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The control of matrix metalloproteinase-2 expression in normal and keratoconic corneal keratocyte cultures

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PURPOSE. *Early phase keratoconic comeas and their cultured keratocytes abnormally produce the* M_r *62,000 form of the matrix metalloproteinase-2 (MMP-2). It is known that platelet derived growth factor (PDGF) and transforming growth factor-β (TGF-β) are involved in the regulation of MMP activity and tissue inhibitor of metalloproteinase (TIMP) production in non-ocular tissues. The purpose of this enquiry was to determine whether these growth factors also play a role in the activity and/or production of corneal MMP-2 and TIMP, and whether their activity could account for the existence of the* M_r *62,000 form of MMP-2 in keratoconic corneas.*

METHODS. *Confluent cultures of normal and early-phase keratoconic corneal keratocytes were established and incubated in serum-free media in the presence or absence of PDGF and TGF-β. The proteins secreted by these cells over periods of 7 days were harvested for analysis. The total protein produced was determined spectrophotometrically. MMP-2 was visualised by SDS-gelatin polyacrylamide gel electrophoresis and assayed using radiolabelled type IV collagen as substrate. The enzyme inhibitors, TIMP-1 and TIMP-2, were quantified by dot blot immunoassay.*

RESULTS. *The addition of PDGF or TGF-β to the culture medium of keratoconic corneal keratocytes had no significant effect on overall protein production, MMP-2 activity or on the amounts of TIMP-1 and TIMP-2 secreted. These observations also applied to normal corneal keratocytes, with the exception that PDGF induced expression of the* M_r *62,000 species of MMP-2.*

CONCLUSIONS. *PDGF may be involved in the production of the* M_r *62,000 species of MMP-2 that is abnormally produced by early-phase keratoconic corneal keratocytes.*
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KEY WORDS. *Metalloproteinases, Gelatinases, Keratoconus, Corneal keratocytes, Platelet-derived growth factor (PDGF), Transforming growth factor β (TGF-β)*

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INTRODUCTION

Keratoconus is a chronic, progressive, non-inflammatory ocular disease in which the central cornea becomes thin, conical, and frequently scarred. Histopathological features of keratoconic corneas include fragmentation of the epithelial and Descemet's basement membranes, disruption of Bowman's layer and fibrillation of the anterior stroma (1). Matrix metalloproteinase-2 (MMP-2) is a member of the family of extracellular enzymes known as matrix metalloproteinases. This enzyme will cleave type IV basement membrane collagen and is the major protease secreted by corneal keratocytes (2,3). On the basis of these observations,

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we have hypothesised that the pathological features of keratoconus are a direct result of the activity of this enzyme.

Under normal circumstances, the production and activity of MMP-2 are under stringent control; in the diseased state a regulatory mechanism may have failed.

MMP-2, of M_r 66,000, accounts for approximately 95% of the total protease secreted by corneal keratocytes in culture (3). Although there are reports that cultures of keratoconic corneal keratocytes produce more MMP (4) and gelatinase activity (5, 6) than cultures of normal corneal keratocytes, it has also been concluded that there are no quantitative differences in either the amount of MMP-2 protein secreted or its activity (7, 8). A possible reason for this discrepancy may be a failure to differentiate between early phase, non-scarred and late phase, scarred keratoconic corneas: It has been shown that the specific activity of the MMP present in the culture medium of early-phase keratoconic corneal keratocytes is significantly higher than that of the MMP-2 in the culture medium of normal corneal keratocytes, after incubation periods of 4 days (3). Furthermore, in addition to the M_r 66,000 species of MMP-2, the early-phase keratoconic corneal keratocytes abnormally produce the MMP-2 of apparent M_r 62,000 that is generally considered to represent the activated enzyme (9, 10).

Cells that secrete matrix metalloproteinases co-secrete proteins known as tissue inhibitors of metalloproteinase (TIMPs) that bind, inhibit and thus regulate MMP activity. The best characterised of these proteins are TIMP-1 (M_r 28,000) which complexes only with activated MMP, and TIMP-2 (M_r 21,000) which may complex with either active or inactive MMP-2 (11, 12). Although MMP-2 may bind two molecules of TIMP (either TIMP-1 or TIMP-2), when complexed with TIMP-2 in a 1:1 ratio, the enzyme is apparently active with respect to gelatinolytic activity but inactive with respect to autocatalysis (13).

Growth factors, particularly platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β), have been implicated in the control of MMP and TIMP expression. PDGF is a disulphide bonded dimeric hetero- or homopolymer (PDGF-AA/AB/BB). The two PDGF receptors, α and β have been found on epithelial cells, stromal keratocytes and endothelial cells in the human cornea (14, 15). It has also been reported that PDGF may have a limited beneficial effect on the promotion of corneal endothelial wound healing (16). In support of its involvement in this process, it has been found that PDGF enhances synthesis of fibronectin and hyaluronic acid in mouse embryo-derived AKR-2B cells (17), that it is important in the transcriptional regulation of collagenase (MMP-1) in human dermal fibroblasts (18, 19) and that it regulates MMP-9 activity in aortic smooth muscle cells (20). The latter enzyme, MMP 9, is also produced by corneal epithelial cells.

Immunohistochemical studies have indicated that TGF-β exists in all 3 layers of the cornea (21), but reports that it stimulates MMP-2 and MMP-1 production (22-25) have been disputed (26, 27). With respect to TIMP, this growth factor may increase TIMP- 1 production by upregulating transcription and increasing mRNA stability (23, 27). Physiologically TGF-β may be a significant factor in inducing synthesis of corneal extracellular matrix after injury (28-30). This possibility is supported by a study in which a significant reduction of anterior corneal fibrosis and fibronectin production was found following injury inflicted in the presence of TGF-β blocking antibodies (31).

The experimental findings documented above suggest that PDGF and TGF-β may play a role in controlling ocular MMP/TIMP systems. This study was therefore carried out to determine whether PDGF and TGF-β can influence corneal MMP-2/TIMP production and MMP-2 activation, and whether keratoconic corneal keratocytes abnormally respond to these growth factors.

METHODS

Experimental material

Normal corneal tissue that had been rejected for transplantation because of minor endothelial defects was obtained from the Bristol Corneal Transplant Service Eye Bank. Keratoconic corneal tissue, from patients undergoing penetrating keratoplasty was obtained from Bristol Eye Hospital Theatres and from Mr A. Tullo, Manchester Royal Infirmary. The extent of scarring of the keratoconic corneas was obtained from the medical notes or, in the absence of this information, by visual assessment. A total of 7 normal corneas and 4 keratoconic corneas (1 non scarred, 3

mimimally scarred) were used to generate the cell cultures for the experiments described.

Tissue culture

To prepare the keratocyte cultures, the anterior surfaces of the corneal were scraped to remove their epithelial cells. They were then diced, placed in 25 cm^2 tissue culture flasks and incubated at 36 °C under 95% O_2 and 5% CO_2 in minimum essential culture medium (Gibco) containing 10% fetal calf serum (FCS) and an antibiotic antimycotic mix (Sigma Chemicals Co). The media was changed every 3-4 days over a period of 2-3 weeks. To obtain uniformly confluent cultures, the logarithmically growing keratocytes of each cornea were treated with trypsin to detach them from their extracellular matrix, suspended in medium and divided between 4-6 new flasks. Once these cell cultures had reached confluence, they were used for experimentation.

The effect of growth factors on MMP-2 and TIMP production

Sets of keratocyte cultures, derived from the same cornea, were incubated in serum-free media containing either PDGF or TGF-β within the concentration range 0.001 to 10 $ng.ml^{-1}$ for periods of 7 days. The culture media were then harvested for analysis.

The preparation of media samples for analysis

The media samples were analysed directly for MMP-2 activity by SDS-gelatin polyacrylamide gel electrophoresis. Additionally, for the [^{3}H] type IV collagen assays and the immunoprecipitation dot blots, their protein fractions were concentrated by ammonium sulphate precipitation (70% w/v) and resuspended in a small volume (100 µl) of 0.05M Tris HCl, pH 7.4 containing 10% glycerol.

Protein estimation

The total amount of protein secreted by confluent cultures of normal and keratoconic keratocyte cultures was estimated spectrophotometrically. The samples and solutions of bovine serum albumin (BSA) of know protein concentration (4-500 $\mu g.ml^{-1}$), were placed in the wells of a 96 well quartz plate. Their optical densities were recorded at 225nm using a Spectromax plus spectrophotometer. The protein concentrations of the samples were determined from the BSA calibration curves.

MMP-2 assays

The following methods were used to visualise and assay the MMP-2 secreted by confluent corneal keratocyte cultures.

a) SDS-gelatin polyacrylamide gel electrophoresis (zymography)

The method of Unemori and Werb (32) was used to visualise the keratocyte secreted gelatinase activities on SDS gelatin polyacrylamide gels. The samples were applied to the gels without boiling or reduction and run at 80mV for approximately 2 hours on a Biorad mini gel apparatus. After developing the activity by incubating the gels in 0.05M Tris HCl buffer, pH 7.2 containing 5mM $CaCl_2$ for 16 hours at 37 °C and subsequently staining with Coomassie blue, the areas of the cleared bands, representing the gelatinase proteins, were measured using a Biorad GS-690 imaging densitometer.

b) Using (^{3}H) Type IV collagen as substrate

To determine the amount of TIMP-free, activated MMP-2 present in the various keratocyte secreted protein preparations, aliquots of the concentrated protein preparations were incubated, in duplicate, with [^{3}H] type IV collagen. The assay mixtures contained 20 µl sample, 60 µl buffer (0.1M Tris HCl, 5mM $CaCl_2$ pH 7.8) and 20 µl of radiolabelled type IV collagen substrate (4000 dpm in 10mM acetic acid).

Negative controls contained no proteinase, and positive controls contained a commercial preparation of activated MMP-2. The reaction mixes were sampled before and after incubation at 37 °C for 16 hours. The aliquots removed (2 x 20 µl), were added to an equal volume of buffer containing 5mM EDTA at 4°C. Foetal calf serum (final concentration 5% v/v) and ammonium sulphate (final concentration 70% w/v) were then added to all samples and after 1 hour at 4°C, they were centrifuged at 15,000 rpm for 10 minutes. To

determine the amount of free radioactivity in the supernatants, samples of known volume were pipetted into scintillation fluid and counted using a LKB scintillation counter.

Dot blot immunoassays

The following procedure was used to quantify the relative amounts of TIMP-1 and TIMP-2 secreted by normal and keratoconic keratocyte cultures.

A Biorad 96 well vacuum blotter was used to absorb the keratocyte secreted proteins onto Immobilon membranes (Millipore). The wells were loaded with aliquots of each sample (3 x 10 µl) and 200 µl 0.05M Tris HCl buffer pH 7.4. Standard solutions of TIMP-1 and TIMP-2 were also included on each blot. After washing the membranes *in situ*, they were removed from the apparatus and incubated in TBS-T blocking buffer containing BSA (0.1M Tris HCl, pH 7.4, 150mM NaCl, 0.1%Tween, and 5% bovine serum albumin), for 2 hours. The membranes were further incubated at 4°C for approximately 16 h with primary antibody (5 µg ml^{-1} TIMP-1 or TIMP-2 antibody in 0.05M Tris HCl, pH 7.4 containing 5% FCS and 0.05% sodium azide), extensively washed with TBS-T and placed in a buffered solution of peroxidase conjugated secondary antibody. After 1 hour at room temperature, the membranes were again extensively washed in TBS-T, rinsed in 0.05M Tris HCl, pH 7.4 and incubated with diaminobenzidine (DAB) substrate solution [18 ml 0.05M Tris HCl, pH 7.4, 2 ml DAB stock (prepared as recommended by Sigma Chem. Co.), 15 µl H_2O_2 (30% v/v)]. The samples containing TIMP-1 and TIMP-2 developed a brown colour over a period of 15 minutes. After rinsing and drying, the membranes were scanned using a Hewlett Packard Desk Scan IIC. Each sample was assayed in duplicate or triplicate.

Statistical analysis

Values are generally expressed as mean ± standard error of the mean. The quantities of protein secreted by normal and keratoconic keratocyte cultures were compared using the two-tailed Student's t-test for unpaired data. The quantities of protein secreted by normal and keratoconic fibroblasts cultured in the presence of PDGF or TGF-β were compared using the one-tailed Student's t-test for paired data.

RESULTS

1) The effect of growth factors on keratocyte protein secretion

The estimated concentrations of total soluble protein secreted by a number of confluent keratocyte cultures derived from the 7 normal and 4 keratoconic corneas over a period of 7 days, were 656 ± 219 µg.ml^{-1} (n = 34) and 388 ± 211 µg.ml^{-1} (n = 13) respectively. These values are, from the unpaired, two-tailed Students t test, significantly different ($p<0.001$) and agree with the findings of other authors (33-36).

The amount of protein secreted over the same time period by these keratocyte cultures in serum-free media containing PDGF or TGF-β (0.001, 0.01, 0.1, 1, and 10 ng.ml^{-1}) was subsequently measured. With the omission of keratoconic corneal keratocytes cultured in the presence of TGF-β, these experiments were carried out in duplicate or triplicate. The averaged results are given in Table I. Analysis of these data indicated that PDGF may have marginally increased the amount of protein produced by normal corneal keratocyte cultures. From regression analysis of growth factor concentration versus the total protein produced by normal corneal keratocytes incubated with PDGF or TGF-β, and keratoconic corneal keratocytes incubated with PDGF, r values of 0.77, 0.57, and 0.09 respectively were obtained. Overall however, though the quantities of protein secreted by the normal and keratoconic corneal keratocytes cultured in the presence of PDGF remained significantly different ($p<0.05$), the quantities of protein secreted by the normal corneal keratocytes in the presence of PDGF and TGF-β were not significantly different ($p>0.2$).

2) The effect of growth factors on MMP-2 activity

After incubating the normal and keratoconic corneal keratocytes in the presence or absence of PDGF or TGF-β, at varying concentration, for 7 days, the zymographic gelatinase activity profiles of the MMP-2 present in samples of the concentrated culture media were obtained. Subsequently, the relative amounts of the M_r 66,000 and M_r 62,000 forms of MMP-2 visualised were estimated by densitometry. TGF-β had no observable effect on the proportion of the total MMP-2 presenting as the M_r 62,000 zymographic activity in

the normal or keratoconic keratocyte cultures (data not shown).

The results pertaining to the inclusion of PDGF in the keratocyte culture media are given in Table II. Although the proportion of MMP-2 recovered as the M_r 62,000 species in keratoconic corneal keratocyte cultures was not affected by PDGF, this growth factor apparently enhanced the contribution of the M_r 62,000 MMP-2 to the total MMP-2 produced by normal corneal keratocytes. For the full PDGF concentration range (0-10 ng.ml^{-1}) the calculated regression coefficient was 0.52; for the PDGF concentration range 0-1 ng.ml^{-1}, the calculated regression coefficient was 0.88. A representative zymographic activity profile of the MMP-2 secreted by normal corneal keratocytes cultured in PDGF at varying concentration is shown in Figure 1.

Despite the appearance of the M_r 62,000 zymographic MMP-2 activity in the concentrated media samples of the keratoconic corneal keratocytes and the normal corneal keratocytes treated with PDGF, the proteolytic activities measured by assaying these samples against radiolabelled type IV collagen, were negligible (Tab. III). This indicated that activated MMP-2, free of TIMP, was not present in these samples.

3) The effect of growth factors on TIMP secretion

Dot blot immunoassays were used to quantify the amounts of TIMP-1 and TIMP-2 secreted by normal

TABLE I - EFFECT OF GROWTH FACTORS ON TOTAL PROTEIN SECRETION BY CONFLUENT CULTURES OF NORMAL AND KERATOCONIC CORNEAL KERATOCYTES

Growth factor concentration (ng.ml^{-1})	Number of experimental cultures	Normal keratocyte cultures		Keratoconic keratocyte cultures
		Total protein secreted in presence of PDGF (µg.ml^{-1})	Total protein secreted in presence of TGF-β (µg.ml^{-1})	Total protein secreted in presence of PDGF (µg.ml^{-1})
0.001	2	508 ± 3	522 ± 88	459 ± 196
0.01	2	488 ± 191	680 ± 246	435 ± 233
0.1	2	453 ± 12	605 ± 213	309 ± 110
1.0	3	606 ± 259	532 ± 74	359 ± 79
10	3	643 ± 263	693 ± 124	408 ± 51

TABLE II - EFFECT OF PDGF ON THE PERCENTAGE RECOVERY OF MMP-2 AS THE M_r 62,000 ZYMOGRAPHIC ACTIVITY IN NORMAL AND KERATOCONIC CORNEAL KERATOCYTE CULTURES

PDGF concentration (ng.ml^{-1})	Percentage recovery of MMP-2 as the M_r 62,000 zymographic activity (± S.E)	
	Normal keratocyte cultures	Keratoconic keratocyte cultures
0	4.7 ± 2.6 (n=7)	29.5 ± 12.6 (n=4)
0.1	11.9 ± 4.3 (n=8)	25.3 ± 10.1 (n=4)
1.0	18.1 ± 5.6 (n=7)	27.2 ± 8.6 (n=6)
10	17.2 ± 4.4 (n=7)	29.3 ± 7.1 (n=4)

and early-phase keratoconic keratocyte cultures over an incubation period of 7 days in the absence or presence of PDGF or TGF-β. Plots of increasing reference TIMP-1 and TIMP-2 solution volume against optical density were linear over the range of densities recorded for the samples tested.

Statistical analysis of the data obtained indicated that the amount of TIMP-1 produced by keratoconic corneal keratocytes (Tab. IV) may have been slightly less than that produced by normal corneal keratocytes (p=0.035). This was not the case for TIMP-2 (p=0.2) and neither PDGF nor TGF-β had any influence over the production of these MMP inhibitors (Tabs. V and VI).

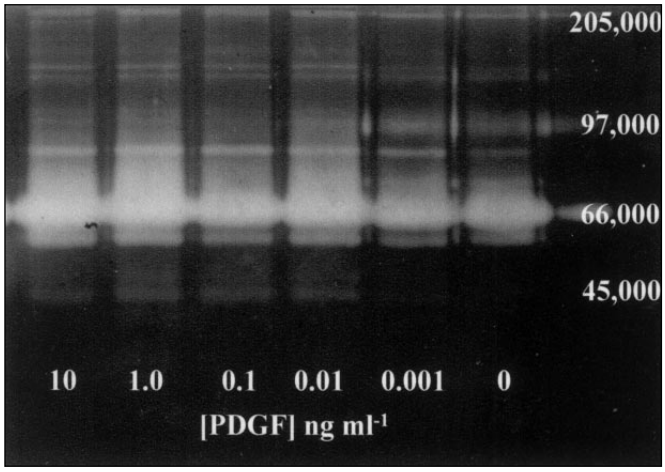

Fig. 1 - *Zymographic activity profiles of the MMP-2 secreted by normal corneal keratocytes maintained for 7 days in serum free medium supplemented with PDGF (0-10ng.ml^{-1}).*

TABLE III - EFFECT OF PDGF AND TGF-β ON THE PRODUCTION OF ACTIVATED MMP-2 BY CULTURES OF NORMAL AND KERATOCONIC CORNEAL KERATOCYTES

Growth factor concentration (ng.ml^{-1})	Radioactivity (DPM) liberated from [^{3}H] Type IV collagen after 18 hours incubation at 37 °C			
	Normal corneal keratocytes		Keratoconic corneal keratocytes	
	+PDGF	+TGF-β	+PDGF	+TGF-β
0	58	45	35	25
1	21	39	39	57
10	48	56	28	26
No enzyme	57	57	29	29
Crab collagenase	281	281	335	335

DISCUSSION

PDGF and TGF-β have been implicated in the regulation of MMP and TIMP expression in various tissues (18-25, 29, 30). These growth factors may also modulate the synthesis of components of the extracellular matrix (17, 18), and effect corneal wound healing responses (16, 28, 31). In consideration of these observations and an hypothesis that the MMP-2 secreted by corneal keratocytes may be involved in keratoconus (3), we have investigated the effects of PDGF and TGF-β on the amount of total

TABLE IV - COMPARATIVE ESTIMATION OF THE QUANTITY OF TIMP-1 AND TIMP-2 SECRETED BY NORMAL AND KERATOCONIC CORNEAL KERATOCYTE CULTURES

Keratocyte culture	Relative intensities of scanned dots ± S.E.	
	TIMP-1	TIMP-2
Normal	160 ± 3.8 (n=8)	194 ± 3.6 (n=8)
Keratoconus	153 ± 1.3 (n=5)	197 ± 10.3 (n=5)

TABLE V - RELATIVE ESTIMATION OF TIMP-1 CONCENTRATION IN THE MEDIUM OF NORMAL AND KERATOCONIC CORNEAL KERATOCYTES AFTER AN INCUBATION PERIOD OF 7 DAYS IN THE PRESENCE OR ABSENCE OF PDGF AND TGF-β

Growth factor concentration ($ng.ml^{-1}$)	Normal corneal keratocyte cultures		Keratoconic corneal keratocyte cultures	
	Relative amount of TIMP-1 recovered (%) in presence of growth factors			
	PDGF (n=3)	TGF-β (n=2)	PDGF (n=3)	TGF-β (n=1)
0	100	100	100	100
0.001	101	103	98	ND
0.01	100	101	97	ND
0.1	100	101	97	ND
1	101	94	98	97
10	107	94	100	100

TIMP concentration without the addition of growth factor = 100%
(ND = no data)

TABLE VI - RELATIVE ESTIMATION OF TIMP-2 CONCENTRATION IN THE MEDIUM OF NORMAL AND KERATOCONIC KERATOCYTES AFTER A PERIOD OF 7 DAYS IN THE PRESENCE OR ABSENCE OF PDGF AND TGF

Growth factor concentration ($ng.ml^{-1}$)	Relative amount of TIMP-2 recovered (%)			
	Normal corneal keratocyte cultures		Keratoconic corneal keratocyte cultures	
	PDGF (n=3)	TGF-β (n=2)	PDGF (n=3)	TGF-β (n=1)
0	100	100	100	100
0.001	102	103	97	ND
0.01	101	101	94	ND
0.1	100	101	96	ND
1	104	103	96	112
10	102	104	101	ND

TIMP concentration without the addition of growth factor = 100%
(ND = no data)

protein, MMP-2, TIMP-1 and TIMP-2 produced by keratocytes of normal and keratoconic corneas in culture.

In addition to MMP-2, the proteins secreted by cultured corneal keratocytes include structural components of the corneal stromal matrix (e.g. collagen, proteoglycan, fibronectin). With the exception of the proportion that has polymerised to form a basal matrix in the culture flask, these proteins are present in a soluble form in their culture media. The data presented here indicate that the amount of soluble protein recovered in the media of confluent cultures of normal corneal keratocytes, after an incubation period of 7 days, was significantly greater than that recovered from the media of similar cultures of keratoconic corneal keratocytes. However, despite the reports that PDGF and TGF-β may modulate fibronectin and collagen production (17, 18, 28, 31, 37), the addition of these growth factors (0.001 to 10 $ng.ml^{-1}$) to the culture media of either normal or keratoconic corneal keratocytes had no apparent effect on the total quantity of soluble protein secreted by these cells over an incubation period of 7 days.

MMP-2 exists as the proenzyme of M_r 66,000 in normal, healthy corneas and in the medium of their cultured keratocytes (2, 3). In early-phase keratoconic corneas and the medium of their cultured keratocytes, the M_r 62,000 form of this enzyme, generally considered to represent N-terminal truncated, activated enzyme (9, 10), becomes a prominent entity (3). With respect to the effect of PDGF and TGF-β on the activation status of this enzyme, the results presented here indicate that these growth factors had no influence over the proportion of MMP-2 presenting as the M_r 62,000 activity in the culture medium of keratoconic corneal keratocytes. However, although TGF-β had no effect upon the zymographic activity profile of the MMP-2 secreted by normal corneal keratocytes either, the addition of PDGF to these cells significantly enhanced the amount of MMP-2 recovered as the M_r 62,000 species. The involvement of PDGF in MMP-9 activation in aortic smooth muscle cells has been established (20) and the mechanisms may be the same, possibly to the extent that the cytokine, IL-6, in combination with PDGF, might further increase the production of the M_r 62,000 species of MMP-2. This remains to be investigated but if PDGF exerts, under normal circumstances, some control over the production of the M_r 62,000 species of MMP-2, then it would appear that this regulatory mechanism has either failed or been bypassed in keratoconic corneas.

To ensure that MMP-2 remains as the proenzyme in healthy tissue *in vivo*, the secreted enzyme is held as a complex with TIMP-2 and/or TIMP-1. To determine the relative amount of 'free' activated MMP-2 present in the medium of normal and keratoconic corneal keratocytes incubated in the presence or absence of PDGF or TGF-β, aliquots of the keratocyte secreted protein fractions were assayed against [^{3}H] Type IV collagen. Despite the presence of the M_r 62,000 form of MMP-2 in some of these media samples, little or no activity could be measured. The lack of detectable MMP-2 activity in keratoconic MMP-2 preparations has been reported previously (3, 8) and indicates that either the M_r 62,000 form of the enzyme remains completely saturated with TIMP, or that it is not the form that is active *in vivo*. In support of the latter of these possibilities, there exists an M_r 43,000 form of activated MMP-2 that lacks both N-terminal and C-terminal peptides and is unable therefore to bind TIMP (11).

Whether or not the M_r 62,000 form of MMP-2 is itself capable of causing tissue disruption *in vivo*, the enzyme autoactivates when TIMP is absent or limiting. For this reason, the failure of PDGF or TGF-β to induce the production of MMP-2 that exhibits peptidase activity *in vitro* indicated that these growth factors did not inhibit TIMP production. Dot blot immunoassays were used to quantify the relative amounts of TIMP-1 and TIMP-2 synthesised by normal and early-phase keratoconic keratocyte cultures maintained in the presence or absence of PDGF or TGF-β for a period of 7 days. The data obtained indicated that early-phase keratoconic corneal keratocytes may secrete less TIMP-1 than normal corneal keratocytes, but provided no evidence for the possibility that PDGF or TGF-β influence TIMP-1 and TIMP-2 production by cultured keratocytes.

In conclusion, the data obtained indicate that the rates of TIMP-1 or TIMP-2 synthesis are not affected by the inclusion of PDGF or TGF-β in the growth media of corneal keratocytes. Whereas TGF-β had no detectable influence over MMP-2 expression in normal and keratoconic keratocyte cultures, in the former, PDGF may effect production of the M_r

62,000 MMP-2 that is abnormally present in keratoconic corneas. The significance of this finding remains to be explored.

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