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Matrix metalloproteinase gelatinase B (MMP-9) is associated with leaking glaucoma filtering blebs☆

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Abstract

The goal of glaucoma filtering surgery is to create a low resistance pathway for aqueous outflow. The result is a blister or 'bleb' on the conjunctiva, from which fluid drains into the vasculature. Filtering surgery results may be compromised if blebs develop leaks, a problem that surfaces more frequently when antimetabolites are used to control the wound healing response. We investigated the role of tissue remodelling enzymes of the Matrix metalloproteinase (MMP) family in the development of bleb leaks. Our design was a case series. We enrolled glaucoma patients with leaking blebs, glaucoma patients with overhanging blebs and normal eyes. Leaking bleb tissues ($n=11$) and bleb leak fluid were collected from patients undergoing bleb revision surgery. Overhanging bleb tissues (from non-leaking blebs, $n=3$), normal conjunctiva ($n=8$), and aqueous humour ($n=4$) were collected for comparison. Samples were analysed for MMP content and proteinase activity by the methods of zymography, western blotting, immunohistochemistry, and in situ zymography. Our main outcome measures were presence and activity of MMP in sample.

Zymography revealed the presence of a high molecular weight caseinase and a 92-kDa gelatinase of a size appropriate for the proenzyme form of gelatinase B (gelB; MMP-9), in extracts from leaking bleb tissue, but not in bleb leak fluid or aqueous humour samples. In contrast, a 65-kDa gelatinase of a size appropriate for gelatinase A (MMP-2) proenzyme was observed in all samples. All proteinases disappeared when 10 mM EDTA was added to the development buffer, consistent with their identity as MMPs. Western blotting and immunohistochemical analyses confirmed the identity of the 92 kDa proteinase as gelB, and further revealed its absence from extracts of overhanging bleb tissue and normal conjunctiva. In situ zymography demonstrated strong gelatinolytic activity in leaking bleb tissue, but not overhanging bleb tissue or normal conjunctiva.

MMP-g may be involved in the mechanism of formation of bleb leaks. Precise description of the cascade of events leading to bleb leakage may allow the design of therapeutic interventions to prevent, stabilize or reverse bleb leakage.
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[1] Commercial relationships: Drs Chintala, Fini and Schuman have a patent interest in the use of matrix metalloproteinase inhibitors in the treatment of bleb leaks.

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1. Introduction

Glaucoma filtration surgery is performed to control the intraocular pressure (IOP) when medical therapy or other measures fail. The procedure lowers the IOP by creating a fistula between the anterior chamber and the subconjunctival space, creating a filtering bleb (Azuara-Blanco and Katz, 1998). The aqueous humour percolates through the bleb and can then be absorbed through veins and conjunctival lymphatics or pass directly into the tear film in cases where the conjunctiva is thin. The major determinant of glaucoma filtering surgery success is the conjunctival wound healing response. Excessive post-operative fibrosis can lead to filtration failure by resealing of the surgically-created outflow pathways (Cordeiro et al., 2000; Khaw et al., 2001). On the other hand, too little wound healing may eventually lead to bleb leaks, which compromise the ability to maintain an appropriate IOP and provide an avenue for infection (Skuta and Parrish, 1987).

A popular method to control fibrosis is by application of anti-metabolites such as mitomycin C at the time of surgery to the area of conjunctiva destined to become the bleb (Greenfield et al., 1998). This improves the early surgical outcome, but appears to increase the likelihood of late bleb leaks (Lamping et al., 1986; Wilensky, 1992; Chen et al., 1997; Scott et al., 1998). A number of modalities exist to treat bleb leaks (Gehring and Ciccarelli, 1972; Awan and Spaeth, 1974; Joiner et al., 1989; Pederson, 1989; Weber and Baker, 1989), including surgical procedures such as conjunctival patch grafts (Wilson and Kotas-Neumann, 1994), and scleral patch grafts in association with conjunctival advancement (Dunnington, 1950; Melamed et al., 1991; O'Connor et al., 1992; Morris et al., 1998). The reason for the existence of many different procedures to reverse bleb leaks is the fact that none is a cure in all cases. Successful closure of late-onset bleb leaks often requires surgical revision of the bleb (Ritch and Belcher, 1993; Wadhwani et al., 2000). Bleb leaks are the result of conjunctival tissue dissolution, suggesting the involvement of proteinases.

Matrix metalloproteinases (MMPs) are the major effectors of tissue remodelling in vertebrates (Woessner, 1998). Molecular substrates for the MMPs include all classes of extracellular matrix (ECM) proteins, as well as a variety of other molecules involved in determining tissue structure and controlling tissue remodelling (Woessner, 1998; Vu and Werb, 2000). The MMP gelatinase A (gelA; MMP-2) is present in bodily fluids, including the aqueous humour (Ando et al., 1993). GelA can typically be extracted from tissues whether or not they are undergoing remodelling. However, most other MMPs are not present in tissues constitutively, but are synthesized locally by resident cells upon demand, or are released by invading inflammatory cells (Fini et al., 1998a,b). Regulation of MMP expression is very important for controlling MMP activity and inappropriate or over-expression of MMPs causes diverse pathologies across all organ systems (Parks and Mecham, 1998; Chintala et al., 1999). In this study, we investigated the possible role of proteinases in the development of late bleb leaks after glaucoma filtering surgery.

2. Materials and methods

Conjunctival tissue and bleb leak fluid was obtained from patients during bleb revision surgery with the written informed consent of the patients. Bleb leakage was confirmed by Seidel testing. The clinical protocol received institutional review board approval at New England Medical Center Hospitals, Boston, MA. The clinical history of patients from whom tissue specimens were obtained, and the uses made of the tissues in this study, is provided in Table 1. Control conjunctival tissue was also obtained from seven cadaver eyes through the National Disease Research Interchange (NDRI, Philadelphia, PA) and was used for both zymography and western blotting (Table 2).

2.1. Preparation of tissue extracts

Freshly-collected tissue was placed in a 1.5 ml tube on ice and immediately transferred to the laboratory. To prepare extracts, tissues were homogenized in 40 μl of radioimmunoprecipitation (RIPA) buffer (1% nonidet P40, 20 mM Tris–HCl, 150 mM NaCl, 1 mM Na_3VO_4, pH 7.4) on ice. Homogenates were centrifuged at 10 000 rpm for 5 min at 4°C and the supernatants were collected. The total protein concentration in each sample was determined using the Bradford assay (Bio-Rad Laboratories, Hercules, CA).

2.2. Zymography

Zymography was used to analyse for the presence of specific proteinase species, including MMPs. This gel electrophoresis technique enables characterization of proteinases by their electrophoretic mobility in the presence of SDS, and their ability to degrade a substrate copolymerized in the gel matrix after removal of SDS. Proenzymes as well as their proteolytically cleaved forms can usually both be visualized because many proenzymes renature into an active configuration after SDS treatment. The procedure is popularly used to detect MMPs, but also detects enzymes of other families. Tissue extracts were fractionated on SDS polyacrylamide gels containing co-polymerized gelatin or casein substrates. The procedure was performed according to our standard lab protocol (Fini and Girard, 1990) with minor modifications. Aliquots of tissue extracts containing 20 μg total protein were mixed with SDS gel-loading buffer (Laemmli, 1970) then loaded without reduction or heating onto 10–12% SDS polyacrylamide gels containing 0.1% gelatin or 0.1%

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