Introduction

The concept of a resorbable matrix replaced by autologous tissue is currently gaining acceptance as an effective procedure for tissue reconstruction in numerous organ systems. This technique involves the use of a biodegradable scaffold that the host tissue can use to remodel and regenerate. One particular degradable biomaterial, small intestinal submucosa (SIS), has stimulated research investigation and clinical interest in the last few years. SIS is derived from the submucosal layer of the porcine jejunum and consists of the stratum compactum layer of the tunica mucosa, the tunica muscularis mucosa, and the tunica submucosa [5]. This biomaterial is primarily an acellular extracellular matrix composed of collagen, proteoglycans, glycoproteins, and glycosaminoglycans [8]. It also contains natural growth factors including basic fibroblast growth factor (basic FGF) and transforming growth factor beta (TGF-beta) [31].

SIS has several interesting physical and biological properties: it has good tensile properties [33], is nonimmunogenic [5], is resistant to infection [6], and when used as a xenograft material promotes wound healing by providing a scaffold for tissue ingrowth [24]. As a result, the collagen matrix is degraded and is replaced by an autologous tissue that closely resembles the native structure [5, 11].

Preliminary evaluation of the biocompatibility of the small intestinal submucosa (SIS) biomaterial with the rabbit cornea

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SUMMARY

The early stages of the ocular response to porcine small intestinal submucosa (SIS) transplantation were assessed in a model of anterior keratectomy in the rabbit. After surgery, the operated eyes (n = 17) were examined on a regular basis over a 15-day period and animals were sacrificed at predetermined times for histopathologic evaluation. In all but one animal, the biomaterial was well tolerated, and the corneal response was restricted to a localized neovascularization. The grafted material was epithelialized within 7 days and incorporated into the corneal tissue without inducing granuloma formation. In one rabbit, the corneal inflammation was severe but the biomaterial was maintained in place. Although further studies with longer follow-up periods are needed, these preliminary observations suggest that porcine SIS can be incorporated into the corneal tissue during healing process and may represent a promising graft material for lamellar keratoplasty.

KEY-WORDS : small intestinal submucosa - corneal tolerance - lamellar keratoplasty - rabbit.

RÉSUMÉ

Étude préliminaire de la biocompatibilité d’une greffe lamellaire de sous-muqueuse intestinale dans la cornée du lapin. Par F. GRIGUER, I. RAYMOND et A. REGNIER.

La réaction oculaire à une greffe cornéenne lamellaire de sous-muqueuse intestinale de porc (small intestinal submucosa : SIS) a été étudiée sur un modèle de kératectomie superficielle chez le lapin. Après la chirurgie, suivi clinique des yeux opérés (n = 17) a été fait régulièrement pendant 15 jours et les animaux ont été sacrifiés à des temps prédéterminés afin de réaliser une étude histopathologique des globes oculaires. Les résultats cliniques montrent qu’à l’exception d’un cas, la greffe lamellaire de SIS a été bien tolérée et n’a entraîné qu’une néovascularisation localisée. Le greffon de SIS a été épithélialisé en 7 jours et il s’est intégré dans le stroma receveur sans entraîner de réaction inflammatoire de type corps étranger. Dans un cas, la réaction inflammatoire de la cornée a été marquée mais le greffon n’a pas été rejeté. Bien que des études à plus long terme soient nécessaires, ces résultats préliminaires suggèrent que la sous-muqueuse intestinale est un biomatériau qui peut s’intégrer au processus de cicatrisation de la cornée et pourrait en cela servir pour la kératoplastie lamellaire.

MOTS-CLÉS : sous-muqueuse intestinale - tolérance cornéenne - kératoplastie lamellaire - lapin.
Since 1989, xenogeneic porcine SIS has been successfully used in laboratory animals for arterial or venous replacement [4, 6] and reconstruction of urinary bladder [21 22], cranial dura mater [12], meniscus [13], abdominal wall [11] or Achilles tendon [7]. Porcine SIS has recently become available on the veterinary market and is currently used as a biological dressing for skin wounds in small animals and horses. Although recent reports disclosed encouraging results with the use of porcine SIS for tectonic keratoplasty in one dog [26] and a small series of cats [15], the fundamental question of SIS biocompatibility with the cornea remains unanswered. In an attempt to address this question, a study was designed to evaluate the early phase of the ocular response to a lamellar grafting of SIS in a rabbit model of keratectomy wound. Results from this study were reported as a clinical research abstract at the British Small Animal Veterinary Association Congress in Birmingham, 6-9 April 2000.

Materials and methods

TEST MATERIAL

Under the lamellar flow hood, the grafts were prepared from a sterile sheet of SIS (7.0 cm x 4.0 cm) available for veterinary surgery (Vet Biosist®, Cook Veterinary Products). The dehydrated sheet was divided into discs, measuring 7.0 mm in diameter, with a corneal trephine. The discs were placed in sterile individual vials containing a sterile solution of corticosteroids and gentamicin at a concentration of 100 µg/ml (Gentalline®, Chauvin Opsia SA) and gentamycin at a concentration of 100 µg/ml (Gentalline®, Schering-Plough). The vials with the enclosed disc of SIS were stored at 4°C until use.

ANIMALS AND SURGICAL TECHNIQUE

Seventeen healthy male New Zealand White rabbits without ocular disease and weighing 2.5 to 3 kg were used in accordance with the ARVO Resolution on the Use of Animals in Research. They were kept in individual cages without ocular disease and weighing 2.5 to 3 kg were used in accordance with the ARVO Resolution on the Use of Animals in Research. They were kept in individual cages under standard animal room conditions, fed dry pellets and water ad libitum. All rabbits were anesthetized intramuscularly with 50 mg/kg body weight ketamine hydrochloride (Kétamine 1000®, Virbac Animaux de Compagnie) and 5 mg/kg body weight xylazine hydrochloride (Rompun®, Bayer Pharma). To supplement the general anesthesia, topical 0.4% oxybuprocaine (Novésine®, MSD-Chibret) was applied to the eye selected for grafting.

The eye to be operated was selected at random for each rabbit. A pediatric eyelid speculum was used to expose the globe and stay sutures of 4/0 silk were placed at 6 and 12 o’clock to stabilize the globe. Each animal underwent a central lamellar keratectomy. A 7.0 mm corneal trephine with depth adjustment to 0.2 mm was used to standardize the incision. The circumscribed epithelium and stroma were removed with a Peaufique corneal dissector. A disc of SIS, similar in size to the keratectomy, was removed from the storage medium and placed over the corneal defect with its serosal side (tunica submucosa) facing the recipient bed. The graft was secured to the edge of the corneal defect with 12 simple interrupted 10/0 nylon sutures. Chloramphenicol ophthalmic ointment (Ophtalon®, TVM) was applied on the operated eye immediately following grafting and twice daily for 7 days after surgery.

CLINICAL FOLLOW UP

The recipient eyes were examined by slitlamp biomicroscopy on postoperative days 1, 2, 3, 4, 5, 6, 7, 10 and 15. According to their severity, conjunctival injection and chemosis, oedema of the peripheral cornea and iritis, characterized by miosis and iridial hyperemia, were scored as follows: 0 = absent; 1 = mild; 2 = moderate, and 3 = severe. The extent of corneal vascularization was assessed on the basis of the farthest vascular ingrowth from the limbus as follows: 0 = no corneal vascularization; 1 = 2-mm vascular ingrowth; 2 = 4-mm vascular ingrowth, and 3 = 6-mm vascular ingrowth. Biomicroscopic examination also evaluated the integrity and appearance of the grafted material.

HISTOPATHOLOGIC STUDIES

The animals were sacrificed with an intravenous overdose of barbiturate at 2 (n = 2), 4 (n = 2), 7 (n = 4), 10 (n = 3), and 15 days (n = 6) postoperatively. After enucleation, the operated eyes were fixed in 10% neutral buffered formaldehyde. The corneas with a rim of sclera were dissected from the fixated globes and mounted in paraffin. Sections 6 µm thick were stained with haematoxylin and eosin (H & E), Masson’s trichrome, and periodic acid Schiff (PAS) stains to be examined by light microscopy.

Results

SURGICAL TECHNIQUE

In all cases, surgery was uneventful and technically not difficult because the SIS was tough and thick enough to hold the 100 sutures without tearing.

GROSS AND BIOMICROSCOPIC FINDINGS

Postoperatively, none of the 17 rabbits showed evidence of discomfort and photophobia. Consistent degrees of conjunctival injection and chemosis were evident in all operated eyes in the early postoperative period. The conjunctival injection and chemosis reached a maximum at day 1, with a mean score of 1.65 and 1.35 respectively, and then gradually diminished to disappear by days 10-15 (Fig. 1). In all but one animal (rabbit 12), no corneal oedema was observed and the cornea around the SIS graft remained transparent throughout the follow-up period. There was no evidence of stromal melting around the keratectomy area in any of the operated eyes for the 15 days of the experimental period. In 11 of 13 (approximately 85%) of the cases that were followed for at least 7 days, vessels could be seen invading the peripheral cornea from 4 to 6 days after surgery (Fig. 1). Vascular sprouts arose from the circumlimbal vessels and formed a small band, approximately 5 mm wide, in the superotemporal quadrant.
The neovessels were superficial and few in number. They progressively grew, perpendicular to the limbus and toward the region of the graft, to a length of 2-4 mm which persisted throughout the experiment. Moderate signs of iritis, manifested as iris vascular injection and/or miosis, were present in all eyes for the first 4-6 postoperative days (maximum mean score = 1.53) and subsequently regressed without sequela (Fig. 1).

During the first 48 hours following surgery, there was no visible change in the appearance of the grafts (Fig. 2A). From postoperative days 3-4 to 6-10, the xenografts appeared opalescent and swollen (Fig. 2B). Their superficial layers were deliquescent and sloughed off, forming small whitish debris at the surface of the grafted area (Fig. 2C). Nevertheless, all grafts were retained on the wound bed and became progressively less opaque by postoperative days 8-10. In parallel, their surface became smoother and brighter. By postoperative day 15, the grafts were partly resolved and the grafted areas were sufficiently transparent to see the iris and pupil (Fig. 2D).

In one animal (rabbit 12), a severe corneal oedema (clinical score = 3) with neovessels invading the stroma and a marked uveitis (clinical score = 3) were observed 2 days after surgery. Uveitis improved by approximately 10 days and left no visible signs. On completion of the experiment, the cornea was markedly vascularized (vessels reached 6 mm in length) and slightly oedematous. The grafted area was opaque and partly covered with fibrovascular tissue (Fig. 3).

### HISTOPATHOLOGIC FINDINGS

On the 2nd and 4th postoperative days, a discrete superficial infiltration of the stroma consisting of polymorphonuclear (PMN) cells and scarce mononuclear cells occurred around the wound bed. The superficial layers of the graft appeared as loose collagen sheets coming off from the remainder of the graft material. Epithelial cells surrounding the keratectomy area had begun to proliferate and migrate down the side of the wound and over the external surface of the graft. Few epithelial cells were also present between the loose superficial layers of the graft and at the periphery of the graft-host interface where they appeared as flat, endothelial-like cells.

Seven days after SIS implantation, the stromal inflammatory infiltration had almost disappeared in the middle of the wound and decreased at its periphery. The superficial layers of the graft had been expelled, and the anterior surface of the remaining material was covered with 1 to 2 layers of squamous-like epithelial cells (Fig. 4). By this time, young and activated fibroblasts were observed in the stroma close to the graft-host interface and islets of newly grown epithelium were sometimes found entrapped within the graft (Fig. 4). Thin and superficial vessels extending from the dorsal limbus toward the corneal apex were identified.

In all but one (rabbit 12) specimens obtained on the 10th and 15th postoperative days, a mild residual inflammatory cellular infiltrate was present around the suture sites. At 10 days, the grafts were totally covered with an epithelium composed of one to two layers of squamous cells and a single layer of basal cells that were cuboidal and lightly stained (Fig. 5). Activated keratocytes were present in great number just under the grafted material and were identified between the collagen fibres of the graft remnants (Fig. 5). By 15 days post-grafting, PAS staining sections showed that a new basement membrane was present under the epithelium (Fig. 6). At that time, the corneal thickness was restored and the newly formed collagen fibres appeared more regularly oriented than those of the graft remnants and grossly parallel to the corneal surface (Fig. 7). Histology of the cornea from rabbit 12 showed a severe infiltration of the graft material and surrounding stroma by PMNs and a few mononuclear cells. Remnants of the SIS appeared fragmented and disorganised, and epithelial coverage of the graft was incomplete (Fig. 8).

At each time point of the follow-up, the other structures of the anterior and posterior segments appeared to be histologically normal.
Discussion

Deep ulcers are a common problem in small animal ophthalmology and require prompt diagnosis and adequate treatment. In addition to medical therapy a number of surgical options can be used, each having both advantages and limitations. Conjunctival grafts are probably the most commonly used surgical techniques. Although these procedures have been shown to be effective, they can also lead to scarring and pigmentation of the recipient cornea [18]. Corneoscleral transposition, autologous sliding lamellar keratoplasty, or homologous lamellar corneal transplantation is also useful when there is a risk of perforation, as each of these techniques is aimed to replace the damaged stroma and promote healing with re-epithelialisation [16, 35]. These three techniques have the advantages to allow for better cosmetic appearance and functional vision than conjunctival grafts [16]. As the use of fresh or preserved homologous lamellar grafts may be limited by the difficulty of harvesting and/or storing donor tissue preparation [17], some investigators have evaluated the possibility of repairing corneal ulcers with various biological membranes that are more readily available than homologous corneal grafts. For instance, preserved homologous amniotic membrane has been used in humans to treat deep corneal ulcers [25], and transplantation of preserved xenografts of pericardium [2], renal capsule [1] or amniotic membrane [3] was a successful treatment of experimental corneal defects in dogs. Despite these results, major limitations for clinical application of these techniques appear to be the preparative and storage procedures to which the transplants should be submitted before use [25]. Such drawbacks might be eliminated by using a biomaterial ready to use, such as the SIS marketed for veterinary surgical applications.

In the present study, the lamellar grafting of SIS was found to produce a mild-to-moderate degree of inflammatory adverse reactions, both clinical and histopathologic. Transient conjunctival inflammation and iritis were consistently observed in the early postoperative phase. This non specific response was likely due to the trauma of surgery since it is well known that the rabbit eye has a high sensitivity to the surgical trauma and rapidly responds by an inflammatory reaction, including conjunctival hyperemia, chemosis, iridal hyperemia, and miosis [10, 34]. With the exception of one case, no obvious symptoms of graft rejection were observed since the corneas remained clear throughout the follow-up period and had a developing reaction to SIS that was confined to a moderate neovascularization. Neovessels were produced from a small area of the peripheral cornea and were apparent in 3 to 6 days. This rate of vascularization coincides with other reported observations on corneal angiogenesis induced by xenografts in rats [9] and biodegradable polymers in rabbits [19]. Histologically, a slight degree of PMN infiltration was observed around the keratectomy area in the early postoperative days. There was no evidence of granuloma formation, a finding which is coherent with previous reports on body wall [11] and bladder wall response [20, 22] to SIS implantation. It is likely that there were at least two routes for the leukocytic infiltration. In the first one, the grafted material, although sutured to the cornea, did not provide an effective barrier against the influx of PMNs from tears. In the other one, soluble components within SIS might act as chemoattractants to stimulate the inflammatory reaction. As such, TGF-beta is known to have chemotactic properties to stimulate corneal leukocytic infiltration [32] and angiogenesis [27]. In the current study, the leukocyte infiltration preceded neovascularization and initiation of angiogenesis was very likely a consequence of the leukocytic infiltration, since corneal angiogenesis is reportedly facilitated by the presence of leukocytes in the stroma [28]. This response might be amplified by a variety of growth factors contained in SIS. For instance, it has been shown that basic FGF can stimulate corneal angiogenesis without inducing an inflammatory reaction [19].

The ocular reaction following SIS grafting was severe in only one operated eye (rabbit 12) and overall, many of the clinical features of this case paralleled those found in posterior xenograft rejection [9]. Nevertheless, the SIS graft was maintained in place, and the ocular inflammation became less intense 10 days after surgery. Histologically, the components of the inflammatory infiltrate consisted primarily of PMN leucocytes with fewer lymphocytes and plasma cells. These histopathological features are different from those observed in rejecting corneas, that are characterized by an accumulation of lymphocytes and monocytes [30]. Although current speculation is that there is no inherent danger of an immunologic reaction, because SIS biomaterial does not contain live cells [23], our observation raises the question whether SIS may elaborate an immunogenic response when implanted in the cornea. Further studies will be needed to elucidate this hypothesis. Pre-operative SIS biomaterial contamination or post-operative infection may be other possibilities to explain the inflammatory reaction in this animal. Although these hypotheses cannot be dismissed, the risk of bacterial contamination was minimized by adding gentamicin to the storage solution and applying chloramphenicol ophthalmic ointment during the first postoperative week.

This study also examined the early phases of corneal epithelium and stromal reconstruction following SIS transplantation, and our preliminary results suggest that this biomaterial was incorporated into the cornea during a wound-healing response. During the first postoperative days, the SIS graft seems to serve both as a patch and a graft for re-establishment of the epithelial cells because epithelialisation can take place over, inside and underneath the grafted material. Although the biomaterial was secured to the keratectomy wounds with the more densely compacted side (stratum compactum) external, epithelialisation of the graft from neighboring corneal epithelium was not facilitated in the first postoperative days since the newly formed epithelium was eliminated with the superficial layers of the graft. In comparison, a 7 mm keratectomy wound in the rabbit is spontaneously covered by epithelial cells in approximately 66 hours [37]. In the current study, the epithelium was reestablished above the grafted material at day 7 and stratified from days 10 to 15. At this early stage after grafting, the number of cell layers was less than in a normal rabbit corneal epithelium but despite this, the epithelium showed signs of persistent adhesion, both clinically and histologically. In some animals, islets of ecto-
FIGURE 2. — Appearance of 7-mm diameter SIS grafts sutured into lamellar corneal beds of rabbit eyes: (A) 2 days postoperatively; (B) 4 days postoperatively; (C) 10 days postoperatively; (D) 15 days postoperatively.

FIGURE 3. — Appearance of the cornea from rabbit 12, at postoperative day 15. Neovascularization from corneoscleral limbus, mild residual stromal oedema, and granulation tissue over the grafted area are present.
FIGURE 4. — Histologic section of a rabbit cornea 7 days after SIS grafting. The entire graft is covered with new epithelium. Some epithelial cells are entrapped within the graft (arrow). (H & E stain, 400 X).

FIGURE 5. — Histologic section of a rabbit cornea 10 days after SIS grafting. Stratification of the epithelial layers (Ep) has begun. There is proliferation of keratocytes at the host-graft interface (arrowheads) and within the graft (Gr) remnants. (H & E stain, 400 X).

FIGURE 6. — Histologic section of a rabbit cornea 15 days after SIS grafting. A PAS positive material yielding a distinct linear red staining (arrows) is present along the epithelium-graft interface. Numerous keratocytes have colonized the graft remnants (Periodic acid Schiff stain, 400 X).

FIGURE 7. — Masson’s trichrome stained section of a rabbit cornea 15 days after SIS grafting. The dark blue fibre bundles of the graft remnants (arrows) can be distinguished from the faint blue fibre bundles of the newly formed collagen (asterisks). (400 X).

FIGURE 8. — Histologic section of the cornea from rabbit 12. The SIS graft is largely degraded and fragmented, and the newly formed collagen is disorderly distributed. New vessels and an intense inflammatory infiltrate of PMNs with occasional mononuclear cells have reached the grafted area which is not completely covered with healed epithelium. (Masson’s trichrome stain, 400 X).
pic epithelium were found within the stroma as a result of the epithelial sliding inside and under the graft during the immediate post-operative period. How the presence of ectopic epithelium may affect the organisation of the stromal scar and consequently the potential recovery of corneal integrity remains to be determined. After 15 days, the SIS biomaterial was partly substituted by a relatively clear corneal stroma, and histologically the scar tissue was organised with keratocytes and collagen fibres with an overall sagittal orientation, parallel to the corneal surface. Since orientation of keratocytes reflects the direction of repair and the potential recovery of corneal integrity [29], the regular arrangement of corneal fibroblasts in the scar tissue suggests that lamellar contiguity across the wound might be obtained over time and result in a degree of stromal organisation close to normal. In addition, an effect of SIS on the proliferative and migratory behavior of stromal keratocytes is not unlikely since infiltration of the grafted material by numerous fibroblasts was observed 10 to 15 days after surgery.

Several characteristics may explain why SIS biomaterial allows for the reconstruction of the corneal epithelium and stroma. Its epithelialisation is likely to be due to its collagensous nature, which resembles the surface of a stromal wound bed on which corneal epithelial tissue heals. Collagen I, one of the components of the SIS extracellular matrix [8], is also a biologic signal that was shown to allow rapid and sustained epithelialisation in vivo [36]. Although this process has to be further investigated, it is possible that SIS modifies the proliferative and migratory behavior of stromal keratocytes and thus the synthesis of collagen. Among the many factors that can influence such processes, TGF-beta deserves special attention because it can increase the synthesis of fibronectin and collagens, and their accumulation around cells [14].

In conclusion, with limit of time of this experiment our results suggest that SIS biomaterial causes minimal adverse tissue reactions and allows corneal healing when used for tectonic keratoplasty purpose. Therefore, we believe these findings are encouraging and warrant further study to assess the long term outcome of the grafted material with respect to its potential use for corneal reconstruction.

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