Transplantation of Newborn Lacrimal Gland Cells in a Rat Model of Reduced Tear Secretion

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Abstract

Background: Decreased lacrimal gland output may cause dry eye syndrome. Using a rat model, we examined the feasibility of transplanting lacrimal gland cells from newborns.

Objectives: To restore lacrimal gland function in eyes with compromised tear production.

Methods: A model of dry eye in adult rats was developed by unilateral surgical removal of the main lacrimal gland. Tear secretion in both eyes was then assessed by masked Schirmer's test. Lacrimal gland tissue from newborn rats was transplanted into the fibrous connective tissue in which the lacrimal gland had been embedded. Masked Schirmer's test was repeated 4, 8 and 12 weeks after transplantation.

Results: Schirmer's test performed in 13 rats 10 days after unilateral lacrimal gland excision revealed significantly less wetting on the side with excised gland compared with the normal side ($P < 0.003$). The lack of secreting cells on the operated side was verified histologically. The reduction in tear secretion on the operated side remained significant for 8 weeks on average. In the six rats with transplanted lacrimal gland tissue however, there were no differences in tear reduction between the two eyes at 4, 8 or 12 weeks after the operation ($P = 0.81$, 0.56 and 0.8, respectively).

Conclusions: Transplantation of lacrimal gland tissue from newborn rats effectively restored eye wetting in this new model. Further research is needed to evaluate this new approach for treating lacrimal gland dysfunction. Using this model might also facilitate evaluation of potential clinical treatments for dry eyes.

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Dry eye syndrome is a common complaint among middle-aged and older people. The reported prevalence of this condition ranges from 14.4% to 33% [1,2]. In the Beaver Dam Eye Study the overall prevalence was 14.4%, and varied from 8.4% in subjects younger than 60 years to 19% in those older than 80 [2]. Symptoms range from transient mild irritation with minimal ocular surface disease to severe and disabling irritation, persistent itching, redness, ocular fatigue, heavy eye sensation, and visual disturbance with sight-threatening corneal complications [3,4].

Dry eye syndrome results from either decreased tear production or excessive tear evaporation. As the watery component decreases, the tear film becomes unstable, causing progressive deterioration of the ocular surface. The main lacrimal gland as well as secretory components of the ocular surface, including the cornea, conjunctiva, Meibomian glands, and glands of Krause and Wolfring, act as a functional unit. Any compromise of this unified function can cause impairment of the normal lacrimal support of the ocular surface, eventually resulting in dry eye syndrome [5,6]. In cases of decreased tear production caused by Sjogren's or non-Sjogren's syndromes, the primary pathogenesis involves the lacrimal gland [5-8].

Because many cases of dry eye syndrome can be attributed to failure of lacrimal gland secretion, transplantation of lacrimal gland tissue into a non-functioning lacrimal gland might alleviate problems of dry eye, provided that the transplanted cells can proliferate, repopulate the non-functioning lacrimal gland, and become functional. To examine the feasibility of this idea, we used a rat model of dry eye to evaluate the effect of transplantation of lacrimal gland tissue from newborn rats into the connective tissue in which the lacrimal gland had been embedded.

Materials and Methods

Animals

The 32 female adult (90 days old) and 13 newborn (1 day old) Lewis rats used for this study were handled according to the ARVO Resolution on the Care and Use of Laboratory Animals. The research project was approved by the Tel Aviv University Committee for Animal Use in Research. The adult rats were fed *ad libitum*, and the newborns were kept with and fed by their mothers. Before surgery, and before undergoing Schirmer's test, the rats were anesthetized by intraperitoneal injections of 40 mg/kg ketamine hydrochloride (Ketaset, 100 mg/ml; Fort Dodge Animal Health, IA, USA) and 8 mg/kg xylazine (Chanazine, 20 mg/ml; Chanelle Pharmaceuticals Manufacturing, Galway, Ireland). At the end of the experiment the rats were killed by intraperitoneal injection of a lethal dose of pentobarbital (Nembutal Veterinary, Sanofi Sante Animale, Paris, France).

Rat model of reduced tear secretion

All the adult rats underwent unilateral excision of the main lacrimal gland. Surgical procedures were carried out using a surgical microscope (Wild M690, Heerbrugg, Switzerland). The main extraorbital lacrimal gland in the rat is located lateral to the parotid, sublingual and submaxillary glands and lateral to the multilocular adipose tissue, above the neck muscles, just under the subcutaneous tissue. A skin incision was made between the right eye and ear. After dissection of the subcutaneous tissue, the right main lacrimal gland was identified and exposed. A curette was used to remove the gland parenchyma through a 2 mm incision. The subcutaneous tissue was then sutured with 10/0

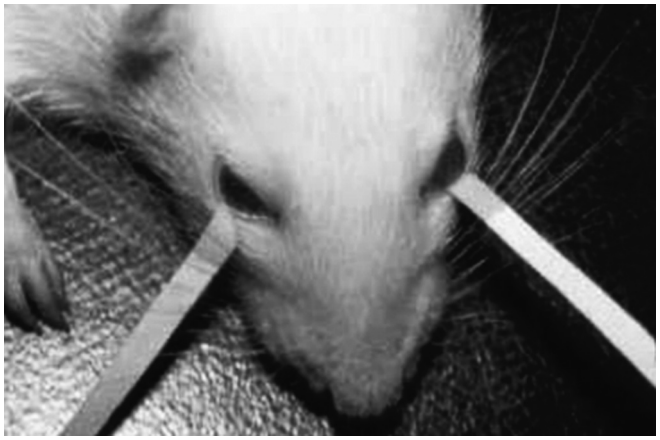

Figure 1. Schirmer's test performed in the rat's eyes

Ethilon sutures (Johnson and Johnson, Ethicon, NJ, USA), and the skin with 8/0 Vicryl sutures (Johnson and Johnson).

Wetting of each eye was assessed by a masked modified Schirmer test, using 2 mm-wide test strips cut from Schirmer test paper to fit the size of the rat's eye. The test strips were inserted 1 mm into the lower fornix for 5 minutes and the wetting distance was measured [Figure 1]. Wetting distances of the eye on the side of the excised lacrimal gland and of the control fellow eye were recorded 10 days and 4, 8 and 12 weeks after surgery.

Transplantation of lacrimal tissue from newborn rats

The lacrimal glands of newborn rats were exposed and their parenchyma was removed and placed in 0.9% saline. After unilateral excision of the lacrimal glands of the adult rats, the fibrous connective tissue that normally surrounds the gland parenchyma was filled with the lacrimal gland parenchyma from the newborn rats and sutured with four 8/0 Vicryl sutures. Vicryl 8/0 sutures were used to close the skin wound.

Experimental design

The effect of unilateral removal of the main lacrimal gland on wetting of the eyes was evaluated in 19 adult rats. Of these, 13 underwent masked Schirmer's test 10 days after the gland was removed and were then euthanized, and in 7 of them the tissue from the area of the excised gland was examined histopathologically to verify the loss of glandular cells. In the other six adults, masked Schirmer's test was performed 4, 8 and 12 weeks after surgery to evaluate the long-term effect of removal of the main lacrimal gland on wetting of the eyes.

After confirming the feasibility of the model by showing diminished wetting on the excised lacrimal glands side, we examined the effect of transplantation of lacrimal gland parenchyma and compared it to a non-implanted control group. Group 1 comprised seven adult rats in which excision of the contents of the right main lacrimal gland was followed immediately by transplantation of lacrimal gland tissue from newborn rats into the fibrous connective tissue that had surrounded the gland. Group 2, a non-implanted control group, comprised six adult rats that underwent unilateral excision and suturing without transplantation. This group was used to examine the possibility of spontaneous regeneration of glandular cells. In both groups, the left eyes were used as normal controls. The results of Schirmer's tests performed in the eye on the side of the operated lacrimal gland at different times after transplantation were compared to those performed at the same time in the control eyes. At the end of the follow-up period, the rats in both groups were killed and tissues from the area of the excised gland were taken for histopathologic examination.

Results

Rat model of dry eyes

The results of Schirmer's test performed in eyes after excision of the parenchyma of the main lacrimal glands and in normal eyes are shown in Figure 2. In the 13 rats tested 10 days after the operation, significantly less wetting of the Schirmer paper strip was seen in eyes on the side of the excised main lacrimal gland than in the control fellow eyes ($P < 0.003$, Student's *t*-test). Histopathologic examination of tissue taken 10 days after surgery from the area of the excised gland revealed no secreting cells.

Examination of the other six rats with excised lacrimal glands showed that the reduction in tear secretion was long-lasting, with significantly less wetting of the eyes on the operated side, 4 weeks and 8 weeks after surgery, than on the side of the normal fellow eyes ($P < 0.04$ and $P = 0.01$, respectively) [Figure 2]. Twelve weeks after gland excision there was still less wetting on the operated than on the non-operated side, but the difference was no longer significant.

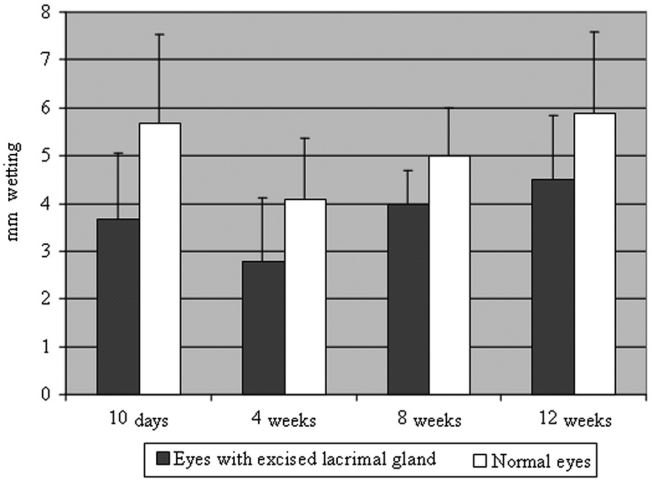

Figure 2. Result of Schirmer's test performed in rat eyes on the side of the excised lacrimal gland parenchyma and in the fellow normal eyes. Significantly less wetting of the Schirmer test paper strip was seen in eyes on the side of the excised main lacrimal gland than in the control fellow eyes 10 days and 4 and 8 weeks after surgery ($P < 0.01$, Student's *t*-test). Twelve weeks after the gland excision, less wetting was found as compared with the normal eyes but the differences were not statistically significant ($P = 0.56$).

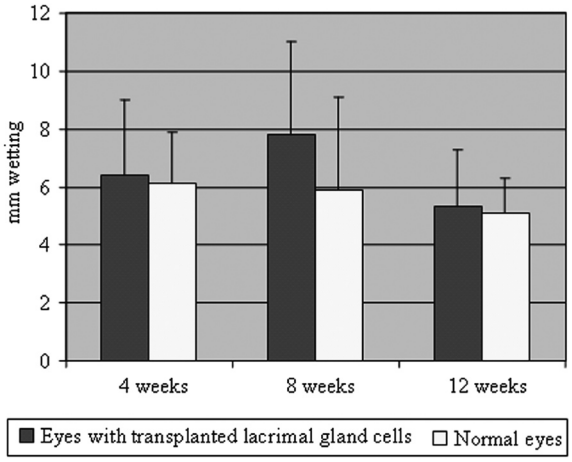

Figure 3. Results of Schirmer's test (in millimeters) after excision of the parenchyma of the right main lacrimal gland followed by transplantation of lacrimal tissue from newborn rats, compared to results in fellow eyes with intact lacrimal glands. At 4, 8 and 12 weeks after the transplantation, there was no statistically significance difference in the Schirmer test results between the groups.

Transplantation of lacrimal tissue from newborn rats

Results of Schirmer's test in the seven rats of group 1 (with transplanted lacrimal tissue) are shown in Figure 3. At 4, 8 and 12 weeks after transplantation, there were no significant differences between the mean test results in the seven eyes on the operated side and fellow eyes (n = 7; P = 0.81, 0.56 and 0.8, respectively). This finding apparently indicates that after transplantation the new lacrimal gland functioned well and that the transplantation was effective.

Histopathologically, 12 weeks after the procedure the transplanted lacrimal gland tissue within the surrounding fibrous connective tissue showed normal acinar morphology [Figure 4]. In contrast, in group 2 rats, which had undergone excision of the lacrimal gland parenchyma and suturing of its surrounding tissue but not transplantation, no spontaneous glandular cell regeneration was detectable.

Discussion

The main lacrimal gland, together with the ocular surface epithelium, tear film, eyelids and pre-ocular tear film, functions to preserve the quality of the refractive surface of the eye, resist injury and protect the eye from dryness [5]. Decreased lacrimal gland output causes damage to the ocular surface, especially the cornea [8]. Although it is generally assumed that the contribution of the main lacrimal gland to the normal basal tear film is limited, some reports indicate that excision of the main lacrimal gland is followed by development of dry eye syndrome [9]. Currently it is accepted that since both the main and the accessory lacrimal glands are innervated they each contribute to reflex secretion [10]. Moreover, tear production is severely diminished by general or local anesthesia, neurotrophic keratitis and reduced ocular sensitivity [11]. Thus, the so-called reflex tearing is probably an outcome of augmented sensory stimulation.

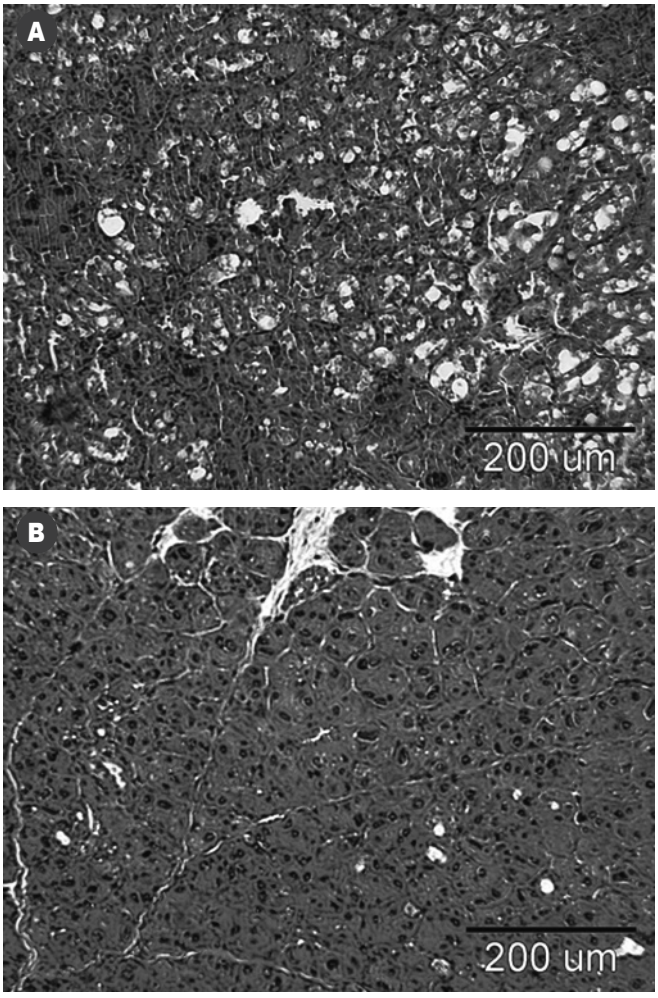

Figure 4. [A] Histologic appearance of the lacrimal gland of a normal eye with acinar structures that contain lumens (hematoxylin & eosin × 100). **[B]** Histologic appearance of normal acinar appearance 12 weeks after transplantation of transplanted newborn lacrimal gland tissue in the connective tissue that surrounded the lacrimal gland (hematoxylin & eosin × 100).

To investigate the effect of the main lacrimal gland in our rat model of reduced tear secretion, we eliminated the fraction of tears produced by the main lacrimal gland by removing the gland parenchyma. This caused reduced wetting of the eyes in both the short term (10 days after the operation) and the long term (8 weeks after the operation). To assess tear production of the transplanted cells we used Schirmer's test, commonly employed in a clinical setting to evaluate tear production. Using a similar rat dry eye model, Fujihara et al. [11] showed that bilateral lacrimal gland extirpation decreased the Schirmer test score by at least 50%.

Other suggested models of dry eyes include prevention of blinking [12,13] and repeated daily instillation of 1% atropine sulphate into rabbit eyes [14]. Recently, a neurturin-deficient mice model showed phenotypic changes and ocular surface inflammation that mimic human keratoconjunctivitis sicca. This model supported the importance of a functional ocular surface/central

nervous system/lacrimal gland sensory/autonomic neural network in maintaining ocular surface health and homeostasis [15]

Our rat model imitates the natural mechanism of reduced tear secretion in dry eye syndrome caused by impaired functioning of the main lacrimal gland due to damage or atrophy. Most of the tear volume in the rat comes from the main lacrimal gland. Additional sources of tears are the Harderian glands, as well as small intraorbital glands which, like the extraorbital gland, produce lacrimal fluid but with less protein. Harderian glands are unicellular conjunctival glands that do not exist in humans. They are located in the medial orbit, lateral to the ethmoid bone, and they secrete mainly mucoid material [16]. The Harderian glands are an important source of saliva and a site of immune response. This gland was not excised in our study because we did not wish to achieve complete absence of tears.

The present model has some limitations. The loss of lacrimal gland function caused by excision of the main lacrimal gland parenchyma differs from the degenerative pathologic processes seen in the main lacrimal gland. The latter are atrophic and progress slowly and gradually, whereas the postoperative loss of function in our model occurs abruptly. Secondary influences of the local regulatory and neural systems in these two situations might therefore differ. It is possible that the immediate transplantation resulted in normal function of the gland, but a delayed transplantation after fibrosis would not be integrated in the adult tissue. Another limitation is the fact that we examined the functioning of the main lacrimal gland only and ignored that of accessory glands.

In patients with severe keratoconjunctivitis sicca, transfer of the autologous submandibular gland to the temporal fossa can provide a continuous endogenous source of ocular lubrication. Such surgical options have been reported by some centers in recent years [17]. A rat model for microvascular transplantation of the submandibular gland, used to assess the feasibility of salivary tissue transfer, showed that the SMG could be transplanted to the eye for treatment of xerophthalmia [18]. In a clinical setting, SMG transplantation for dry eyes was reported to reduce conjunctival and corneal neovascular hyperemia and improve comfort, and the biomicroscopic staging was stable or showed a trend toward improvement [18]. Significant symptomatic relief and an increase in baseline secretion have been reported [17,18]. However, the tears resulting from such transplantation represent condensed SMG saliva, whose quality is intermediate between normal tears and normal SMG saliva. Levels of secretory proteins in these tears are high, demonstrating that the gland maintains an active salivary function [17].

The results of the current study demonstrate that transplantation of lacrimal gland tissue from newborn rats into the lacrimal gland of the connective tissue in which the lacrimal gland was formerly embedded effectively restored lacrimal gland function in adult rats for at least 2 months. Histologic comparison between a transplanted lacrimal gland and tissues in the area of an excised lacrimal gland in a non-transplanted rat clearly showed normal acinar morphology in the former but no gland morphology in the latter. Because the decrease in tear production in this model was significant only when examined at 4 and 8 weeks but not at 12 weeks, we cannot exclude the possibility that some lacrimal gland tissue had regenerated, and it is not clear whether the effects of the excision of the lacrimal gland parenchyma and its function last for more than 2 months. Another explanation may be the compensatory reaction of the accessory tear glands. However, since the rats that underwent excision without transplantation showed no spontaneous glandular cell regeneration after 12 weeks, it is possible that the transplanted glandular tissue was still effective at that time.

Transplantation of tissue from newborns is allogenic in nature. The primary cause of late allogenic failure is an immune-mediated inflammatory process that results from accelerated atherosclerosis and vascular occlusion. This usually develops some months after transplantation and is caused by proliferation of intimal smooth muscle cells induced by a growth factor secreted by lymphocyte-activated macrophages [19]. Systemic immunosuppressive treatments are needed to prevent graft rejection. The possibility of allogenic graft rejection may have reduced the lacrimal gland's function. This is evident by the reduction of Schirmer's values from week 8 to week 12 (from 7.8 to 5.3 mm, respectively). However in our study, not only at 4 and 8 weeks, but also 12 weeks after transplantation there were no significant differences between the mean test results in the seven eyes on the operated side and fellow eyes.

The transplantation of the newborn lacrimal gland cells was into the fibrous connective tissue that normally surrounds the gland parenchyma. In a pilot study of two rats in which the newborn lacrimal gland was transplanted into the empty space after complete removal of the lacrimal gland including its envelope, histopathologic evaluation after 12 weeks showed loss of the implanted gland tissue with only few glandular acini left within fibrotic tissue and inflammatory reaction. This process might represent late allogenic failure.

Selection of the treatment modality for patients suffering from dry eyes depends largely on the severity of the syndrome. The mainstay of treatment is the use of topical tear substitutes [9,20]. Many types of eye drops have been suggested [14,21-23]. Recently, cyclosporine (Restasis®) emulsion was approved by the Food and Drug Administration as therapy for dry eyes. This drug, along with some others, such as steroids, tetracycline and autologous serum, treats the inflammatory aspect of the dry eye syndrome. However, it is effective only if the lacrimal gland dysfunction is not complete and if the inflammation-related degeneration is not too advanced. [24,25]. Other suggested treatments are hot compresses with eyelid massage, discontinuing systemic medication such as beta-blockers, and modification of the patient's environment in an effort to reduce the evaporation of tear film. In more severe cases, humidifier or moisture shields on spectacles, punctual occlusion, or lateral tarsorrhaphy can be used. In cases of very severe dry eyes, even those modalities do not resolve the severe symptoms of dry eyes, and some patients seek a solution by restoring the normal tear film. Regeneration

SMG = submandibular gland

of the gland's natural function as suggested in the present study is an option. However, some questions have yet to be answered, such as: how to reach the source of the lacrimal gland cells, and should rejection be prevented by systemic or by local immunosuppression? Obviously, the indications for this invasive procedure of lacrimal gland transplantation are limited. It would most likely not be effective in cicatricial diseases where the lacrimal ductules have been scarred, or when the new gland is likely to be involved in the disease process that caused the damage or dysfunction of the patient's own gland (e.g., systemic immune conditions such as Sjogren's syndrome and graft versus host disease). It could be a treatment option only for severely suffering patients such as in post-radiation lacrimal gland damage.

In conclusion, we used a new rat model of reduced tear secretion induced by unilateral excision of the main lacrimal gland parenchyma, and utilized Schirmer's test to compare the wetting of the eye on the operated side with that in the eye on the side with an intact lacrimal gland. The use of this model in which there is an actual reduction in tear production enabled us to demonstrate in rats the feasibility of transplanting lacrimal gland tissue from newborns as a possible treatment for dry eyes. Restoration of the function of the main lacrimal gland in this way might suggest a new approach to the treatment of severe dry eye conditions. Further studies are needed to evaluate this possible solution for lacrimal gland pathology causing dry eyes.

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