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Wound Healing as a Barrier to Successful Filtration Surgery

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Summary

Trabeculectomy fails to control the intra-ocular pressure adequately in a proportion of patients. Approaches to solving this problem have involved modifications of surgery, histological studies of tissue from failed and functioning blebs, animal studies, and *in vitro* investigations of some of the basic processes of wound healing. This paper reviews the current state of investigations in these disciplines with particular reference to wound healing in this specialised site.

Why do trabeculectomies fail? This question, asked by many an ophthalmologist, could perhaps be written as "why do my trabeculectomies fail?" It is perhaps the wrong question, the correct question should be – why do they succeed at all? The natural response of the body to a wound is repair with, eventually, restoration of the status quo. A successful trabeculectomy converts the anterior chamber from a closed system having a one way valve in the angle to an open system bypassing the trabecular meshwork and allowing free exit of aqueous into the sub-conjunctival space.

The question may be re-written once again. It could be, "why do some trabeculectomies fail?" The paragraphs that follow outline this problem and act as an introduction to the experimental studies reviewed in this paper.

Background

It has been known for many years that some types of eye requiring glaucoma surgery would be more likely to have a successful outcome following surgery than others. Thus failure (to establish a filtration bleb and lower intra-ocular pressure to less than 21 mmHg) is more likely in the young patient,[1] in eyes undergoing re-operation, in blacks and in eyes with secondary glaucoma.[2,3] These high failure rates have stimulated many different surgical approaches although no method has yet proved ideal. Failure to control intra-ocular pressure may be deemed as occurring early when it occurs within the first few postoperative months, or late – varying from months to years after the operation. It is to "early failure" that problems with wound healing apply.

Morphology of the Filtration Bleb

Studies of the evolving filtration bleb show that in the first post-operative weeks the conjunctiva and sub-conjunctival tissues are oedematous.[4] A review of sequential photographs of the evolving bleb reveals that initial tissue swelling and hyperaemia slowly settles over the first post-operative weeks. Typically the conjunctiva overlying the trabeculectomy flap shows greatest elevation and least hyperaemia. Under normal circumstances a steady state is reached with slight elevation of the conjunctival bleb and

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dilatation of draining veins (taking an increased fluid load away from the drainage site). This picture is complicated with a limbal based flap because the conjunctival incision sections all the draining conjunctival veins. Until deficient collaterals have opened up to drain fluid circumferentially away from the sclerostomy, the conjunctival incision site shows persistent hyperaemia and congestion.[5] Drainage blebs that subsequently go on to fail show a greater than average degree of conjunctival hyperaemia and (in those eyes with a limbal based flap) forward movement of the suture line towards the sclerostomy. This movement indicates subconjunctival fibrosis with contracture. The development of a Tenon's cyst with a high domed area of conjunctival elevation surrounded by markedly hyperaemic conjunctival blood vessels occurs as an acute event with pain, discomfort and marked ocular hypertension.[6]

Histology

1. *Functioning Blebs*

There are few histological studies of the functioning bleb.[7] The existing material shows varying amounts of sub-epithelial fibrous tissue. However, this does not give any indication as to the changes occurring in the acute post-operative period.

2. *Failed Blebs*

Several studies have looked at the histological characteristics of filtration blebs removed at the time of re-operation.[7,8] One study noticed a difference in the appearance of the trabeculectomy blebs failing early (within the first three months) and late.[8] The characteristic appearance of early failure was the hypercellular response. These specimens showed many activated fibroblasts with microfilaments and stress fibres. This appearance agrees with the clinical features of wound edge contracture and persisting hyperaemia, and is associated with a failure of absorption of aqueous. It seemed that early failure is associated with an exaggerated healing response in the conjunctiva and sub-conjunctival tissues.

The appearances seen with "early failures" are quite different from the conjunctiva obtained at re-operation in cases of late failure. These tissue specimens had a bleb wall of "microkeloid" appearance with collagen lamellae and few cells. Normal capillaries were seen outside the drainage bleb. In these eyes failure would appear to be associated with an increased resistance of aqueous flow through the collagen lamellae possibly because of the deposition of ground substance.

Early failure of filtration surgery would therefore appear to be caused by a massive cellular response within the conjunctiva. Such a response is seen typically in eyes having previously undergone intra-ocular surgery, as well as in young patients, black patients and in eyes with secondary glaucoma. Possible causes for this response may be the pre-existing state of the conjunctiva as well as the quality of aqueous leaving the eye.

The glaucomas noted above form a significant proportion of glaucoma patients undergoing filtration surgery. In order to understand the processes involved, we set out to develop an animal model of filtration surgery which consistently failed.

Animal Models

Early researchers used animal models to experiment with new surgical procedures,[9,10] but functioning blebs were unusual and the operations tended to fail. Animals occasionally remain useful for investigation of surgical techniques such as the formation of an internal sclerostomy using the YAG laser.[11] A more bizarre use of animal eyes was described by Holth who in 1922, during a discussion on the availability of human eyes for histology, stated "As a rule I have obtained permission to hold post mortem examination on promising that the appearance of the face shall not suffer. This I have achieved by using rabbit eyes preserved in formal as prosthesis".[12]

Since modifications of the techniques of trabeculectomy have not significantly improved results,[13] the main interest in glaucoma filtration surgery is the pharmacological modification of the wound healing response so that a drainage bleb is maintained. Animal models are suitable for such investigations as they allow the study of the normal healing pattern by both clinical and histological examination,[14,15,16,17] which can be performed at important time points during the healing process. An animal that has a rapid failure and closure of the sclerostomy is suitable for such investigations because the number of

time points for collection of data is low and the histological appearance at each time point is consistent. Experimental animals also tend to be genetically similar which further reduces variation in the response. Animals have the additional advantage that experimental studies of aqueous dynamics may be performed using invasive techniques.[14,15] These factors enable data to be compared in different groups of animals in which wound healing has been modified by using pre-, per-, or post-operative drugs or irradiation.

In recent years both monkeys and rabbits have been used frequently in such investigations. Both species have suitably sized globes. Monkeys have the advantage that the anatomy is closer to that of humans,[18] but expense of purchase and upkeep restricts the numbers used in studies. They are also difficult to examine without anaesthesia and the anaesthetic may have an unquantified effect on the intraocular pressure and facility of outflow. Capturing the animal for examination can also cause complications such as hyphaema, which may influence the wound healing process.[17] Rabbits on the other hand are much less expensive so that greater numbers can be used. They are also docile and easily examined without anaesthesia, and without stress to the animal or surgeon. Depending on the surgical method used, hyphaema may not be a problem.[14,19]

A major disadvantage of both species is that the eyes are not glaucomatous. Attempts have been made to induce glaucoma artificially,[20] but this itself may be introducing a wide range of unknown factors which may influence the wound healing. A further disadvantage is that it is not possible to assess the vision in animals in the same way as humans so the effects of surgery and additional drugs may not become apparent in an animal population.

Wound healing in animal models

Wound healing in animal models of filtration surgery has been investigated in several centres, including our laboratory.[14,21] The aim has been to identify the normal healing process and the cells responsible for failure so that cells could be targeted and their behaviour modified using drugs or irradiation. In our laboratory, New Zealand white rabbits were operated upon. The features of wound repair were similar to those observed by other centres in cats,[22] monkeys[16,17] and rabbits[15] when examined by light and electron microscopy.

The surgical trauma to the tissues following filtration surgery initiates an acute inflammatory reaction as seen in other tissues outside the eye.[23] Inflammatory mediators arise from the damaged tissues and from extravasted blood, and include serous proteins released into the wound site due to increased vascular permeability,[23] and from aqueous which is modified following the breakdown of the blood-aqueous barrier.[24] The secondary aqueous contains proteins and mononuclear cells.[21] The aqueous passes through the sclerostomy and forms a subconjunctival bleb, and infiltrates the surrounding tissues causing oedema. The increased vascular permeability within the subconjunctival tissues also contributes to the oedema initially.

The aqueous contains fibrin, and this forms a lining on the inside of the bleb wall and can also be identified within the oedematous subconjunctival connective tissues.[21,22] Inflammatory cells are present in large numbers during the initial inflammatory phase and many undergo autolysis. In particular, there are many polymorphonuclear leukocytes, lymphocytes and the occasional eosinophil. Macrophages are numerous and actively phagocytic. They may be identified ingesting erythrocytes, cellular debris and traumatised connective tissue.[21]

Active fibroblasts may be identified as early as three days postoperatively.[21] These cells have some of the features of spindle shaped fibroblasts with stress cables in a few cells.[21] Multiplication of the fibroblasts occurs[15,17,22] and at 10 days nests of dividing fibroblasts are present. Many have the typical features of myofibroblasts[21,22] with stress cables, actin filaments, prominent endoplasmic reticulum, dilated cisternae, and microtendinous projections.[25] The fibroblasts appear to arise principally from the episcleral tissues, subconjunctival connective tissue and from the area immediately adjacent to the extra-ocular muscles.[21]

Synthesising fibroblasts are present at the bleb margin at 10 days and are actively secreting collagen.[15,17,22] As the cellular and tissue debris is phagocytosed by the macrophages, and the inflammatory cells become less evident, the main cell type becomes the fibroblast in a hypercellular scar[17,22] (Fig 1a and b). The bleb

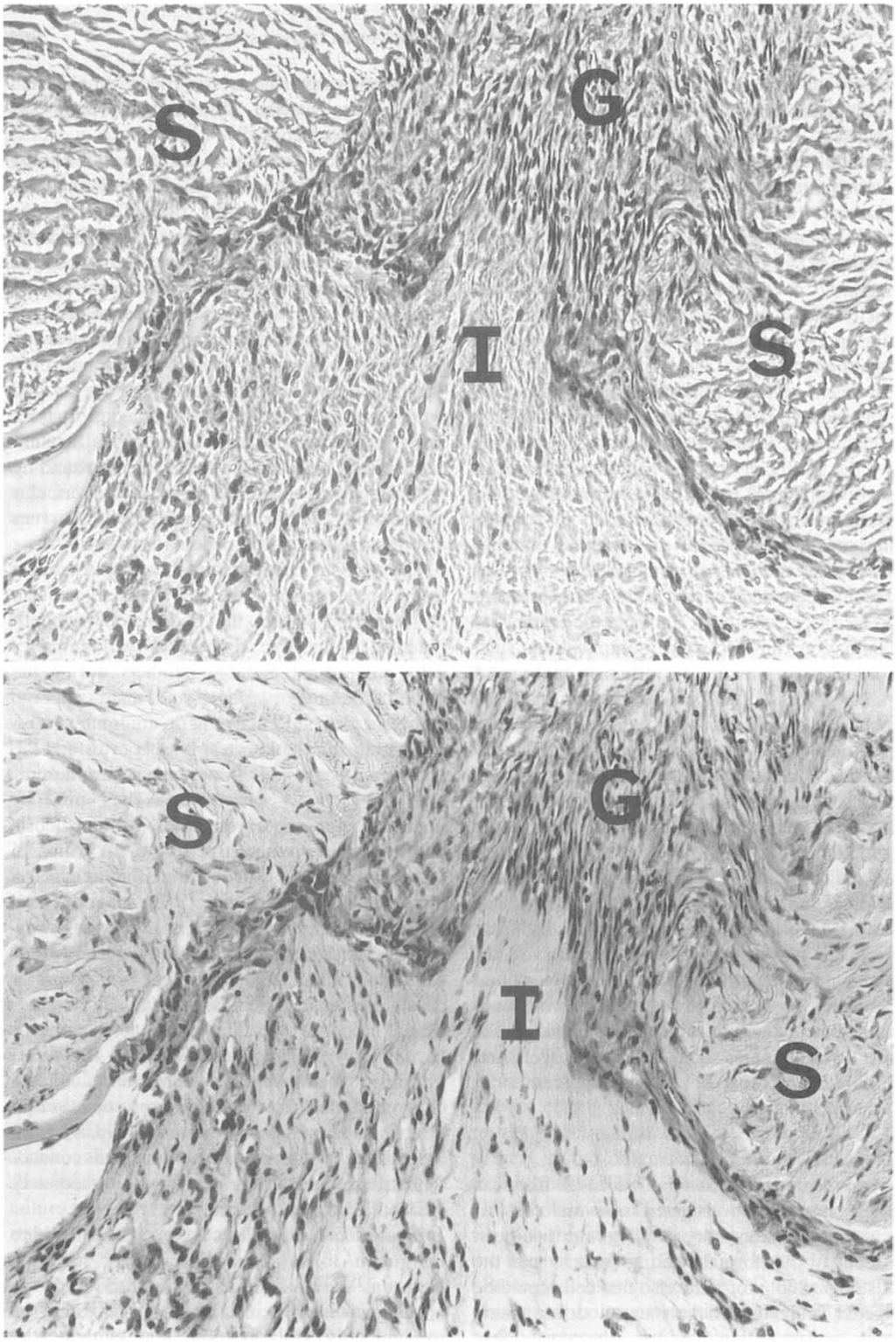

Fig. 1. *Photographs showing the appearance of the sclerostomy at 10 days post-operatively in a rabbit eye. a.) The thermosclerostomy is filled with granulation tissue (G) which adheres to the iris (I). Neither the iris nor the sclera (S) appear to contribute significantly to the granulation tissue. (Haematoxylin and eosin x20). b.) Phase contrast of the same field shows the different orientations of the collagen fibres and demonstrates the sharp borders between the three tissues.*

collapses due to combination of bulk filling by fibroblasts and collagen, and contraction by the fibroblasts.[15,17,21,22]

Remodelling of the newly formed scar tissue occurs and the collagen, which was initially randomly orientated, becomes organised. At 17 days post-operatively, the prominent cell type is the synthesising fibroblast.[21] At 30 days the number of fibroblasts is reduced, The subconjunctival tissues and scar within the sclerostomy have re-established their normal contours.[21,22] Eventually the bulk of the scar is formed by dense collagen with a few scattered fibroblasts.

Modification of wound healing in animal models

Following glaucoma filtration surgery there needs to be sufficient healing of the conjunctiva so that the conjunctival wound does not leak but not so much that the sclerostomy closes. It is fortunate that following the majority of trabeculectomies in patients, this balance is achieved without additional treatment, and a drainage bleb develops.

Filtration surgery tends to fail in animal experiments which indicates that the behaviour of animal eyes is very different to that of humans. Because of this difference, it may not be possible to achieve a permanently draining bleb in an animal even with adjunctive therapy and observations of reduced intraocular pressure supported by altered behaviour of cells may indicate that a drug would be beneficial in humans. If we expect to achieve a perfect result in animals then it is likely that potentially helpful agents for humans will be missed.

Several agents have been used in experiments:

Beta irradiation

In experimental skin wounds, treatment with beta irradiation reduces the numbers of fibroblasts, reduces the amount of collagen formed and delays wound contraction. There is, however, no long term effect on the appearance of the scar tissue.[26] These features are also seen in animal models of filtration surgery. Beta irradiation delays the scar formation and the blebs may be present for a few days longer,[21,27] but in our laboratory there has been no definite evidence to suggest it improves the results of surgery and the histological appearance in the long term is not altered.[21]

Steroids

The reported effect of steroids in animal models is variable. Laval and Coles[28] found that fibroblasts were suppressed in partial thickness incisions but not in full thickness sclerostomies, Seetner and Morin[15] observed a slight delay in wound healing in histological studies and these findings have also been observed in our laboratory. Greater success has been reported with Triamcinolone,[29] but this has not been supported by other centres.[30]

5-Fluorouracil

5-Fluorouracil has been studied in greater detail than other agents because of the encouraging results of the initial investigations. In these studies the time over which the bleb remained functional was increased and a lower intraocular pressure was maintained for longer.[31] The disadvantages of the drug are the side effects, which include persistent corneal epithelial defects, delayed conjunctival healing and systemic toxic effects. Further studies using slow release systems have aimed to reduce these complications.[32,33] They have achieved some success while maintaining the desired effect of increasing the life-span of the bleb.[33,34]

Other drugs

Several other drugs have shown some success in animal models, including bleomycin,[33] which extends the time that the bleb is present by both macroscopic and microscopic examination. D-penicillamine has been examined both alone[19,20] or in combination with dexamethasone[19] or beta-aminoproprionitrile (BAPN).[20] D-penicillamine and dexamethasone were studied in rabbits[19] by assessing the intraocular pressure. However, since a decrease in intraocular pressure occurs following other types of anterior segment surgery in the rabbit,[21,35] the data requires cautious interpretation. BAPN and D-penicillamine used in monkeys with artificially induced glaucoma had a temporary (three days) improvement in the intraocular pressure control.[20] The lack of histological data limits interpretation.

Conclusions from animal studies

Studies using animal models show that wound healing following glaucoma filtration surgery is similar to the pattern of wound healing which occurs in all tissues.[23] There is an inflammatory

phase followed by repair. The factors involved in wound repair are multiple and highly complex, but the main cell type involved in fibrosis is the fibroblast. It is this cell which is responsible for the failure of glaucoma filtration surgery by bulk filling of the bleb cavity and the sclerostomy, and by contraction.

Animal models are expensive and time consuming to establish and only limited numbers of agents which might modify wound healing have been studied. Full investigation of these agents requires detailed clinical examination, including light and electron microscopy. There is a wide range of chemicals that might have an influence on wound healing, and therefore it is necessary to examine the effect of potential agents in tissue culture so that a greater number of substances may be tested before selecting a drug for trials in animals. Experiments have been performed in several centres, including our laboratory.

In Vitro Studies

Fibroblast behaviour can be conveniently considered in the following categories:

Activation
Migration
Multiplication
Contraction
Secretion of matrix

It is somewhat artificial to separate these functions since many of them occur simultaneously. However, each aspect can be regarded as a potential target for the the inhibition of fibrosis and each can be studied separately in tissue culture.

Activation

The process of fibroblast activation is exceedingly complicated. It is poorly understood how an inactive fibrocyte, surrounded by collagen and other matrix components, is converted into an active fibroblast which partakes in wound healing.[36] The fibroblasts which contribute to the fibrosis in a filter do not originate from all the surrounding tissues, and the major response is made by cells in the episclera and Tenon's capsule. Fibroblasts from different sites in the body behave differently,[37] and presumably the different matrix components in different tissues affect the capacity of fibroblasts to respond to activating stimuli, ie. cells within dense collagen (sclera) are less easily able to respond than cells in loose connective tissue (Tenon's capsule). In general terms these stimuli appear to be the numerous products of acute inflammation[38] which include macrophage, lymphocyte, and platelet derived substances.

Anti-inflammatory agents have had some success in improving the success rate of trabeculectomies. Steroids have been shown *in vitro* and *in vivo* to have diverse effects on inflammatory cells, fibroblasts, and collagen formation,[39] and dexamethasone has been clearly shown to be beneficial in trabeculectomies performed for the first time.[40] However, in reoperations its role is relatively limited.[3] Other anti-inflammatory agents such as indomethacin, which inhibits the enzyme cyclo-oxygenase, have been tried as single therapy with no success.[4] The reason for the failure of drugs acting at a single site, to improve the surgical success rate is that there are multiple simultaneous cascades of mediators acting at the site of inflammation. Blocking of a single channel can be circumvented in a complicated pathway and may therefore not affect the ultimate process. Clearly, combination anti-inflammatory therapy which acts simultaneously on a number of "inflammatory pathways" is indicated, as suggested by Molteno,[41] but this has had limited success.[42] An alternative is to use a single drug which is active at a number of different sites of the inflammatory pathways.

Migration

Fibroblast migration is an essential component of wound healing, and interference with this process is likely to have a beneficial effect on trabeculectomies. Exuberant scar formation is a serious problem in many branches of medicine eg. the healing of burns, keloid formation, and intra-abdominal adhesions, and a great deal of work has been done on fibroblast migration at numerous sites in the body, including migration in the vitreous. However, until relatively recently, little was known about the migration of fibroblasts in relation to filtration surgery. In particular, the role of aqueous humour in relation to fibroblast activity was poorly defined. Aqueous has been ascribed both as having a degenerative effect on collagen[43,44] as well as a fibrosing effect.[45] Herschler's laboratory has reported the presence of a fibroblast growth in-

hibitory factor present in normal aqueous humour and absent in glaucomatous aqueous.[46,47]

In a pilot study in our laboratory we have found that normal rabbit aqueous humour is powerfully chemoattractant to rabbit Tenon's fibroblasts,[48] ie. chemical substances in the aqueous attract fibroblasts. This activity was primarily chemotactic in that the cells migrated down a concentration gradient of the chemoattractant, but there was also a chemokinetic component as shown by an enhanced random migration in the mere presence of the chemoattractment. Both are likely to be important in fibrosis. This rather surprising result has now been confirmed by studies using human aqueous.[49] Initially using aqueous humour taken from patients at the start of cataract extractions, we demonstrated a powerful chemoattractant effect on both rabbit and human ocular fibroblasts. Further analysis has shown that the effect is primarily one of chemotaxis with a smaller chemokinetic component. The chemotactic effect is attributable to heat stable and heat labile components, all of which are over 30,000 molecular weight, and which are resistant to strong alkalis but not strong acids. The exact identity of these components has not been defined, but fibronectin, which has been shown to occur in bovine aqueous,[50] and platelet derived growth factor, which has been shown to occur, at least in proliferative vitreoretinopathy, in the vitreous,[51] may play a role.

Comparison of the chemoattractant activity of the control aqueous specimens, derived from patients with cataracts, with specimens from patients undergoing repeat drainage surgery after previously sustaining failed surgery, demonstrated significantly greater activity in the latter group ($p < 0.01$). Patients who were undergoing conventional trabeculectomy after a failure of medical treatment (secondary trabeculectomy) or were undergoing trabeculectomy as a primary procedure, had aqueous chemoattractant activities midway between the controls and the reoperations. The results of a typical experiment are shown in Figure 2 where rabbit Tenon's fibroblasts have been used as the indicator cells. Almost identical results have been obtained with human ocular fibroblasts.

Our studies have not demonstrated whether these changes in the aqueous predated the unsuccessful glaucoma surgery or were caused by it. A prospective study on patients undergoing a first trabeculectomy would be needed to clarify this point. However, because of the relatively low failure rate of trabeculectomies, numerous patients would have to be included. What is important is that all the specimens of aqueous

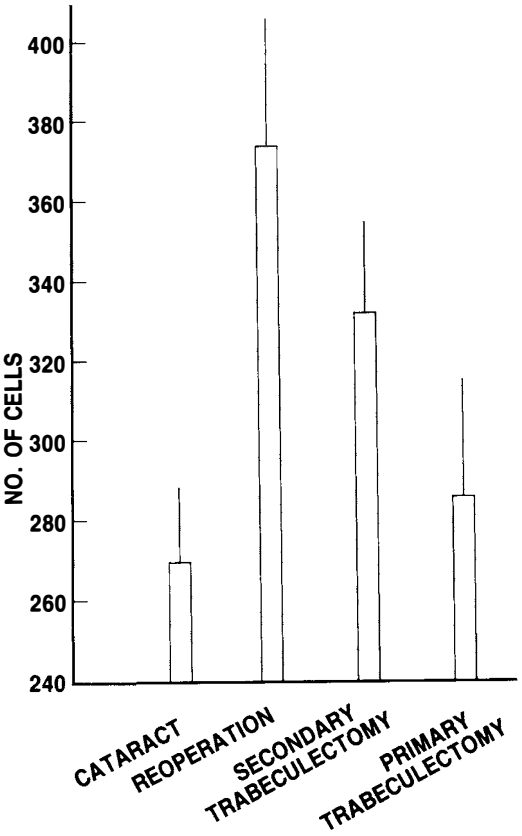

Fig. 2. *Graph showing the chemoattractant activity of human aqueous humour. 4, 10, and 20% concentrations of aqueous humour from each patient have been used as the chemoattractant. The number of rabbit Tenon's fibroblasts migrating at each of these concentrations has been counted and then added together for each patient, to give a migration score, shown on the ordinate. For cataractous aqueous n=11; reoperations n=9; secondary trabeculectomies n=13; primary trabeculectomies n=8; bars are SEM. The chemoattractant activity of the aqueous humour of patients undergoing reoperation is significantly greater than the control cataractous specimens (p<0.01 Analysis of variance).*

humour that we examined were powerfully chemoattractant to fibroblasts, and pharmacological antagonism of this property of aqueous humour is a logical field to explore.

We have been examining a number of potential substances in our laboratory. Colchicine[52] and Cytochalasin B[53] have been extensively studied in relation to wound healing at other sites in the body. We have confirmed that both of these substances inhibit the migration of ocular fibroblasts to fibronectin (which we used as a standard in all our migration studies). We have also demonstrated that when aqueous is used as a chemoattractant, the migration of fibroblasts is similarly inhibited.[54] Oral colchicine was advocated by Molteno[41] as part of his anti-fibrotic regime and it has also been shown to have therapeutic activity in a model of proliferative vitreoretinopathy.[55]

A product of the Yew tree, named taxol, which affects the cytoskeleton of cells, has been used in an experimental model of proliferative vitreoretinopathy.[56] It was also shown to have a high threshold for retinal toxicity. We have demonstrated that taxol powerfully inhibits the migration of Tenon's fibroblasts to both aqueous humour and fibroblasts.[54] This substance also has anticontractile effects (see below) and it is worthy of further investigation in animal models of filtration surgery.

Other substances currently under investigation which have anti-chemoattractant activity include prostaglandin E_2, cyclic-AMP, ritodrine hydrochloride, salbutamol, and trifluoperazine. Some of these will be further studied in the animal model mentioned above.

Multiplication

A great deal of work has been done on drugs that inhibit cell replication.[31,33] Extensive clinical experience has been gained with many of these substances which have been used as anti-tumour and anti-leukaemic agents. Potential substances for use in filtration surgery and in the vitreous were initially screened *in vitro.* These drugs have activity on all rapidly proliferating cells, which accounts for many of their side effects (see above). No specific fibroblast anti-proliferative agent has yet been developed and consequently no anti-proliferative agent is yet at the stage of development where its routine use in every case of drainage surgery could be contemplated.

Contraction

Wound contraction is a fundamental feature of healing wounds and contractures can be serious complications of burns, tendon repairs, and anastamoses of vessels and visci. There is considerable evidence that interfering with wound contraction can retard wound healing,[57] and drugs with anti-contractile activity may prevent the failure of trabeculectomies. As described above, many of the fibroblasts at the site of a failing filtration bleb are myofibroblasts with features of contractile cells. Fibroblasts are thought to generate their contractile force by one of two mechanisms. Either they act like little muscles (hence myofibroblasts) or the movement of the cell membrane functions like that of a tank track in generating traction.[58] However, unlike a tank track, both the top and bottom membranes of a single cell move in a retrograde direction. Whatever the mechanism of cell traction, there is no doubt that fibroblasts are able to generate considerable forces, which are sufficient to detach the retina,[59] and cause contractures across joints.

Various models have been developed to study fibroblast contraction, and include placing cells in collagen gels. After a number of days of incubation, the volume of the cell-populated gel shrinks and the amount it shrinks is regarded as an indication of cell contraction. Similar models have used bovine vitreous as a gel.[60] The problem with these experiments is that the system is not specific enough for cell contraction, since cell proliferation, cell attachment and cell migration are all implicated. Nevertheless, many substances have been tested in systems of this type and have been found to have anticontractile activity. These include taxol, cytochalasin B, and colchicine.[56,61]

Other models of cell contraction have been based on the techniques used to study skeletal muscle *in vitro.* This involves chemically skinning fibroblasts using either glycerol[62] or a detergent, Triton X-100,[63] which leaves the cytoskeletal contractile apparatus exposed. Agents with activity on this contractile mechanism can then be studied. This system is not very physiological since skinning the cells kills them. Also, removing the cell membrane removes the potential for drugs which work via the cell membrane to inhibit the contractile process.

We have developed a more physiological

system for studying fibroblast contraction *in vitro*.[64] This involves observing single living cells under phase contrast microscopy. The cells are in the normal medium in which they grow in tissue culture. Application of adenosine triphosphate (ATP) to the fibroblasts leads to their rapid contraction, which can be reversed and repeated up to at least seven cycles. The cells are photographed and their areas are computed using an image analyser. We have found that taxol and colchicine, but not cytochalasin B have anticontractile activity in this system.[54] These drugs which also have anti-migratory activity may be useful in combination chemotherapy to stop the failure of trabculectomies.

Matrix secretion

Fibroblasts secrete collagen, glycosaminoglycans and mucopolysaccharides which form the connective tissue matrix. A number of drugs interfere with the process of formation of the matrix and have been used in the hope of altering wound healing.

Beta-aminoproprionitrile (BAPN) is a lathyrogenic agent which prevents the covalent cross-linking of collagen by irreversibly inhibiting the enzyme lysyl oxidase. BAPN has been shown to inhibit skin incision healing in rats[65] and to decrease keloid formation.[66] D-penicillamine is also a lathyrogen which interferes with the cross-linking of collagen. Both of these substances have been used after experimental filtering surgery in cynomolgus monkeys[20] with artificially induced glaucoma, as mentioned above. A decrease in the intra-ocular pressure was observed for a few days longer in the treated monkeys compared to controls, but the mechanism of the reduction of pressure is unclear in the absence of histological data. The authors concluded that either their dosing regime was inadequate or that these agents which act only on collagen cross-linking and thus the production of mature collagen, provide too limited a protection against the closure of the filtration opening. They suggested that agents that inhibit the formation of other matrix components eg. glycosaminoglycans may be more effective. However, specific agents directed at these substances are not yet available.

Elevated levels of intracellular cyclic-AMP have been shown *in vitro* to lead to a selective decrease in collagen production,[67] but have not yet been used successfully *in vivo*. Likewise, various amino acids and analogues, essential for collagen production, are being investigated as inhibitors of fibrosis.[68]

From the lack of success of BAPN and D-penicillamine in experimental filtration surgery it seems likely that attempts to inhibit solely collagen formation may be directed too late in the healing process, and that success is more likely by interfering with earlier steps in the process.

Conclusions from in vitro studies

Pharmacological antagonism of wound healing in relation to trabeculectomies, using a combination of agents, active at each of the stages mentioned above is likely to have more chance of improving the success rate of filtration surgery than the use of a single drug. Alternatively, a single drug with a number of different modes of action may achieve the same effect. Of the current drugs under consideration *in vitro* in our laboratory, taxol appears to have the most potential as a therapeutic agent. However, this will require validation in animal models of glaucoma surgery, before its use can be considered in man.

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