Thalidomide prevents Corneal Angiogenesis in an Alkali Burn model of Rabbit Corneal Neovascularization

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Abstract

Objectives: To determine if Thalidomide has a similar effect in an alkali burn model of corneal neovascularization, we evaluated the effect of Thalidomide on corneal angiogenesis after alkali corneal burns in rabbits.

Methods: Twelve rabbits received alkali burns to one cornea. Post injury, four rabbits received oral Thalidomide, 200 mg/kg/day and eight rabbits received placebo (powdered sugar) for thirty days. Assessments were made to quantitate the degree of corneal neovascularization (NV), the number of clock hours (CH) of limbus involved in NV, the longest NV pedicle (NVP) length and the duration of time required for NV to develop.

Results: Thalidomide clearly decreased the area (A) of total NV in the test group ($p<0.003$), the number of CH involved with NV ($p<0.006$) and the longest NVP length ($P<0.010$). There was no significant difference in the rate of development of initial NV between the test and the control groups ($p<0.418$).

Conclusions: These findings indicate that Thalidomide is an effective inhibitor of corneal angiogenesis in an alkali burn induced corneal neovascularization model in rabbits.

Clinical Relevance: Thalidomide might be used as a modulator of corneal angiogenesis following corneal alkali burns, to improve the outcome of corneal transplants (JPMA 53:183; 2003).

Introduction

Chemical injuries are potentially devastating ocular surface injuries with possible permanent visual impairment.[1-8] Because of their ability to penetrate the eye, alkalis can damage not only the corneal and conjunctival surface, but also the corneal stroma, endothelium and other anterior chamber structures.[1,7-12] The ultimate outcome is related to the degree of injury to the limbal stem cells, which are vital for subsequent re-epithelialization.[13-17] Complications include poor re-epithelialization, sterile ulcerations, bacterial keratitis, progressive corneal thinning with risk of perforation, elevated intraocular pressure (IOP), chemical iritis, cataract formation, goblet and mucin cell dysfunction resulting in tear film abnormalities and the development of severe corneal neovascularization. Corneal transplantation to replace scarred corneal tissue following alkali injury is complicated by a higher incidence of rejection because of the presence of corneal pannus.[18] A large part of the post injury treatment plan is dedicated to decreasing inflammation and preventing the development of neovascularization using topical immunosuppressive and antiangiogenic agents.

Thalidomide was developed in the 1950's as a sedative. It was nontoxic in rodent models. In 1961 McBride[19] and Lenz[20] described an association between Thalidomide use in pregnant women and limb defects in their offspring. It has been postulated that the limb defects occurred as a result of the direct inhibitory effect of Thalidomide on angiogenesis in the developing limb bud.[21-24] It has been reintroduced into the US market for erythema nodosum leprosum and the skin manifestations of lupus erythematosus. In the past decade, there has been renewed interest in the antiangiogenic activity of this drug. This property is being investigated for activity against cancers of breast, ovary and prostate and multiple myeloma and preventing graft rejection.[25-30] This inhibitory property of the drug has been clinically shown to be beneficial in various ailments of the eye.[31]

D'Amato et al showed that in a dose of 200 mg/ Kg/ Day Thalidomide produced significant inhibition of corneal angiogenesis in the basic fibroblast growth factor and induced corneal neovascularization model in rabbits.[32] Later, Kruse et al showed the same effect when they induced corneal neovascularization with vascular endothelial growth factor.[33] No data was reported on the effects of oral Thalidomide on alkali or chemical injury induced corneal neovascularization. We studied the efficacy of Thalidomide in preventing corneal angiogenesis in an alkali corneal injury model in rabbits.

Materials and Methods

Twelve male New Zealand white rabbits of average age weight 3.31 kg were used in this study. All animals were handled in accordance with the National Institute of Health guidelines on the care and use of animals in research, the Association for research in Vision and

Table. Effects of Thalidomide on alkali injury model of rabbit corneal neovascularization.

	Earliest evidence of corneal neovascularization (Days)		Clock hours of corneal neovascularization		Longest neovascular pedicle length (mm)		Total corneal area involved in neovascularizationon(mm2)	
Rabbits	Test	Con	Test	Con	Test	Con	Test	Con
1	5	6	2.0	11.0	4.5	6.0	20.23	141.11
2	9	5	4.0	8.0	2.0	3.0	24.05	86.39
3	5	7	7.5	10.0	2.5	6.0	56.45	124.19
4	6	6	5.0	11.5	4.0	7.0	49.74	129.59
5	-	5	-	10.0	-	5.5	-	121.22
6	-	5	-	12.0	-	5.75	-	141.37
7	-	6	-	5.0	-	6.0	-	41.23
8	-	5	-	8.5	-	5.0	-	96.93
Mean	6.25	5.63	4.63	9.50	3.25	5.53	37.62	110.25
S.D.	+1.893	+0.744	+2.287	+2.284	+1.190	+1.168	+18.148	+34.059
p-value	<0.418		<0.006		<0.01		<0.003	

Ophthalmology statement for the use of Animals in Ophthalmic and Vision research, and the Brookdale Hospital Medical Center, New York, Institutional Animal Care and Scientific Research Committee guidelines. Adequate anesthesia was obtained by using IM Ketamine and Xylazine. A circular pledgett, 7.0 mm in diameter, was soaked in 0.3 cc of a 1.0 N solution of NaOH and allowed to sit on the central cornea for one minute following which the cornea was thoroughly irrigated with BSS.[34] The eye then received one drop each of Occuflox, Cyclogyl and Pred-forte eye drops and was patched with Tobradex ointment. This being the standard clinical management of such an injury. The post injury care for all the twelve rabbits included Occuflox and Cyclogyl drops till the epithelial defects healed and Pred-forte drops QID for the entire duration of the study. The rabbits were randomly divided into a test and a control group. There were four rabbits in the test group and eight in the control group. From day one post injury the rabbits in the test group received 200mg/kg/day of Thalidomide in a blinded fashion while the control rabbits were fed placebo consisting of powdered sugar. The rabbits had daily examinations to assess epithelial healing, the presence of infectious keratitis, symblepharon formation and the development of corneal neovascularization. Special emphasis was placed on the number of days required to develop the first evidence of significant corneal pannus (defined as at least 0.25 mm of anterior corneal encroachment), the total number of clock hours of limbus involved in neovascularization and the length of the neovascular pannus invading the cornea from the limbus. At the end of the study period of thirty days an assessment of the total area involved in neovascularization was performed using the following equation:[32]

$$C/12 \times 3.1416 [r^2 - (r - L)^2] \text{ where,}$$

C = the number of clock hours at the limbus involved in the neovascular response,

L = length of the longest neovascular pedicle from the limbus onto anterior cornea, and

r = radius of the cornea.

Following this the rabbits were euthanised, the results assimilated and the study unmasked. Data was entered and analyzed using the computer software SPSS version 10.0 for Windows. Independent samples' t-test was used to determine the efficacy of Thalidomide in preventing corneal neovascularization after alkali burn.

Results

Twelve rabbits were used for this study with an average weight of 3.31 kg. There was no significant difference in the first appearance of significant corneal neovascularization. The test group had significantly less clock hours of the limbus involved in neovascularization than the control group. The test group had a significantly smaller neovascular pedicle length compared to the control group. At one-month post injury the test group displayed lesser corneal area involved in neovascularization than the control group. This difference was highly significant, (Table).

Discussion

Alkali burns to the cornea result in a severe corneal neovascular response. The mechanism of this response has been elucidated to some extent. Hypoxic, chemical, thermic and mechanical alterations of the cornea induce an activation of corneal cytomembranes, thus initiating the cyclooxygenase-dependent synthesis of prostaglandins with consecutive vasodilation and increase of vascular permeability as well as histamine liberation resulting in corneal edema. The lipoxygenase-dependent synthesis of leukotrienes induces chemotaxis and diapedesis of polymorphonuclear leukocytes into the corneal stroma. Surviving and regenerating epithelium has an increased

capacity of synthesizing 12(S)-HETE and 12(R), a known direct and indirect angiogenic factor.[35] The lipoxygenase and cytochrome P450-dependent activities increase in a time-dependent manner.[36] There is also an increase in the number of Langerhan's cells that may act as Antigen Presenting cells.[37] These inflammatory cells are then the main source of newly synthesized leukotrienes maintaining the chemotaxis, and prostaglandins with angiogenetic activity. Cyclooxygenase- and lipoxygenase-inhibitors such as Prednisolone can inhibit these activities at two different levels, leading to an approach of successful therapy of corneal diseases inducing neovascularization.

Thalidomide, after hydrolysis is converted to active metabolites, specifically binds to GC-promoters and inhibits expression of b_2 and b_3 integrin subunits by the leukocytes.[38-40] b_2 integrins mediate leukocyte adhesion to the endothelium. In rat models, Thalidomide has an immunosuppressive effect pronounced enough to replace corticosteroids after lung transplantation.[25] It decreases Tumor Necrosis Factor - alpha (TNF-a) production by human monocytes and blocks the VEGF-induced down regulation of caveolin-1, vital for the propagation of endothelial cells.[41,42] Thus, Thalidomide helps in reducing the angiogenic effects of inflammation.

The ultimate usefulness of the eye is dependant on the degree of neovascularization both in determining if a penetrating keratoplasty will be necessary, and if done, the success rate.[18] Present modalities for the treatment of corneal alkali burns include copious irrigation, monitoring of tear pH, debridement of necrotic tissue and the promotion of re-epithelialization with the use of tear substitutes, punctal occlusion, occlusive therapy, bandage contact lenses and tarsorrhaphy. Progestational steroids, citrate, fibronectin and epithelial growth factor have been studied and found to be helpful. Autograft of limbal conjunctiva is effective in restoring corneal surface integrity in unilateral cases. Topical and systemic steroids are presently our only treatment for retarding neovascularization. However steroids do not work sufficiently well in moderately to severely burnt corneas.

This study clearly demonstrates the efficacy of Thalidomide, in conjunction with topical corticosteroids in decreasing the severity of this response when considering the various parameters studied: (1) the number of clock hours involved, (2) the longest neovascular pedicle length, and (3) the total corneal surface area involved in the neovascular response. There was no significant difference in the number of days required to detect the earliest neovascular response in the test and the control groups leading one to believe that Thalidomide did not control the initial neovascular stimulus. This might be due to the multifactorial nature of neovascularization as stated above. This raises interesting questions about the appropriate dose of Thalidomide especially in the immediate post injury period.

There may be some concern about the reproducibility of corneal neovascularization with alkali injury model as compared to the FGF-b and VEGF models. However, our model of injury has successfully been reported previously.[34] Thalidomide has been effective in decreasing corneal neovascularization in both FGF-b and VEGF models of corneal angiogenesis and we decided to assess the same in an alkali injury model, which portrays the cases in clinical practice.

Even though our study group is small, the results sufficiently express the potent antiangiogenic properties of Thalidomide.Topical prednisolone, a known anti-angiogenic drug, was used to remain true to the standard clinical management of such an injury. Our results show that the efficacy of the present management is greatly enhanced after the introduction of Oral Thalidomide. The brief period and the small sample size warrant further investigation to determine the appropriate duration and dosage for this drug.

In summary, Thalidomide in conjunction with topical steroids significantly inhibits the development of corneal neovascularization in the rabbit eye model that has suffered severe alkali corneal injury. Thalidomide does not appear to retard the initial development of micropannus seen for the initial six to ten days, but the overall severity of the neovascular response is significantly decreased. Needless to say, if Thalidomide is ever considered for human testing, its use has to be carefully monitored and restricted to male patients, and female patients who understand the possible complications.

Acknowledgements

We thank Syed Iqbal Azam, Senior Instructor, CHS Department, Aga Khan University, Karachi and Sajjad Akhter, M.D for their help in performing the statistical analysis for this article. We would like to thank Dr. Peter Andrulis of Andrulis Pharmaceuticals for providing the Thalidomide for this study.

The project was funded by George F. Hyman, M.D., Chairman, Department of Ophthalmology, Brookdale Hospital Medical Center, Brooklyn, NY 11212, USA.

The authors do not have any commercial, propriety or financial interest(s) in the products or companies mentioned in this article, nor are they paid consultants for any of the companies involved.

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