Inhibition of Matrix Metalloproteinase Activity in the Chick Sclera and Its Effect on Myopia Development

Hsin-Hua Liu, Alex Gentle, Andrew I. Jobling, and Neville A. McBrien

**PURPOSE.** To investigate the contribution of matrix degradation in the two-layer avian sclera to the development of myopia.

**METHODS.** Tissue inhibitor of metalloproteinase-2 (TIMP-2) was used to inhibit chick scleral collagen degradation with 3H-proline, a marker for this effect. Ex vivo scleral culture experiments confirmed TIMP-2 doses for in vivo experimentation. Ocular growth and refractive response to exogenous TIMP-2 (11.25, 2.25, and 0.45 picomoles, plus vehicle only) were monitored in 7-day-old chicks during the induction of myopia over 4 days with a translucent occluder. Collagen degradation was assessed, in whole sclera and in separated scleral layers by using the same paradigm (11.25 picomoles TIMP-2; vehicle only).

**RESULTS.** Approximately 60% of collagen degradation was inhibited with low (2 nM) doses of TIMP-2 in the ex vivo sclera. Degradative activity in the in vivo chick sclera increased significantly (46%) during myopia development, with all the altered activity confined to the fibrous layer. Addition of TIMP-2 significantly reduced (by 46%) this accelerated scleral collagen degradation, also by acting in the fibrous layer. TIMP-2 had no significant effect on 3H-proline incorporated in the cartilaginous scleral layer and cornea. Despite inhibiting collagen degradation TIMP-2 had no significant effect on myopia development.

**CONCLUSIONS.** Increased collagen degradation is a feature of scleral remodeling in chick myopia development, but is confined to the fibrous scleral layer. Significant inhibition of this collagenolytic activity with TIMP-2 has little effect on refractive error development, suggesting that collagen degradation in the chick's two scleral layers that mediates scleral remodeling contributes little to the development of myopia in the chick. (Invest Ophthalmol Vis Sci. 2010;51:2865–2871) DOI: 10.1167/iovs.09-4322

The development of myopia is accompanied by progressive changes in the structure of the sclera, which, as the outer coat of the eye, is a key factor in the induction of any significant change in eye size. Alterations in the scleral structure of human high myopes have been well documented, the major characteristic being significant scleral thinning, particularly at the posterior pole of the eye. Such morphologic changes have been shown to have both biochemical and mechanical correlates, with the thinned sclera containing less collagen and proteoglycans and being more distensible. Despite differences in scleral structure and physiology, studies in both avian and mammalian models of myopia have been complementary in showing that scleral remodeling in myopia is a tightly regulated, active process, resulting in changes in the amount of scleral tissue. It is presumed that the interaction between scleral remodeling and altered tissue biomechanics is ultimately causal in the development of myopia.

Tissue remodeling constitutes a balance between the degradation of existing tissue and the synthesis of new tissue, and it has been shown that in myopia there is a shift in the balance between degradation and synthesis. In the two-layer chick sclera, the inner cartilaginous layer thickens during myopia development, whereas the outer fibrous layer thins in a manner more akin to the fibrous mammalian sclera. This morphologically complex observation in the chick suggests a complicated balance between synthesis and degradation in the separate scleral layers. Adding a further layer of complexity, study results suggest that there is communication between the chick's two scleral layers that mediates scleral remodeling.

Studies in birds and mammals, including humans, have implicated members of the matrix metalloproteinase (MMP) enzyme family and their natural regulators, the tissue inhibitor of MMPs (TIMP), in the control of degenerative activity in the sclera of the myopic eye. MMP-2 has been most strongly implicated in this process of scleral remodeling, although it is likely that other MMPs and non-MMP- proteases are also involved. The various members of the MMP family are secreted in an inactive, or latent, form then activated extracellularly through a process at the cell membrane involving membrane-type (MT) MMPs and TIMPs. Activated MMPs are then able to catalyze the breakdown of the extracellular matrix, with MMP-2 itself capable of breaking down both collagens and proteoglycans. MMP-2 is further regulated through the capacity of TIMPs to bind the active form of the enzyme and inhibit its activity. However, there are no completely specific TIMP-MMP relationships and the four known TIMPs each regulate the activity of a spectrum of MMPs.

Altered degradative activity in the sclera of chicks with developing myopia has been linked to both an increase in the production of MMP-2 and a decrease in the production of its TIMP regulators, with the net effect of these changes being increased MMP-2 activity. However, the nature and extent of the contribution of this increased MMP-2 activity to the structural changes that occur in chick sclera during myopia development are less clear. For example, although studies demonstrated increased levels of latent MMP-2 and decreased TIMP activity across the whole sclera of eyes with developing myopia, this phenomenon was subsequently shown to result from increased MMP-2 gene expression and decreased TIMP-2 expression in only the fibrous layer of the chick sclera. This finding led to the theory that MMP-2, produced primarily by the fibrous sclera, participates in remodeling of both the fi-
brous and cartilaginous sclera. 12 Specifically, it is thought that increased MMP-2 degradative activity works in concert with reduced fibrous matrix synthesis to produce fibrous scleral thinning, at the same time remodeling the cartilaginous matrix sufficiently to incorporate increased amounts of newly synthesized matrix components, thus facilitating scleral thickening. It is presumed that this combination of processes facilitates myopia development in the chick. 9, 12 To date, however, support for this theory has been limited. Proteoglycan degradation in the whole chick sclera is certainly more rapid in eyes with developing myopia and is consistent with the increased levels of MMP activity. 18 However, although it has been shown that inhibition of proteoglycan synthesis across the whole sclera prevents the development of myopia, 19 the contribution of degradative activity in the sclera to the development of myopia remains to be demonstrated.

In the present study, we investigated the role of MMP-related scleral degradative activity in the development of myopia in the chick. Recombinant TIMP-2 was used to inhibit MMP activity, with scleral collagen degradation used as a marker for this activity. First, it was confirmed, through an ex vivo scleral explant culture, that collagen degradation is regulated in chick sclera. Second, it was confirmed that scleral collagen degradation is regulated in vivo within the two layers of the chick sclera. Finally, the capacity of MMP inhibition to control the rate of progression of myopia was investigated across a range of doses.

**Materials and Methods**

**Experimental Subjects**

Day-old chicks (Gallus gallus domesticus, 44 in total) were obtained from the Research Poultry Farm and Hatchery (Research, VIC, Australia) and maintained in a temperature-controlled brooder. Illumination of the brooder floor during the light cycle was approximately 300 lux, and the chicks were exposed to a 12/12-hour light/dark cycle. Food and water were available ad libitum. All experimentation complied with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research.

**Ex Vivo Inhibition of Collagen Degradation**

Posterior scleral buttons (n = 16) were isolated from eight of the chicks as they reached 7 days of age, as described in a later section, and transferred to Dubecco’s modified Eagle’s medium (Invitrogen-Gibco, Carlsbad, CA) supplemented with 25 mM HEPES (Sigma-Aldrich, St. Louis MO), penicillin-streptomycin (100 U/mL; Invitrogen), and 10% fetal bovine serum (JRH, Melbourne, VIC, Australia). Newly synthesized collagen was labeled via the addition of 10 Ci/mL L-[2,3,4,5-3H]-proline (PerkinElmer, Waltham MA) for 20 hours at 37°C in a humidified incubator (5% CO2, and 95% air). The scleral buttons were allocated to one of four groups (n = 4 each group) and then placed in fresh culture medium, with no 3H-proline. In three of these groups, scleral MMPs were activated through the addition of 1 mM aminophosphonacetic acid (APMA; Sigma-Aldrich), with one group (no APMA) serving as the control for MMP activation. Samples were incubated for an additional 24 hours after the addition of TIMP-2 to vehicle and before being snap frozen in liquid nitrogen and stored at −80°C until assayed.

**Comparison of In Vivo Group Treatments**

<table>
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<tr>
<th>Treatment</th>
<th>1</th>
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<th>4</th>
<th>5</th>
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<td>0.45</td>
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<td>No injection</td>
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Y, yes; N, no; IP, intraperitoneal; SC, subconjunctival.

**In Vivo Inhibition of Collagen Degradation**

**Myopia Induction.** Myopia was induced by monocularly depriving the remaining 36 animals (7 ± 1 days of age) of form vision for 4 days, with hemispheric translucent diffusers with approximately 25% transmission. The diffusers were attached to the left eye via Velcro rings, stuck to the feathers around the eye with cyanoacrylate adhesive. Six groups of chicks were assessed in total (Table 1). Four groups of monocularly deprived chicks (n = 6 in each group) were used to investigate the in vivo effect of TIMP-2 on ocular growth and myopia development (group 1, 11.25 picomoles TIMP-2; group 2, 2.25 picomoles TIMP-2; group 3, 0.45 picomoles TIMP-2; and group 4, vehicle). Of the above groups, only the chicks in group 1 (11.25 picomoles TIMP-2) and group 4 (vehicle) were also given 3H-proline, to assess scleral collagen degradation. In addition, two further monocularly deprived groups (n = 6 in each) were used to analyze degradation patterns in separated scleral layers (group 5, 11.25 picomoles TIMP-2; and group 6, no injection). 3H-proline was administered to both of these groups. TIMP-2 doses were based on previous in vivo pirenzepine delivery data, which suggested that only 2.5% of the subconjunctival drug reaches the posterior sclera.21

**In Vivo 3H-Proline and TIMP-2 Delivery.** In chicks in those groups that received 3H-proline (groups 1, 4, 5, and 6), radiolabeled collagen precursors (180 μCi 3H-proline, 180 μCi 0.63 micro moles) were injected intraperitoneally, 24 hours before the induction of myopia, as previously reported.7 Subsequent intraperitoneal injections of unlabeled collagen precursors (220 μL 1-proline, 0.51 micro moles) were administered, at the start of monocular deprivation, to dilute tissue pools of the radiolabel and limit any further labeling of collagen during induction of myopia.

Daily monocular subconjunctival injections of either vehicle (1.5% carboxymethylcellulose in 0.9% saline; group 4) or recombinant human TIMP-2 (11.25, 2.25, or 0.45 picomoles in vehicle; groups 1, 2, 3, and 5) were delivered to the deprived eye at the start of the myopia induction period. The animals were placed under 2.5% isofluorane anesthesia, and 1 drop 0.5% proparacaine was added before the injections. Vehicle/TIMP-2 injections were delivered daily over the 4-day monocular deprivation period. The 90-μL injection volume was equally distributed between the superior (45 μL) and inferior (45 μL) conjunctiva. Conjunctival resistance to the needle did not change during the injection period, and there were no signs of bleb leak during injection, as monitored through the operating microscope.

**Ocular Biometry.** After a full 4 days (i.e., four light/dark cycles) of monocular deprivation, measurements were taken on the morning of the fifth day. Animals were anesthetized with ketamine (75 mg/kg) and xylazine (5 mg/kg) and maintained on a heating pad during measurements. Keratometry was performed by using a calibrated one-position

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**Table 1. Comparison of In Vivo Group Treatments**
keratometer (Bausch & Lomb, Rochester, NY), modified with an +8-D lens. Values recorded were the average of three readings in each of the horizontal and vertical meridians. Retinoscopy was performed by two experienced observers using a streak retinoscope, to an accuracy of ±0.5 D in both the vertical and horizontal meridians. Ultrasonography was performed with a 10-MHz probe driven by a pulser-receiver (Panas- metrics, Waltham, MA) and data acquisition controlled by a computer running a custom-designed program (written in LabView; National Instruments, Austin TX). The recorded data consisted of six separate measurements, each the average of 20 incoming waveforms. Data were converted from time to distance and then analyzed with the software.

**Tissue Collection.** Scleral tissue was collected immediately after the ocular measures were obtained. The animals received an overdose of pentobarbital sodium (150 mg/kg). The eyes were enucle- ated and the extraneous orbital tissue removed, before being separated into anterior and posterior segments through a circumferential cut 1 mm behind the scleral ossicles. A posterior scleral button was isolated from the geometric center of the posterior segment, with an 8-mm trephine, followed by the careful removal of retinal and choroidal tissue with a cotton bud. To determine degradation in the fibrous and cartilaginous sclera (groups 5 and 6), scleral buttons were placed in phosphate-buffered saline (pH 7.4) and the scleral layers gently teased apart with fine forceps, under a dissecting microscope. A 5-mm central corneal button was also isolated as a control. Samples were either immediately placed in liquid nitrogen until assay or were used for ex vivo culture.

**Radiolabeled Collagen and Hydroxyproline Assays**

All scleral and corneal samples were isolated as described and homogenized in sterile water before being suspended in pepsin (2 mg/mL; Sigma-Aldrich) and 0.5 M acetic acid. Tissue was digested for 48 hours at 4°C with gentle shaking. Insoluble material was removed by centrif- uigation. Wells of a filter plate (Multiscreen 0.65-μm pore size Durapore membrane; Millipore, Billerica MA) were prewetted with 100 μL 25% trichloroacetic acid (TCA) before replicate sample homogenates (100 μL) were loaded. A further 100 μL of 50% TCA was added and the plates incubated at 4°C with gentle shaking for 1 hour. The plates were drained with a manifold system and washed three times with 300 μL 10% TCA. The dried filters were punched into scintillation vials containing 500 μL 4 M guanidine HCl in 33% isopropanol. The vials were incubated overnight with shaking, and 10 mL liquid scintillation fluid (Cytoscint ES; Fisher Scientific, Pittsburgh, PA) was added and analyzed (Winspectral 1414; Wallac Turku, Finland).

Radiolabeled collagen data were normalized to the hydroxyproline content of the samples. Sample replicates of 0.5 to 8 μg trans-4-hydroxy-L-proline standards (40 μL; Sigma-Aldrich) were combined with 10 M sodium hydroxide (10 mL) and autoclaved at 120°C for 20 minutes. After cooling, 450 μL chloramine-T (0.005 M in 50% n-propanol and acetate-citrate buffer pH 6.5; Sigma-Aldrich) was added, and the reaction was incubated for 25 minutes at room temperature, before 500 μL Ehrlich’s reagent (1 M p-dimethylaminobenzaldehyde in 2:1 n-propanol:perchloric acid; Sigma-Aldrich) was added and the color developed for 20 minutes at 65°C. Absorbance values of samples and standards were measured on a spectrophotometer (Shimadzu, Kyoto Japan) at 550 nm.

**Data Analysis**

All data were transferred into a spreadsheet (Excel; Microsoft, Redmond WA), where they were processed, before being transferred into another program (Prism; GraphPad Software, La Jolla CA) for analysis and graphing. Ocular biometric data were expressed as either absolutes or the difference between treated and contralateral control eyes and compared via one-way ANOVA incorporating a Tukey post hoc test. Ex vivo scleral radiolabeled collagen data were normalized to scleral hydroxyproline content, expressed as disintegrations per minute per microgram hydroxyproline, and compared via one-way ANOVA incorporating a Tukey post hoc test. In vivo radiolabeled collagen data were normalized to scleral hydroxyproline content, expressed as either disintegrations per minute per microgram hydroxyproline or as the percentage difference between treated and contralateral control eyes and analyzed through either paired t-test or two-sample t-test (Bonferroni corrected to α = 0.005).

**RESULTS**

**TIMP-2 Inhibition of Collagen Degradation in Scleral Organ Culture**

The IC₅₀ concentrations of TIMP-2, determined in previous studies from this laboratory,²⁰ were verified in an ex vivo culture environment, employing buttons of chick sclera. Significant levels of radiolabeled proline were incorporated in scleral collagen after the culture period, and there was a significant decrease in radiolabeled collagen (1660 ± 29 to 439 ± 11 dpm/μg, −74%; P < 0.001) on the addition of APMA, signifying increased collagen degradation relative to the control (Fig. 1). The addition of both 2 and 10 nM TIMP-2 significantly inhibited this collagen degradation, relative to the APMA data (1131 ± 87 and 1204 ± 120, respectively, one-way ANOVA; P < 0.001), suggesting a 57% (2 nM) and 63% (10 nM) inhibition of collagen degradation with low nanomolar levels of TIMP-2. Radiolabeled proline levels remained significantly less than the no-APMA control in response to both doses of TIMP-2 (P < 0.01).

**TIMP-2 Effect on Ocular Biometry**

Animals deprived and treated with vehicle developed significant amounts of myopia and ocular enlargement in the treated relative to the control eye over the 4-day period (Table 2). The relative myopia found in treated eyes was −13.5 ± 1.1 D (treated minus control eye; Fig. 2A). The relative difference in

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933456/)

**FIGURE 1.** In ex vivo studies, the presence of APMA significantly reduced 3H-proline levels, relative to total scleral hydroxyproline, indicating increased scleral collagen degradation. 3H-proline levels in scleral buttons treated with TIMP-2 were significantly increased, relative to APMA-only buttons, but were significantly reduced relative to the no-APMA control (P < 0.01), indicating that both TIMP-2 concentrations inhibited collagen degradation in the scleral buttons by approximately 50%. **P < 0.01, ***P < 0.001; error bars, ±SEM; n = 4.
vitreous chamber depth (Fig. 2B; 0.38 ± 0.06 mm), the primary structural correlate of myopia, and axial length (Fig. 2C; 0.44 ± 0.07 mm) reflected the refractive findings with significant relative increases.

Increasing doses of TIMP-2 showed no significant effect on the amount of relative myopia induced by monocular occlusion when compared with the vehicle group by one-way ANOVA (Fig. 2A; 0.45 picomoles, −12.0 ± 1.5 D; 2.25 picomoles, −9.8 ± 0.9 D; and 11.25 picomoles, −9.4 ± 1.0 D; P = 0.07). There were also no significant differences in relative vitreous chamber depth and axial length across groups with increasing doses of TIMP-2 (Fig. 2B; 0.35 ± 0.11, 0.32 ± 0.05, and 0.29 ± 0.06 mm, P = 0.34. Fig. 2C; 0.45 ± 0.14, 0.41 ± 0.06, and 0.36 ± 0.09 mm, P = 0.22). There was no significant difference in absolute myopia, vitreous chamber depth, or axial length in the TIMP-2- or vehicle-treated animals, when control eyes were compared by one-way ANOVA (Table 2), indicating that TIMP-2 treatment had no yoking effect in the control eye. No significant relative differences were noted between the groups in corneal curvature, anterior chamber depth, or lens thickness (data not shown).

**TIMP-2 Regulation of In Vivo Collagen Degradation**

Form-deprivation myopia was associated with increased collagen degradation in vehicle-treated eyes relative to contralateral control eyes (Fig. 3A). The TIMP-2 effect on this increased degradation was quantified by paired analysis of the data, showing that radiolabeled proline content was significantly reduced (−46% ± 2%) in myopic, vehicle-treated eyes, relative to their contralateral control eyes (Fig. 3B; P < 0.0001, paired t-test), demonstrating that collagen degradation is accelerated in the whole sclera of eyes with developing myopia. In animals that developed significant degrees of myopia although treated with 11.25 picomoles of TIMP-2, collagen degradation was also accelerated (Fig. 3B; −25% ± 2%, P < 0.0001), but the relative rate of collagen degradation in these animals was significantly less than that in vehicle-treated animals (by 46%; P < 0.0001, two-sample t-test). This result indicates that, with the addition of TIMP-2, inhibition of collagen degradation did not have a concomitant effect on the amount of myopia development. There was no significant difference in absolute proline incorporation between the two groups of control eyes of TIMP-2-treated and vehicle-treated animals (Fig. 3A; two sample t-test P = 0.52), again implying that TIMP-2 treatment had no yoking effect in the control eye. Corneal tissue was also analyzed for collagen degradation, due to its proximity to the site of TIMP-2 delivery and its capacity to affect refractive error, however, no significant difference between the relative collagen degradation in vehicle and TIMP-2-treated animals was found (−10% ± 3% vs. −3% ± 3%, respectively, P = 0.09).
Relative collagen degradation data from separated cartilaginous scleral layers showed no significant difference between TIMP-2-treated and no-injection, form-deprived eyes (Fig. 4A; −4% ± 2% vs. −6% ± 2%; \( P = 0.44 \)), indicating that little or no differential collagen degradation occurs in the cartilaginous sclera during myopia development and that the addition of TIMP-2 does not change this fact. However, data from the fibrous sclera showed that significant differential levels of collagen degradation were present in the fibrous sclera of the treated eyes of form-deprived, no injection animals (Fig. 4B; −44% ± 4%) and that the addition of TIMP-2 significantly reduced this relative rate of collagen degradation in the fibrous sclera (by 50%, to −22% ± 3%; \( P < 0.001 \)). These data indicate that differential collagen degradative activity during myopia development is confined to the fibrous sclera and that inhibition of collagen degradation during myopia development is achievable only in the fibrous sclera.

**DISCUSSION**

In the present study, we have shown that, as in mammals, the development of myopia in chicks is associated with accelerated collagen degradation. In addition, we have shown that this altered degradative activity is confined to the fibrous layer of the sclera.
the chick sclera during myopia development and that there is little differential degradative activity in the cartilaginous layer, which complements a previous finding that MMP-2 expression is predominantly localized to the fibrous layer. Finally, despite the delivery of TIMP-2 to the posterior chick sclera, which caused a significant decrease in collagen degradation in the fibrous sclera, there was little effect on ocular growth rate or myopia development. Taken as a whole, these findings suggest that MMP-controlled scleral tissue degradation does not contribute directly to myopia development in the chick. This conclusion reinforces the implication in a previous study that scleral matrix synthetic activity is perhaps of greater importance in chick myopia.

In chicks, it is generally held that the inner cartilaginous layer produces more extracellular matrix and thickens during myopia development, whereas the outer, fibrous layer reduces its synthetic activity, produces less matrix, and differentiates into cartilage at the interface between the two layers. Thus, the fibrous layer displays many characteristics similar to those of the single-layer mammalian sclera during myopia development. Although the current and an earlier study found accelerated collagen degradation in whole sclera and accelerated scleral proteoglycan degradation during myopia development, the present study further extends our understanding, showing that the fibrous layer of the chick sclera is the major site of altered collagen degradative activity, again suggesting that the fibrous layer changes are similar to those of mammals. Furthermore, although an earlier study suggested that proline incorporation is upregulated in the fibrous chick sclera of form-deprived eyes, paradoxically suggesting that the fibrous layer should thicken during myopia development, the present study demonstrates that increased degradative activity serves to offset the small increase in collagen production. This, in concert with reports of unaltered or reduced proteoglycan production in chick scleras, helps to further explain why the fibrous layer actually thinning during myopia development. In addition, the lack of significant changes in turnover of scleral collagen in the cartilaginous layer, in concert with previous reports of continued production of extracellular matrix, is consistent with the reported thickening of the cartilaginous layer in myopia.

Ex vivo findings in the present study suggested that low-nanomolar levels of recombinant human TIMP-2 produce an approximate 60% inhibition of collagen degradation in the whole chick sclera. Of interest, there was little difference in this effect between the 2- and 10-nM concentrations of TIMP-2, which possibly reflects that MMPs that are less sensitive to TIMP-2 are activated by APMA and engage in collagen degradation. In vivo delivery of TIMP-2 was also effective, in that scleral collagen degradation in the fibrous layer slowed by approximately 50% in response to a 11.25-picomole dose, relative to vehicle controls. On the basis of the reported tissue distribution of pirenzepine after subconjunctival delivery, the 11.25-picomole dose was estimated to be equivalent to 56 nM (approximately five times the maximum ex vivo dose) of TIMP-2 in the 8-mm button of whole posterior chick sclera. However, it is acknowledged that there is little similarity between the molecular structure of TIMP-2 and pirenzepine, and the difference could affect the calculated tissue concentrations. Nevertheless, our in vivo findings were broadly consistent with the outcome predicted by the ex vivo findings (60% ex vivo vs. 50% in vivo).

These in vivo findings are significant in that a previous study has demonstrated that MMP-2 and TIMP-2 expression in the chick sclera is largely confined to the fibrous scleral layer. The current data also serve to demonstrate that at least 50% of collagen degradation in the fibrous sclera of the myopic eye is mediated by TIMP-2-sensitive MMPs, such as MMP-2. Indeed, given that previous studies suggest the involvement of non-MMP proteases in chick scleral remodeling, it would have been surprising to find all scleral collagen degradation to be TIMP-2 sensitive.

Several potential questions arise in comparing the ex vivo and in vivo situations. For example, altered levels of active scleral MMPs during myopia development could differ from those estimated in ex vivo studies, or there may be a change in the interaction between MMPs and TIMPs during myopia development—that is to say, a change in the dose-response characteristics. The use of APMA in ex vivo experiments, to activate scleral MMPs, brought an approximate 3.5-fold increase in MMP activity, matching the theoretical magnitude of increased scleral MMP activity during myopia development, as suggested by reported MMP-2 and TIMP-2 mRNA expression patterns and likely exceeding the actual in vivo increase in MMP activity. Furthermore, despite this evidence of changes in MMP and TIMP activity in the chick sclera in myopia, there is no evidence that the development of myopia results in a change in the interaction between these two groups of molecules. The similarity between our ex vivo and in vivo findings broadly support both the validity of the ex vivo model for estimating in vivo dosages and the notion that myopia does not alter the characteristics of MMP/TIMP interactions.

Unlike the findings in the fibrous sclera, the cartilaginous layer showed no evidence of differential collagen degradation, suggesting that cartilaginous scleral collagen degradation is not a feature of developing myopia. Rada et al. have demonstrated that most MMP-2 (and probably TIMP-2) present in the cartilaginous layer is produced in the fibrous sclera and suggest that fibrous scleral gelatinases participate in the turnover of matrix in the cartilaginous sclera. However, the outcome of the present study is contrary to this suggestion, although the role of fibrous scleral MMPs in mediating proteoglycan degradation in the cartilaginous sclera remains to be demonstrated.

Data from the cartilaginous sclera also demonstrated no response to the delivery of TIMP-2. This finding suggests that either (1) physiological matrix turnover in these tissues is not mediated by a significant, TIMP-2-sensitive mechanism, or (2) there is little overall collagen degradation in these tissues for TIMP-2 to inhibit. Given that studies in the sclera and other cartilaginous systems suggest that normal growth of cartilaginous tissue involves expression of a range of TIMP-2-sensitive MMPs, it seems most likely that the latter option is the case. Although we have no direct measure of delivered TIMP-2 levels in the cartilaginous layer, we assume that significant levels did indeed reach this site for the following reasons: (1) the in vivo data imply that significant TIMP-2 levels reached the fibrous layer, (2) previous studies imply that MMP-2 is highly diffusible from the fibrous to the cartilaginous sclera, and (3) the TIMP-2 molecule is two to three times smaller than MMP-2.

Despite the inhibition of a significant degree of scleral collagen degradation, there was little impact on the development of myopic refractive error. This result essentially suggests that the fibrous scleral matrix structure in chick has little effect on eye size, although it is still possible that communicating factors secreted by the fibrous sclera mediate other matrix changes in the cartilaginous sclera, as has been suggested previously. However, as a previous study found that inhibition of proteoglycan synthesis completely prevented abnormal ocular growth and myopia development, it seems that, in the chick, the process of scleral matrix synthesis is a more important determinant of eye size, than is the process of degrading scleral tissue. This notion in itself is surprising, given that in many tissue systems, turnover and growth of an extracellular matrix is facilitated by degradation of existing tissue, which allows the new tissue to be incorporated. Given the lack of
collagen degradation in the cartilaginous layer, the current findings suggest that during myopia development newly synthesized tissue is laid down on the inner and outer surfaces of the cartilaginous layer, without degradation of existing cartilaginous tissue to allow its incorporation, and that degradative activity in the fibrous sclera facilitates this process at the cartilaginous-fibrous interface. This notion would certainly be consistent with previous reports of increased matrix synthesis in the inner aspect of the cartilaginous sclera and a zone between the cartilaginous and fibrous sclera, where fibrous sclera appears to remodel into cartilage.\(^{22,25}\) It follows that, as the whole chick sclera is weaker in eyes with developing myopia,\(^{29}\) this zone of new matrix synthesis may somehow weaken the scleral matrix and promote subsequent development of myopia. Despite the analogies between the chick fibrous sclera and the mammalian sclera in myopia, it appears that the way in which the two-layer chick sclera remodels to facilitate eye size change is quite different from that of mammals.

References