects and help maintain tissue homeostasis. Chronic exposure to various stresses might overwhelm the tissue defense mechanisms leading to permanent changes in the tissue microenvironment and contribute to the progression of many age-related degenerative conditions. Three main types of stress have been reported to affect the trabecular meshwork (TM)/Schlemm canal (SC) conventional outflow pathway: mechanical, oxidative, and phagocytic. In this review, we summarize the current information available about how the TM responds to these 3 types of stress, and the potential role that such responses may have in the alterations of the TM in aging and glaucoma.

RESPONSE OF THE OUTFLOW PATHWAY TO MECHANICAL STRESS

As shown by Coleman and Trokel[1] in 1969, the TM/SC outflow pathway tissues are constantly exposed to changing levels in intraocular pressure (IOP). These variations in IOP include transient pressure changes up to 10 mm Hg resultant from blinking and eye movement, as well as small cyclic oscillations of approximately 2 to 3 mm Hg per second associated with the ocular pulse. Cells in the outflow pathway sense these variations in IOP in the form of mechanical stretch or distortion. Numerous studies performed in both human enucleated eyes from postmortem donors fixated at different pressures, and eyes from rhesus monkeys subjected to graded levels of physiologic IOP levels in vivo, have documented dramatic changes in the morphology of the outflow pathway under the influence of changing

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IOP. As pressure increases, a progressive distension of the juxtacanalicular tissue and the endothelial lining layer of the SC is observed.[2–7] According to measurements conducted by Grierson and Lee,[8] changes in pressure from 8 to 30 mm Hg could result in a level of stretching of the outflow pathway cells that could reach as much as 50%. This distention of the inner wall of SC is accompanied by an increase in the size and number of vacuoles,[9–11] as well as by a decrease in the complexity of intercellular junctions.[12]

The effect of elevated pressure on the outflow facility has been examined by several laboratories. Initial studies performed in perfused whole enucleated eyes for short time periods, showed a decline in outflow facility in response to increasing IOP.[13–15] However, more recent experiments conducted in the perfused anterior chamber system for longer periods of time have shown that, although the initial response to increasing flow rate correlates with an increase in resistance, the levels of measured IOP gradually return to the original baseline over time.[16–18] These observations suggest the presence in the outflow pathway of an adaptive response to counteract and accommodate changes in IOP, thus providing a homeostatic regulatory mechanism capable of maintaining a physiologic IOP by altering outflow resistance. Such mechanism would require cells in the outflow pathway to sense the mechanical strain originated by oscillations in IOP and/or fluid distortion and to respond to mechanical stress by triggering signals aimed at modulating the flow of aqueous humor (AH). The specific responses of TM cells to mechanical stress and the presence of such potential regulatory mechanisms have been investigated by several groups.

Effect of Mechanical Stress on Extracellular Matrix Turnover

The composition of the extracellular matrix (ECM) in the outflow pathway has been known for some time to play a major role in outflow resistance.[19–21] Therefore, the effects of mechanical stress on the ECM might represent one potential mechanism to counteract pressure fluctuations and regulate outflow facility. It is well known that mechanical stimuli can influence the expression of specific ECM proteins, as well as that of matrix metalloproteinases (MMPs) and other proteins involved in ECM turnover.[22–24] A number of studies have analyzed the effect of mechanical stress on MMP production in TM cells. Application of cyclic mechanical stress to bovine TM cells resulted in increased levels of active MMP-2 and TIMP-1 after 72 hours; levels of MMP-9 and TIMP-2 remained unchanged.[25] Similarly, the levels of MMP-2 and MMP-3 were found to be increased when bovine TM cells were subjected to biaxial static stretch.[26] This effect was reversible with relaxation of mechanical stress. The production of neither TIMP-1 nor TIMP-2 resulted affected under this strain model. Bradley et al[17] examined the effect of mechanical stress in the production of MMPs in both primary cultures of porcine and human TM cells, as well as in perfused and stationary human anterior segment organ cultures. Consistent with previous studies, the authors observed an increase in MMP-2 activity in all 3 experimental models following application of static strain. The authors additionally found a slight increase of MT-MMP, an activator of MMP-2; and a dramatic decrease of TIMP-2 levels in stretched porcine TM cells compared with nonstretched cultures. This increase in MMP-2 and MT-MMP protein levels in response to mechanical stress has been more recently reported to be transduced at least in part by mTOR, the mammalian target of rapamycin.[27] Despite some inconsistencies, all of these studies demonstrate that the MMPs turnover is modulated in the TM in response to mechanical stress.

Effect of Mechanical Stress on Cell Signaling in the TM

In response to changes in IOP, cells in the outflow pathway distend, stretch, and change shape. Despite its obvious importance, very little is known regarding how cells in the outflow pathway transmit and translate these stretch-induced morphologic changes into appropriate signals. Early studies performed by Tumminia et al[28] showed that cultured

human TM cells experience cytoskeletal alterations, involving reversible reorganization of actin filaments after uniaxial strain application. These alterations in the cytoskeleton were accompanied by cyclical changes in the total levels of tyrosine phosphorylation and signaling cascades. Matsuo and Matsuo[29] reported transient elevations in the concentration of intracellular calcium when primary cultures of human TM cells were exposed to elevated hydraulic pressure. Although the mechanisms for such calcium increase have not been studied, it is likely to be mediated through the activation of stretch-activated or mechano-sensitive channels caused by deformations in the cellular membrane as a result of fluctuations in IOP. This increase in intracellular calcium, in turn, might be responsible for the observed enhanced production of nitric oxide (NO) by cultured human TM cells under hydraulic pressure,[30] and for the reported activation of high-conductance calcium-activated channels in response to cell membrane stretch.[31] Cells in the TM could this way modulate outflow pathway permeability through cell volume regulation and contrac-tile responses. In addition, cyclic mechanical stress has been shown to induce the production of other factors capable of affecting outflow facility, such as prostaglandin F2 α.[32]

Our laboratory recently demonstrated the induction of transforming growth factor-β1 and IL-6 in response to cyclic mechanical stress in both primary cultures of human TM cells and perfused human anterior segments.[33,34] The stretch-induced production of IL-6 seemed to be at least partly mediated by transforming growth factor-β1. We additionally found increased permeability through SC cell monolayer after IL-6 treatment, and an increase in outflow facility when porcine perfused anterior segments were injected with the cytokine. These findings suggest that mechanical stress might thus modulate outflow resistance through the induction of factors capable of altering the dynamic interactions between SC and TM cells. In addition, mechanical forces could potentially act directly over the inner wall of the SC as suggested by studies conducted in SC cell monolayers subjected in vitro to hydrostatic pressure gradients.[35,36] Consistent with this concept, Ethier et al[37] have shown that SC endothelial cells experience physiologic levels of shear stress that may help control the caliber of SC through changes in the F-actin distribution.

Effect of Mechanical Stress on Gene Expression in the Outflow Pathway

Signal pathway alterations in response to mechanical stress will ultimately lead to changes in the transcriptional profile of the cells in the outflow pathway. Mitton et al[38] reported a transient loss in the protein levels of α B-crystallin in primary cultures of human TM cells just after application of sustained mechanical stress followed by a recovery phase involving up-regulated transcription of the gene. Up-regulation of myocilin, a glaucoma-linked gene, after static stretch has been reported by Tamm et al.[39] The authors suggested that the lack of the dynamic mechanical stimuli in monolayer cell cultures might account for the lost expression of myocilin in cell culture conditions. The induction of a novel gene, oculomedin, located in the GLC1A locus, was described in cyclically stretched human TM cells.[40,41]

The gene expression profile of TM cells exposed to mechanical forces has been analyzed in 3 different studies: (1) we investigated the genes up-regulated in the TM of human perfused anterior segment subjected to high IOP (50 mm Hg) for 6 hours[42]; (2) Vittitow and Borras[43] identified the genes showing altered expression during 2 to 4 days of elevated IOP in human perfused anterior segments; and (3) Vittal et al[44] studied the response of cultured porcine TM cells subjected to sustained stretch for 12, 24, and 48 hours. The results presented in these studies reflect a high level of complexity in the response of TM cells to mechanical insult. Although changes in the expression of genes potentially relevant for outflow pathway regulation were reported in all cases, there was no consistency among the 3 studies, which may have resulted from the used of different experimental models. Systematic analyses of

changes in gene expression to more physiologic levels of mechanical stress have not been conducted so far.

Finally, although some responses of TM cells to increased mechanical stress may constitute a homeostatic response to restore the normal physiologic conditions of the tissue; long-term activation of these responses may contribute to pathologic alterations. It is well known, for instance, that atherosclerotic plaques in blood vessels tend to form in regions of low or oscillating flow, such as arterial branch points, bifurcations, or inner curvatures, whereas those segments of the vessels that are exposed to steady laminar shear stress tend to be relatively lesion free.[45] Such correlation between the formation of lesions and the pattern of local hydrodynamic forces suggests that decreased flow or increased oscillatory flow may lead to pathogenic alterations of the blood vessels. Similarly, the cellular responses of the TM cells to increased mechanical tension or decreased flow associated with glaucoma could contribute to accelerate the functional decline of the TM overtime.

OXIDATIVE STRESS IN THE OUTFLOW PATHWAY

Oxidative stress results from an imbalance between oxidant production and antioxidant defense mechanisms. Living tissues are exposed to reactive oxygen species (ROS) that are normally generated during the process of energy production in aerobic respiration. One-electron and 2-electron reduction of O_2 generates superoxide (O_{2-}) and hydrogen peroxide (H_2O_2), respectively. In the presence of free transition metals, such as iron and copper, O_{2-} and H_2O_2 generate the extremely reactive hydroxyl radical (OH), which is believed to be the species responsible for initiating most of the oxidative tissue damage. Exposure of O_2 to ultraviolet radiation also results in the generation of 2 energetically excited species termed “singlet oxygens.”[46]

An additional source of oxidative stress in the TM may be NO. NO is a signaling molecule generated enzymatically by isozymes of NO synthase, which are known to be present in the AH.[47,48] The presence of NO in the AH can lead to the formation of several reactive species including the powerful oxidant peroxynitrite.[49,50] Peroxynitrite generation represents a crucial pathogenic mechanism in conditions such as stroke, myocardial infarction, chronic heart failure, diabetes, circulatory shock, chronic inflammatory diseases, cancer, and neurodegenerative disorders.[51] However, little is known about the potential role peroxynitrite in the pathology of glaucoma.

Evidence of Oxidative Stress in the Outflow Pathway

Because ROS are unstable, their presence in living tissues is difficult to quantify. Therefore, measurements of H_2O_2 and other ROS in the AH are controversial.[52–54] However, there is specific evidence of oxidative stress in the TM that has been provided by studying the presence of oxidation products from the main targets of ROS: lipids, proteins, and nucleic acids.

Ultraviolet-spectrophotometry and fluorescent analysis of lipid extracts from AH, TM, and SC from patients with primary open-angle glaucoma showed a significant increase in the accumulation of the primary, secondary, and end-products of lipid peroxidation (diene and triene conjugates, Schiff's bases) in glaucoma donors compared with controls.[55] This observation is potentially relevant, not only because it supports the significance of oxidative stress in the TM, but also because such end-products of lipid peroxidation are known to react with proteins to form adducts that induce protein dysfunctions and alter cellular responses,[56–58] which could contribute to the pathogenesis of glaucoma.

Some studies have also provided evidence for the accumulation of oxidized proteins in the TM. Reactive oxygen and nitrogen species can generate several oxidative modifications of proteins including methionine sulfoxide, formation of free carbonyl groups, polypeptide backbone fragmentation, and cross-linking via S-S bonds.[59,60] Horstmann et al[61] showed in 1983 that ageing of the TM is associated with an increase in methionine sulfoxide content that results from oxidation of methionine residues. More recently, our laboratory reported a 3-fold increase in carbonyl groups in primary cultures of TM cells from old donors compared with those from young donors.[62] The increase in carbonylated proteins was associated with a 7.5-fold decrease of proteasome activity, suggesting that a progressive loss of proteasomal function may contribute to the observed increase in the accumulation of oxidized proteins.

Oxidative damage to nucleic acids in the TM has been recently investigated by Izzotti and collaborators.[63–65] Oxidation of DNA is known to generate adducts of base and sugar groups, single-strand and double-strand breaks in the backbone, and cross-links to other molecules. Among the more than 20 known products resulting from DNA oxidation,[66,67] 8-oxo-2,7-dihydro-2′-deoxyguanosine (oxo8dG) can be easily quantified and is commonly used as a method to assess oxidative damage to DNA.[68–70] Izzoti's laboratory reported significantly higher levels of 8-OH-dG in glaucoma patients compared with age-matched and sex-matched controls. This group found that oxidative DNA damage was significantly increased in the TM of glaucoma patients and correlated significantly with IOP and visual field defects.[63–65]

Additional indirect evidence of the role of oxidative stress in glaucoma is suggested by the observation that constitutive activation of the oxidative stress response in TM cells, including the activation of the transcription factor NF-κB and inflammatory marker endothelial leukocyte adhesion molecule 1 (ELAM-1), may be a defining feature of the diseased phenotype of the TM in glaucoma.[71] Gene array analysis of TM samples from normal and glaucomatous donors are consistent with the up-regulation of ELAM-1 in glaucoma.[72] Increased expression of ELAM-1 has also been reported in the aqueous outflow pathway of porcine eyes with induced glaucoma[73] as well as a result of oxidative stress in cultured TM cells.[74] Emerging pathologic evidence supports a role for chronic inflammation as an underlying mechanism for the molecular alterations that link aging and major age-related diseases such as atherosclerosis, arthritis, osteoporosis, and cardiovascular diseases.[75] Current evidence strongly indicates that oxidative stress and the concomitant generation of intracellular ROS are widely implicated in the chronic activation of an inflammatory response observed in aging tissues.[75–77] Some of the key players involved in the age-related inflammatory process are believed to include the up-regulation of NF-κB, IL-1β, IL-6, and adhesion molecules such as ELAM-1. The reported up-regulation of some of these markers in association with glaucoma supports the concept that oxidative stress may lead to chronic inflammation in the TM, which could contribute to the malfunction of this tissue in glaucoma. Indeed, experimental data in other systems suggests that the suppression of the proinflammatory mediators that are up-regulated in aging helps to delay the progression of several age-related pathologies.[76,78] Therefore, the observed activation of a chronic stress response in the TM deserves special attention because it could lead to new therapeutic approaches on the basis of the suppression of proinflammatory mediators.

Antioxidant Mechanisms in the Outflow Pathway

The TM has been shown to have effective mechanisms to remove ROS[79] including high concentration of reduced glutathione (GSH),[80] as well as superoxide dismutase (SOD) and catalase activities.[81] In addition, other tissues may contribute to eliminate ROS in the AH. GSH peroxidase has been detected in the AH[82] and is believed to be originated mainly from the ciliary epithelium.[83,84] Studies on enucleated calf eyes have shown the ability of the

outflow pathway tissues to completely remove H_2O_2 at physiologic concentrations present in the AH (25 μM). Kahn et al[80] showed that such detoxifying mechanisms may be important in the maintenance of normal levels of outflow resistance. Although exposure to H_2O_2 produced no direct effect on outflow facility in the normal eyes, the same treatment in eyes with the GSH-depleted TM caused a 33% decrease in facility. The authors concluded that, although GSH may not participate directly in regulating AH outflow, a decrease in the ability to prevent TM H_2O_2-induced oxidative damage may lead to an increase in outflow resistance. A decline in the effectiveness of antioxidant defenses has been demonstrated during normal aging and in certain pathologic conditions in many tissues.[85] Evidence of a similar decline in oxidant defenses during normal aging in the TM is still limited. De La Paz and Epstein[86] reported an age-dependent decline in the specific activity of SOD, but not catalase, in normal cadaver human TM. Two-dimensional gel electrophoresis from human TM dissected from both eyes of 16 normal donors ranging in age from 9 to 91 years showed that SOD and GSH reductase were present in all ages on the 2-dimensional maps. Age-related changes in the TM were not apparent, although slight differences were observed between juvenile and adult TM.[87] However, no measurements of enzymatic activity were conducted.

More data are available supporting a decrease in antioxidant defenses associated with glaucoma. Aleksidze et al[88] reported a decrease in the content of ascorbic acid in the AH of glaucoma donors compared with controls. Analysis of trabeculectomy samples from patients with various stages of primary open-angle glaucoma also showed a decrease in GSH content associated with the stage of the disease.[89] Chemoluminescent measurements of total antioxidant redox potential (TRAP) in the AH of 24 patients with glaucoma and 24 cataract patients revealed a 64% decrease of TRAP in the glaucoma donors. This decrease in TRAP was associated with an increase in SOD and GSH peroxidase activities and no change in catalase.[83] Similar increases in SOD and GSH peroxidase activities in the AH of glaucoma patients were reported by Ganea and Harding[90] and have also been observed in other degenerative conditions in which oxidative stress is believed to play a pathologic role such as Alzheimer disease.[91]

Effects of Oxidative Stress on the Outflow Pathway

Despite the potential role of oxidative stress on the pathologic changes of the TM during aging and glaucoma,[92] very little experimental work has been conducted to investigate the effects of ROS on TM cells. Russell et al[93] demonstrated aggregation of the proteins in bovine TM after oxidative stress. More recently, we reported that human TM cells exposed to chronic oxidative stress experienced a marked decline in protea-some activity that was associated with premature senescence and decreased cell viability.[94] This inhibition of proteasome function could contribute to accelerate the accumulation of protein aggregates in the TM. Short-term sublethal oxidative stress has also been demonstrated to have important effects on the cytoskeleton and adhesive properties of TM cells. Zhou et al[95] showed that treatment with 1 mM of H_2O_2 for 10 or 30 minutes significantly reduced the adhesion of TM cells to fibronectin, laminin, and collagen types I and IV. The short-term loss of cell-matrix adhesiveness was associated with increased levels of transcription factor NF-κB and NF-κB-binding activity. Rearrangement of cytoskeletal structures were proposed to be responsible for the observed alterations in TM cell adhesion to the ECM. Although such changes were reversible, the authors suggested that repeated oxidative stress in vivo might result in reduced TM cell adhesion, leading to cell loss, compromised TM integrity, and pathologic consequences.

In addition to pathologic effects, oxidative stress is expected to activate protective responses similar to those reported in other tissues. The accumulation of α B-crystallin after oxidative stress in human and monkey TM suggest the presence of such protective response

mechanisms.[96] However, no detailed studies have been conducted so far to investigate the specific responses activated by oxidative stress in TM cells.

PHAGOCYTIC STRESS IN THE OUTFLOW PATHWAY

TM cells are known to be actively phagocytic; they are capable of ingesting endogenous and exogenous material, thus keeping the trabecular outflow channels free of potentially obstructive debris.[97–101] Phagocytic stress has been shown to have noxious effects in the cells of the outflow pathway. Exposure of TM cells to exogenous material, such as zymosan, blood, or latex beads in perfused and living cat and monkey eyes resulted in detachment of particles-laden TM cells from the beams, and migration of the detached cells through the SC.[98,102,103] Experiments performed in vivo also showed signals of inflammatory response with recruitment of macrophages to the outflow pathway after the phagocytic challenge.[102,103] Similar loss in cohesiveness and detachment has been described in cell culture conditions.[104] Despite the physiologic importance of the phagocytic activity of the TM and the potential role of phagocytic stress in the pathogenesis of glaucoma, the specific molecular mechanisms involved in the response of the TM to phagocytic challenge have received very little attention. Zhou et al[105] observed a transient increase in sensitivity to trypsin and reduced amounts of fibronectin and laminin in bovine TM cells incubated with latex spheres, suggesting a short-term loss of cell-matrix cohesiveness, which coincides with disruption of the cytoskeletal structure and decreased focal contact formation.[106] Whether the reported loss in tissue cellularity associated with aging and glaucoma in the outflow pathway[107–109] might result from the migration of phagocytically challenged TM cells has yet to be established.

CONCLUSIONS

The experimental evidence available to date strongly suggests that mechanical, oxidative, and phagocytic stress may play key roles in both the normal maintenance of the tissue homeostasis and the pathologic alterations of the TM in glaucoma. In addition, the ability of the TM cells to cope with stress is expected to show important levels of variability in human populations as a result of genetic, environmental, and, potentially, epigenetic differences. However, our current knowledge of the cell-specific and tissue-specific responses of the TM to stress is still very incomplete and relies in good part on studies conducted many years ago without the benefit of more modern experimental techniques. The advent of genomic and proteomic technologies has provided new tools to conduct comprehensive studies of the complex responses induced by stress conditions. A more systematic use of such technologies will be desirable to increase our understanding of the impact of stress in the physiology of the TM. A second area of improvement is the development of consistent experimental models to investigate the responses of the TM to mechanical, oxidative, and phagocytic stress. The availability of standard models used simultaneously in different laboratories appears as a necessary step to clarify the large inconsistencies in experimental results reported to date. Acute stress models should help to elucidate the adaptive mechanisms used by TM cells to cope with stress and their relevance in the maintenance of normal levels of AH flow resistance. Chronic stress models may be more technically challenging to generate, but should be particularly relevant in understanding the pathologic alterations of the TM in glaucoma and might lead to the identification of new targets for therapeutic intervention. Chronic models for mechanical stress will require the optimization of the conditions for long-term stress using computer-controlled systems such as those from Flexcell to generate different types of mechanical stress that affect the outflow pathway, including shear stress and periodic stretching. Chronic oxidative stress models have been used in other systems for a long time. The more widely used models include the chronic administration or generation of extracellular sources of ROS, and normobaric hyperoxia (elevated ambient

oxygen).[110,111] Although hyperoxia models require additional equipment not available in all laboratories, they may mimic better the in vivo conditions, since the increased production of ROS occurs at the same intracellular sites where these molecules normally generated by the cells.[111,112] Finally, it would be particularly important to translate the results obtained in vitro to more physiologically relevant experiments in vivo. A major step to accomplish this objective would be the development of methods for specific targeting of gene expression to the TM in vivo in order to generate animal models and investigate the role of different genes and molecular pathways in the normal and pathologic responses of the TM cells to stress.

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