COLLAGEN SHEET AS AN EXTRACELLULAR MATRIX SCAFFOLD FOR THE MANAGEMENT OF CORNEAL ULCERS IN DOGS

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[Received: 12.5.2015; Accepted: 14.11.2015]

The efficacy of collagen sheet of bovine intestinal submucosal origin in the healing of corneal ulcers was studied in six dogs. Surgical manipulations were performed under general anaesthesia and collagen sheet was placed after scarification and/or superficial keratectomy. Temporary tarsorrhaphy was done in all dogs. Oral administration of cephalexin and ocular instillation of ciprofloxacin as primary antibiotic or based on the culture and sensitivity test and flurbiprofen were administered till complete healing. Collagen sheet applied was completely dissolved by 3rd postoperative day. It was well tolerated. and no immune reactions were noticed. Fluorescein dye test became negative by 7th postoperative day and complete epithelization of the corneal defects was occurred by the time. Corneal vascularization developed in all the cases were resolved by the end of the observation period. Complete filling of the corneal defect was seen early in all dogs. In stromal ulcers, the clarity was achieved by 60th day. Corneal pigmentation was the major complication encountered under this study.

Key words: Corneal ulcer, Staphyloma,Collagen sheet, Bovine submucosa.

Corneal ulcers are the most common ocular emergencies in dogs. An ulcer involves the loss of corneal stroma in addition to the loss of epithelium. The brachycephalic breeds like Chinese Pugs are more prone to corneal injury due to their inherited corneal insufficiency and a lack of protective eye consciousness (Raji *et al.,* 2007). In addition, the increased intraocular pressure in them resulted the weakening of the central cornea (Mandell and Holt,2005). Complicated deep ulcers may lead to impaired vision because of corneal scarring or, when corneal perforation occurs, staphyloma and anterior synechia formation (Bedford,1982). Severe ulcerative keratitis may lead to loss of the eye because of endophthalmitis, glaucoma, phthisis bulbi, or some combination of these. In most cases, if prompt and suitable treatment is provided, could achieve reasonable healing even if perforation has occurred (Gilger *et al.*2008). The simple superficial corneal ulcers heal with medical care but the complicated cases were found to take longer course and may adverse the condition (Gilger *et al.*2008). Collagen was found to act as scaffold and thus reduce the healing period of wounds. Collagen sheet prepared from bovine intestinal submucosa is an easily available collagen source and was found effective in enhancing healing of wounds (Raji *et al.,* 2007). Their role in the treatment of corneal ulcers is yet to be established. Hence the study was undertaken with the objective of evaluating the efficacy of collagen sheets of bovine submucosal origin for the management of corneal ulcers in dogs.

Materials and Methods

The study was conducted in six dogs of different age, breed and sex presented with corneal ulcers. The clinical conditions included stromal corneal ulcers with or without complicated by staphyloma (Fig.1).All the dogs were subjected to surgical treatment under general anaesthesia with proper preoperative preparation. Intramuscular injection of atropine sulphate @ 0.045mg/kg body weight and xylazine hydrochloride @ 1.5mg/kg body weight was used for premedication. General anaesthesia was induced with intramuscular injection of ketamine hydrochloride @ 5mg/kg body weight. Following endotracheal intubation, anaesthesia was maintained with inhalation of isoflurane. After initial surgical treatments like debridement / scarification / keratectomy/ iredectomy according to the extent of ulcer, the corneal lesions were

protected with collagen sheet of bovine intestinal submucosal origin prepared in the shape of cornea. Temporary tarsorrhaphy was done to protect the eye and Elizabethan collar was applied to all the cases to avoid self mutilation. In order to counter act infection and inflammation, ciprofloxacin and flurbiprofen eye drops were instilled topically at an interval of 10 minutes four times daily, till complete healing. Topical medications were done up to seventh post operativeday. Cephalexin was given orally at the dose rate of 20 mg/kg body weight twice daily for five days. The postoperative clinical observations were made at weekly intervals for four weeks and later fortnight intervals up to 60th day.

Results and Discussion

After the correction of staphyloma, collagen sheet was placed over cornea to cover the corneal defect. The collagen sheet was retained in position by tarsorrhaphy. Temporary tarsorrhaphy acted as a protective bandage and was useful as a part of treatment for corneal ulcers which was in accordance to Herring (2003), who reported that temporary tarsorrhaphy allowed the postoperative application of topical medication through the medial canthus and improved postoperative healing. It wascalso supported by Moore (2003). The superficial keratectomy removed the acellular zone of hyaline collagen in the anterior stroma thus eliminated the barrier to epithelial healing and strengthened the adhesions of epithelium to the corneal stromal collagen as also reported by Stanley *et al*. (1998) and Moore (2003). Collagen sheet was soaked with gentamicin eye drops, so that it could be applied over the cornea without any air spaces and retained in position (Fig.2). Collagen sheet applied over the cornea was found dissolved completely by third day in all the cases and no remnants were appreciable on the corneal surface. Collagen as a biomaterial graft for corneal repair offered advantages in being inexpensive, readily obtainable and technically straightforward to place surgically as also mentioned by Feathestone *et al*. (2001 and Hollingsworth (2003). No adverse reaction to the material was noticed in any dogs and was well tolerated by them. The advantages of using collagen sheet included good corneal transparency, preservation of corneal integrity and maintenance of vision also narrated by Vanor *et al*. (2007). All the dogs were responded well to the application of collagen sheet after scarification and keratectomy. In every case, the depth and extend of the lesion got reduced and become shallow on 7th postoperative day itself and were completely covered by 28th postoperative day of observation and regained the corneal surface (Fig 3). Factors essential to maintain corneal clarity included lack of blood vessels or

Fig.1 Staphyloma on the day of Presentation

Fig.2 The collagen graft in position

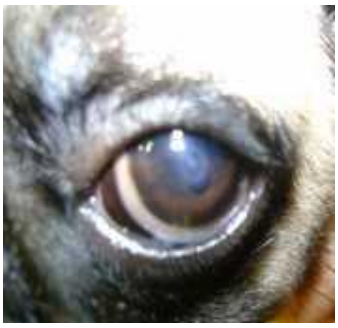

Fig.3.The eye regained corneal clarityon 28th post operative day of presentation

pigmentation, the size and regular arrangement of the collagen fibrils of the stroma and relatively dehydrated nature of cornea as also reported by Morreale (2003). Featherstone *et al*. (2001) reported that during normal stromal healing, type 3

collagen was laid down and was less regularly arranged, thus resulted in corneal scarring and possible visual impairment. Physiological and haematological parameters were within the normal range in all the animals throughout the period of observation. Fluorescein dye test was negative by 3rd postoperative day in three dogs and by 5th postoperative day in one dog and by 7th postoperative day in another dog after the application of collagen sheet. Positive fluorescein test was observed only in one dog on 7th postoperative day and became negative thereafter. The water soluble fluorescein dye does not penetrate the intact lipophilic corneal epithelium, but do stain the intercellular spaces of the corneal stroma when the corneal defects were present as also reported by Helper (1989) and Gelatt (1991). Collagen sheet was acting as a scaffold for the epithelization and resulted in a fast healing. It was in accordance with the observations of Gelatt and Gelatt (1994), that irrespective of the nature of the graft structure, the structure of the recipient bed has to be simulated in the graft as it acts as a scaffold for the host corneal cells to migrate, as a source of corneal stroma and collagen lamellae and not as a source of viable cells. Corneal clarity was regained in two dogs with stromal ulcers and corneal opacity in the centre of the lesion was observed in three dogs and haziness in one dog. Corneal oedema was observed till 28th postoperative day in all the dogs except two, where it resolved after 14th postoperative day. Alteration in the endothelial cells resulted in the cornea absorbing aqueous humor and became oedematous as also mentioned by Gilger *et al*. (2008). Vascularization of the cornea was observed in all the animals and it progressively increased by 7th postoperative day and remained till 14th postoperative day except in one animal where it resolved by 14th postoperative day. Thereafter vascularization was progressively regressed in all dogs. Corneal vascularization is an inportant part of corneal healing as reported by Startup (1984) and Mandell and Holt (2005) also. Corneal vascularization represented an emergency reaction to improve the nutrition of the cells of the cornea in different pathological processes and to support the process of healing as also mentioned by Magrane (1977).

Summary

From the study it was concluded that corneal epithelization and subsiquent healing can be supported with the application of collagen sheet derived from submucosa of bovine intestine. Pigmentation of the cornea was the major complication observed during the study.

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