Doctorate Thesis

Study of Lamellar Corneal Transplantation in Dogs and Cats

Abstract

1997

Soroku Kudo

Surgical corneal therapy is expected to be applied in the clinical practice for the treatment of small animals not only to preserve eye function by covering corneal lesions, but to improve the clarity of the injured cornea and consequently vision improvment. In this experiment, I have performed numerous lamellar corneal allotransplantation to facilitate and improve the procedure to meet the needs stated above. However, the animal rights movement has imposed severe limitations and made it more difficult to secure corneas from dogs and cats needed for these experimental studies. Therefore, as a transplant substitute, esculent porcine cornea were transplanted in to dogs and cats to evaluate modify and improve this experrimental procedure. First the thickness of the each cornea was measured, which is necessary for the successful resection of the corneal lesion, creating grafts and facilitates in the observation and evaluations of long-term follow-up of these experimental dog and cat allotransplants. Next the radius of the curvature of the cornea was determined, which is a necessary step prion to the application of contact lenses to cover the surgical area.

1. Lamellar Corneal Allotransplantation

Lamellar corneal transplantation was performed on both dogs and cats utilizing allografts that has been preserved in glycerine. In the first phase, this basic experimental procedure was performed on three healthy dogs with normal eyes and two healthy cats with normal eyes. In the second phase, this procedure was performed, as part of a clinical study, on healthy dogs and thirteen healthy cats. One eye from each of the experimental animals was allotransplanted and all of these animals initially presented with exciting corneal opacity. This phase was designed to study and evaluate this transplantation procedure efficacy.

The corneal grafts were prepared using the following procedual steps. The eyeballs were enucleated from cadaveric dogs and cats after I had obtained written informed consent from the owners, deceased animals donating these organs for this experimentation. Initially these eyes were rinsed thoroughly with antibiotic solution containing 2% aminobenzyl penicillin. The cornea was then resected to obtained a sclera of 2 mm width. The resected corneas were again rinsed with the same antibiotic solution and they were preserved in sterile glycerine at from 4° to 6 ℃, for period of from six months to two-and-a-half years.

The preparation for the surgical transplantation of the corneal graft began with the soaking of each of the grafts in 100 ml of normal saline. This solution was replaced three times, following twenty minute intervals, to facilitate the washing out of the glycerine preservative from these grafts.

Next the anterior epithelium of the cornea was removed by rubbing the corneal surface with sterile gauze and the graft was trimmed to using a trephine fit in the corneal bed. The lamina propria was resected by horizontally cutting along the circular edge utilizing a golf-club knife to remove Descemet's membrane and the endothelium.

The operation was performed with the experimental animals under general anesthesia and using a surgical microscope.

Eyelid speculum was applied and the operating field was obtained by the application of two to four threads through the conjunctival bulbi. The cornea was resected circularly using a trephine and applying 0.02 ml of fluorescein on its blade after determining the depth of the resection. The incision line, which was stained with fluorescein, was grasped with forceps and incised along the circular line with a golf-club knife.

The corneal graft was applied on the corneal bed and fixed at four points with stay sutures then the circumference was continuously sutured and the four stay sutures were removed. Lincomycin (0.3 ml of 300 mg/ml) solution was injected subconjunctivally. Next therapeutic contact lens were applied over or the eyelids were sutured to cover the post surgical eyeball for our experimental sobject.

An Elizabeth collar was applied to all animal subjects postoperatively. A broadspectrum antibiotic, Ofloxacin, was orally administered twice daily (5mg/kg) and was also instilled into the eyes in an ophthalmic solution (0.3 %). Also 0.5mg/kg of prednisolone, an anti-inflammatory corticosteroid, was injected subcutaneously or given orally for ten to fourteen days, until the corneal epithelium had been covered by the graft. Fluorometrone (0.02 %), a corticosteroid ophthalmic solution, was applied three times daily for two weeks.

The eyelid sutures were removed one to two weeks post-operatively and the corneal sutures were removed after two to six months.

In the five experimental cases that composed the first phase or the basic experiment which consisted transplantation of normal, healthy eyes, the transplanted eyes displayed transitional corneal opacity and neovascularization. However, both the graft and corneal bed cleared up within the first month post-operatively. No rejection reactions were encountered.

The second phase was a clinical study where corneal allotransplantation was performed on a single eye dogs and cats that presented with corneal leisions. These initial conditions included canine superficial corneal dystrophy, superficial corneal scaring and feline corneal sequestration. The grafted cornea generally improved in clarity following the operation. The results showed eleven cases with excellent recovery, seven cases with good recovery, two cases of poor recovery and an unknown case. These results support the contention that this procedure is feasible and appropriate in this type of clinical setting.

2. Measurement of Corneal Thickness

The thickness of the corneal bed tended to vary significantly with each case of corneal transplant and this thickness variation led to a wide variation in post-operative corneal thickness. Thus overall thickness appeared to be significantly influenced by the stage of the healing process, and it is thought that the measurement of corneal thickness may prove to be an important indicator for used pre-operative evaluation.

Therefore, the corneal thickness was ultrasonically measured using a pachymeter for both eyes

of 53 healthy normal dogs to establish a normal standard.

The results indicate that the thickest portion of the cornea was the dorsal part of the corneal margin, which measured 0.636 ± 0.59 mm, followed by the lateral, ventral and medial portions, and the thinnest portion was at the center of the cornea which measured 0.583 ± 0.05 mm.

3. Measurement of the Corneal Curvature Radius

Contact lenses, designed for human use, were applied to cover the dog and cat eyes post-operatively. However, this technique procedure very poor results,as these lenses tended to fall out after a period of several hours to a few days. This development led to the decision to measure the corneal radius curvature of both eyes from 45 dogs using an ophthalmometer.

The results obtained had a mean curvature radius of 8.49 ± 0.19 mm along the vertical axis and 8.58 ±0.18mm along the horizontal axis. There were no detectable variation between the right and the left eyes. A positive correlation ($r=0.58$, $p<0.01$) was discovered between the weight and the corneal curvature radius along both the vertical and horizontal axes.

This result can be applied to determine the appropriate contact lens size for the experrimental animals based upon the specific weight of each of the animals.

4. Lamellar Corneal Heterotransplant

The glycerine-preserved porcine corneal grafts were used for the allotransplantation of lamellar corneal transplants into six healthy eyes of six dogs, to evaluate the clinical applicability of this procedure.

The first experimental surgery was performed on three single eyes of three dogs. The grafts were prepared and preserved (75 days for the first experiments and 150 days for the second, at 4 °C).

The operation was performed utilizing the same procedure as for the allotransplant guidelines.

However, the anti-inflammatory therapy was not carried out to identify problem subjects.These experimental canine subjects were treated with oral antibiotics and antibiotic eye drops for three weeks along the same protocol as for allotransplants. Graft swelling and opacification, corneal bed thickning and opacification, neovascularization and granulation formation were observed post-operatively. Three weeks after the operation, 0.02 % fluorometrone ophthalmic solution was administered three times daily over a period of three weeks. Then, 0.25 % prednisolone succinate eye ointment was applied twice daily for two weeks. In cases in which the granulation did not regress after five weeks of treatment surgical resection of this granulation.

The corneal beds clarified six weeks post-operatively in all the three cases. However, there was one case with ghost vessels, while the rest did not show any regression of neovascularizated vessels. One graft was clear, one was partially clear while the other was opaque. Therefore, two out of three did not gain clarity.

The second experiment was further modified and improved based on the experience and results

gained from the first experiment. In the second experiment, the corticosteroid was initiated a week earlier than for the first experiment. Also 0.02 % fluorometrone ophthalmic solution was initiated two weeks post-operatively and applied three times daily for a three weeks period.

Prednisolone 0.25 % was applied succinate two times daily for a two-week period. As a result, all three cases exhibited live grafting, neo-vessel ghosts, regression in granulation, and clarity in the corneal bed. The graft was partially clear in one case, while the other two had clear grafts. All three animals regained their eyesight.

In the one-week post-operative period, these six corneas became 1.3 to 1.5 times thicker than they were in their pre-operative state. This observed increase in corneal thickness gradually decreased during first three weeks post-operative. The series of processes, including thickening of corneal bed with neovascularization and opacity around neo-vessels followed by granulation formation, were observed. They are proposed as markers or factors for the evaluation and detarmination for the appropriate timing and dosage of the corticosteroids.

Corneal projection using a photokeratoscope demonstrated that one-to-three week post-operative grafts were shaped like a significantly irregular concentric circle with a distorted cornea. However, by eight-weeks post-operative, one of three animals from the first experiment and all three eyes from the second experiment group exhibited grafts with regular concentric circles in which the earlier distortion had been corrected.

The problems I encountered in the allotransplantation and heterotransplantation experimental procedures were solved with the following modifications of the protocol:

1. Rejection reaction can be avoided by using as a graft only the lamina propria, after the removal of the anterior epithelium, Descemet's membrane and the endothelium.
2. Promotion of the regeneration of the corneal epithelium is necessary, by securely suturing the graft to the corneal bed.
3. Systemic administration of corticosteroids started immediately after the operation can prevent the increased thickness of the cornea and neovascularization.
4. Corticosteroid ophthalmic solution should be continuously after the corneal epithelium covers the graft, to improve the clarity of the grafted cornea.

In conclusion, the series of the current experiments suggest that the lamellar corneal heterotransplant using porcine corneal graft can be clinically applied to dogs with success.