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TITLE: Inflammation Modulatory Protein TSG-6 for Chemical Injuries to the Cornea

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13. SUPPLEMENTARY NOTES						
14. ABSTRACT During this study period we initiated testing to evaluate the effectiveness TSG-6 applied intravenously (IV) after corneal alkali burn in the rat model, and completed the setup of equipment and facilities for TSG-6 production for intravenous studies at the Central Texas Veterans Health Care System Research building, after the migration of the Texas A&M Institute of Regenerative Medicine from Temple, Texas to College Station, Texas. We found that lower dose IV TSG-6 applied singly immediately after injury did not provide a statistically significant benefit to the cornea after alkali injury as evaluated by corneal clarity and by biochemical markers of inflammation. Experiments to test the effectiveness of higher dose IV TSG-6, with and without topical TSG-6, are underway.						
15. SUBJECT TERMS cornea, alkali injury, rat, inflammation, chemical burn, TSG-6						
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON	
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1. INTRODUCTION:

This project aims to study the treatment of chemical injury of the cornea with a natural anti-inflammatory protein, TSG-6, which has a novel mechanism of action. Chemical injuries of the eye are difficult to treat, and may lead to severe debilitation or blindness with few patient treatment options. The wartime threat for chemical injury to the eye is evidenced by the thousands of soldiers injured with mustard gas in the Iran-Iraq war. The mechanism of injury after chemical ocular injury includes inflammation secondary to trauma, and may be alleviated with anti-inflammatory agents like TSG-6. TSG-6 works by inhibiting inflammation at the earliest stage, and is effective in the treatment of mild chemical injuries caused by alcohol and mechanical scraping in a mouse model. This study is designed to determine if TSG-6 will be effective with more severe chemical alkali injuries of the cornea, and which would more closely mimic injuries from mustard gas or other severe caustic agents. We will treat rat corneas injured with different concentrations of alkali using topical, anterior chamber, and IV TSG-6 to determine the efficacy and time course of treatment as evaluated by clinical improvement and corneal clearing, and biochemical markers of inflammation.

2. KEYWORDS:

Cornea, alkali injury, eye trauma, chemical injury, anti-inflammatory protein, stem cells, TSG-6, regeneration

3. ACCOMPLISHMENTS:

a. Project Goals

The specific goals of the project for the second year of the project as pertains to the SOW included:

- 1) Specific Aim 2 Subtask 1: Determine the efficacy of topical and intraocular TSG-6 (completed last period)
- 2) Specific Aim 2 Subtask 2: Determine the therapeutic window of topical TSG-6 (topical TSG-6 alone proven ineffective last period)

- 3) Specific Aim 3 Subtask 1: Determine the efficacy and dose response of IV TSG-6 (ongoing)
- 4) Specific Aim 3 Subtask 2: Determine the therapeutic window of IV TSG-6 administration (second and third year goal)

3. ACCOMPLISHMENTS, ctd

b. Goals Achieved

1) Specific Aim 3 Subtask 1: To determine the efficacy and dose response of IV TSG-6, ongoing as of this report. Initial experiments last period for the 1N injury level and a single moderate dose of TSG-6 did not demonstrate a statistically significant benefit with respect to inflammatory markers or corneal opacification as graded by two independent corneal specialists. Based on these results, an amendment was submitted and approved to increase the dose of TSG-6, and to increase the number of injections after application.

Rat corneas were exposed to 0.5N NaOH for 30 seconds using filter paper soaked with NaOH, and then the corneas were thoroughly rinsed with 50cc of BSS. The corneas were photographed for clinical scoring at varying time points up to seven days, and the corneas were collected for cytokine PCR assay for RNA at varying intervals after injury. TSG-6 was delivered through tail vein injection immediately after injury, and at 6 and 24 hours. Initial results do not show a statistically significant benefit, and further testing with increased dosing of TSG-6 are underway. **Appendix 1, p11-16.**

2) Specific Aim 3 Subtask 2: To determine the therapeutic window for IV TSG-6 administration. Once efficacy of IV TSG-6 is established this period, the therapeutic time course of IV TSG-6 application will be determined with TSG-6 produced at the Central Texas Health Care System facility. We believe that TSG-6 given in the intravascular space will reach viable cells better than can be achieved with topical or anterior chamber application after alkali injury and could have therapeutic benefit.

3) The Central Texas Veterans Health Care System (CTVHCS) invested \$100,000 in new equipment and provided new laboratory space Bldg 205, Room 3R34 in support of this project, and to establish a new production site for TSG-6. This investment was necessitated by the move of the Texas A&M Institute of Regenerative Medicine from Temple to College Station, Texas. TSG-6 production is now being accomplished by our team in Temple at the CTVHCS facility for use in IV application of TSG-6 following corneal alkali injury in rats. **Appendix 2, p17-21.**

c. Training Opportunities and Professional Development

Nothing to report

d. Dissemination of Findings and Results

1) Paper presented February 2016 at the World Ophthalmology Congress in Guadalajara Mexico titled: **Comprehensive Profiling of Alkali Injuries to the Cornea. Appendix 3, p22-23.**

2) Research presentation of results to date and future plans, Texas A&M University Health Care System Temple Campus Research Strategic Planning Seminar, September 16, 2016.

3) Paper tentatively accepted for publication pending revision, **Comprehensive Profiling of Alkali Injuries to the Cornea.** Experimental Eye Research.

4) Paper submitted for presentation, acceptance pending, **The Efficacy of TSG-6 for Acute Corneal Alkali Injuries in a Rat Model.** American Society of Cataract and Refractive Surgery Annual Symposium, Los Angeles, California, May 5-9, 2017. **Appendix 4, p24-25.**

e. Plans for Next Reporting Period

1) We plan to continue to test and complete the study of the efficacy of intravascular (IV) TSG-6 in the treatment of corneal alkali injuries. We have amended the study to allow for increased dosing with multiple IV applications. We also plan to study the efficacy of combined IV and topical application of TSG-6, and will determine the therapeutic window of application if efficacy is established.

2) We plan to produce TSG-6 in the newly established laboratory space at CTVHCS with the new equipment purchased by the VA in the quantities required for IV testing.

4. IMPACT

a. Principal Discipline

The results of this work for this report are the most encompassing and complete of any other reported study that we are aware of for chemical alkali injuries to the cornea, and will serve the ophthalmic community in the future as a benchmark model. The complete description of time course of injury with inflammatory cytokine and protein expression, clinical evaluation, and histopathology correlates to 21 days post injury is not duplicated in the literature, and is why we believe our abstract was accepted for paper presentation at the prestigious World Ophthalmology Congress in February 2016. If we are able to establish efficacy of IV TSG-6 in this model, it would provide the first effective pharmacologic treatment for severe chemical trauma to the eye.

b. Other Disciplines

The results of this work, albeit with lack of demonstrated efficacy of TSG-6 to date with topical or anterior chamber application, will help investigators in the field of Regenerative Medicine focus on alternate methods of delivery, and to demonstrate that while TSG-6 has been shown to be effective in a model of mild chemical corneal injury induced by ethanol, it is insufficient to benefit more severe ocular alkali chemical injuries when applied topically or in the anterior chamber, and may require intravenous application with or without topical application to be effective.

c. Technology Transfer

Nothing to report

d. Impact on Society

Nothing to report

5. CHANGES/PROBLEMS

a. Changes

1) During the reporting period, we found that a single modest IV application of TSG-6 was ineffective to reduce inflammatory cytokines or corneal opacification. We amended the protocol to allow for higher doses, and multiple applications post injury to determine if increased dosing of TSG-6 would prove efficacious in the treatment of severe corneal alkali injury. The amended protocol was approved IACUC on 12/7/2015, and subsequently by ACURO on 12/17/2015.

b. Actual/Anticipated Problems

1) There were no actual problems that resulted in delays for this reporting period.

2) We anticipated potential logistical issues related to the move of Dr. Prockop and the Texas A&M Institute of Regenerative Medicine from Temple to College Station, Texas. Inasmuch as Dr. Prockop and his team are consultants on our project, and we had previously shared his laboratory space and equipment, and the TSG-6 that his team formerly produced, his move had potential to impact our study. Fortunately, we have been established our new laboratory in the Research facility at Central Texas Veterans Health Care System (CTVHCS) in Building 205 Room 3R34, and CTVHCS has invested \$100,000 to procure the equipment needed for TSG-6 production. Dr. Prockop and his team will continue to collaborate as consultants even with their move to College Station, and we will continue to collaborate and meet through Skype, conference calls, or onsite visits.

c. Changes in Expenditures

Nothing to Report

d. Changes in Animal Use

No changes to report other than that reported under **a.** above

6. PRODUCTS

a. Publications/papers/presentations

1) Paper presentation **Comprehensive Profiling of Alkali Injuries to the Cornea**, presented at the World Ophthalmology Congress, February 2015.

2) Paper tentatively accepted for publication pending revisions, **Comprehensive Profiling of Alkali Injuries to the Cornea**, in Experimental Eye Research.

3) Research presentation, Annual Texas A&M Health Science Center Temple Campus Research Strategic Planning Seminar, September 16, 2016.

4) Paper submitted for presentation, acceptance pending, **The Efficacy of TSG-6 for Acute Corneal Alkali Injuries in a Rat Model**. American Society of Cataract and Refractive Surgery Annual Symposium, Los Angeles, California, May 5-9, 2017.

b. Websites

1) Central Texas Veterans Research Foundation <http://www.ctvrf.org/research-programs>

c. Technologies/Techniques

Nothing to report

d. Inventions/Patents/Licenses

Nothing to report

7. PARTICIPANTS/COLLABORATING ORGANIZATIONS

a. Individuals

1) Name: Hosoon Choi, PhD

Project Role: Research Scientist

Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 23
Contribution to Project: Dr. Choi has performed rat surgery to treat corneal alkali injury, performed chemokine and cytokine assays, and protein production and purification

2) Name: Casie Phillips
Project Role: Animal Technician, research assistant
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 22
Contribution to Project: Casie has assisted Dr. Choi in animal surgery, performs postoperative care, assists with laboratory techniques, prepares and stains histology specimens

3) Name: Samuel Fulcher, MD
Project Role: PI
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 24
Contribution to Project: Dr. Fulcher performs the duties of the PI, and scores the clinical injuries by grading photographs of the corneal opacity

b. Collaborating Institutions

1) Texas A&M Institute for Regenerative Medicine, Temple, Texas. Dr. Darwin Prockop and his team have served as unpaid consultants.

2) Central Texas Veterans Research Foundation, Temple, Texas. CTVRF is the sponsoring agency.

3) Central Texas Veterans Health Care System, Temple, Texas. CTVHCS is the employer of Dr. Fulcher, and has allotted time for research activities, and has supported the project with new laboratory space and equipment.

4) Baylor Scott and White Health Care, Temple, Texas. BSW is the sponsor of the IACUC, and operates and maintains the vivarium where experiments are performed, and employs the supervising veterinarian who oversees all animal research projects.

8. SPECIAL REPORTING REQUIREMENTS

a. No Collaborative Awards to report

b. Quad Chart, Appendix 5, p26-27.

9. APPENDICES, SEE APPENDICES A1-A5

Appendix 1

Corneal Injury Model

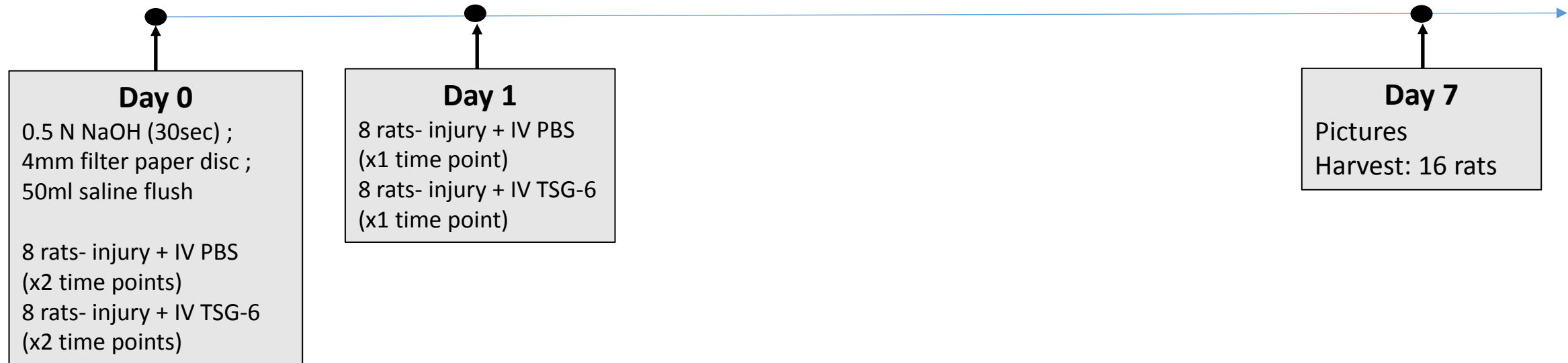
2014-013-R

- **Date of study:**
1-22-16 through 1-29-16
- **Number of animals used:**
8 rats: 0.5 N NaOH injury + 50ml saline flush + IV PBS
8 rats: 0.5 N NaOH injury + 50ml saline flush + IV TSG-6 (0.2mg per time point)
= 16 Lewis rats total
- **IV Injection time points:**
 1. Immediately following injury
 2. 6 hours after injury
 3. 24 hours after injury
- **Harvest day / number of animals:**
Day 7 harvest / 16 rats



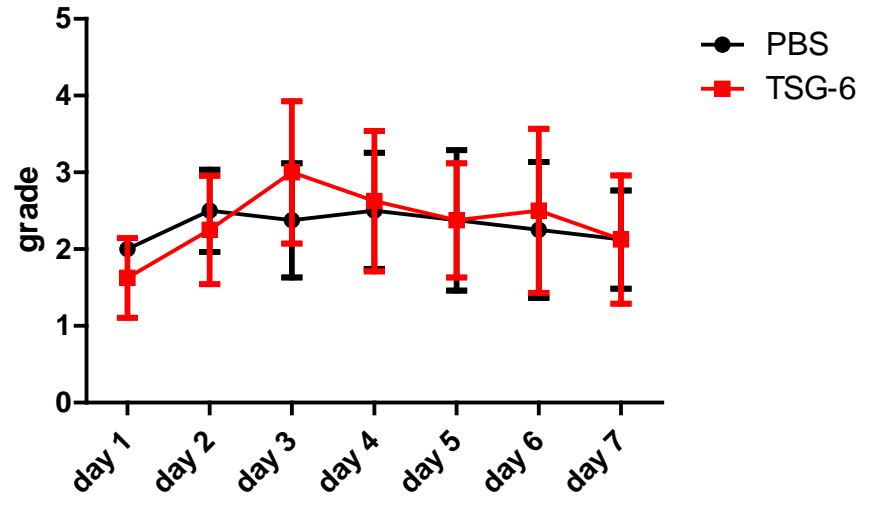
Alkali Injury of Rat Cornea

data reflects 1-22-15 injury date

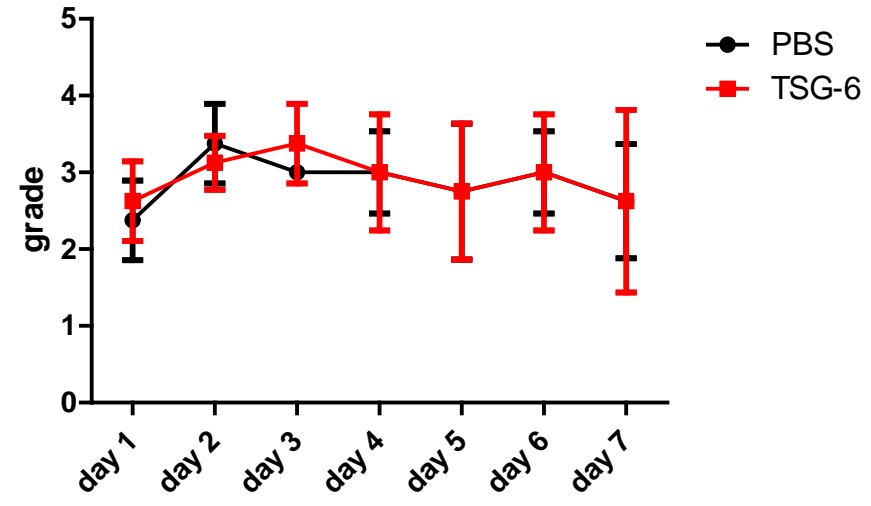


* Photographic data was recorded daily for each rat until time of harvest

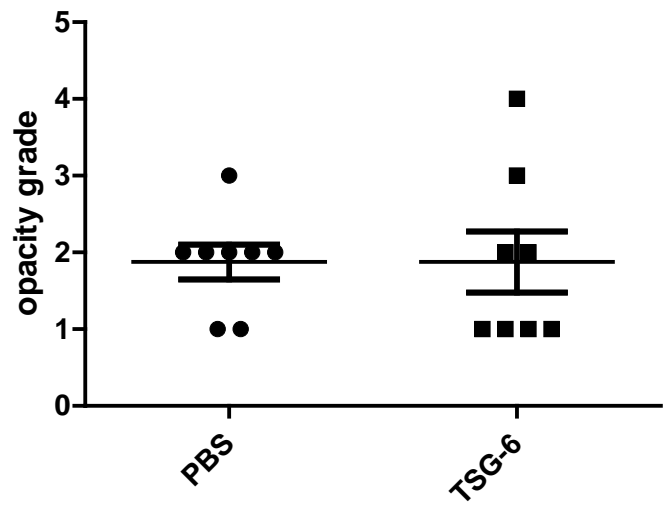
Opacity (Dr. Fulcher)

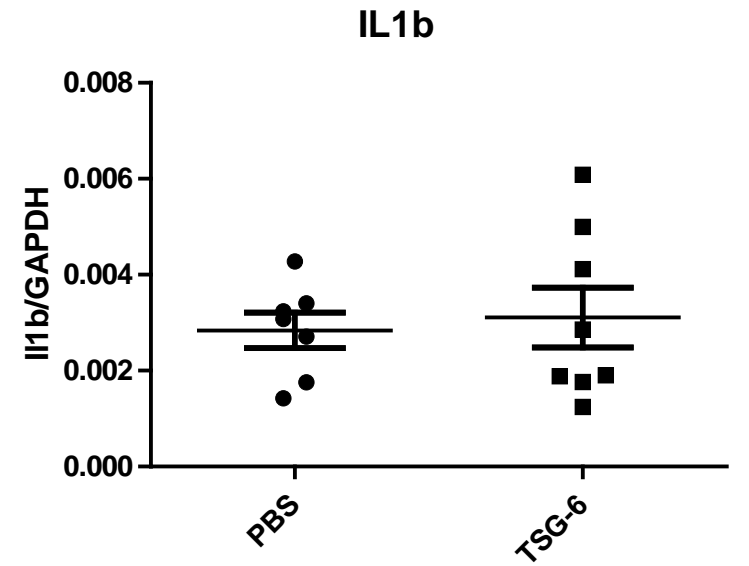
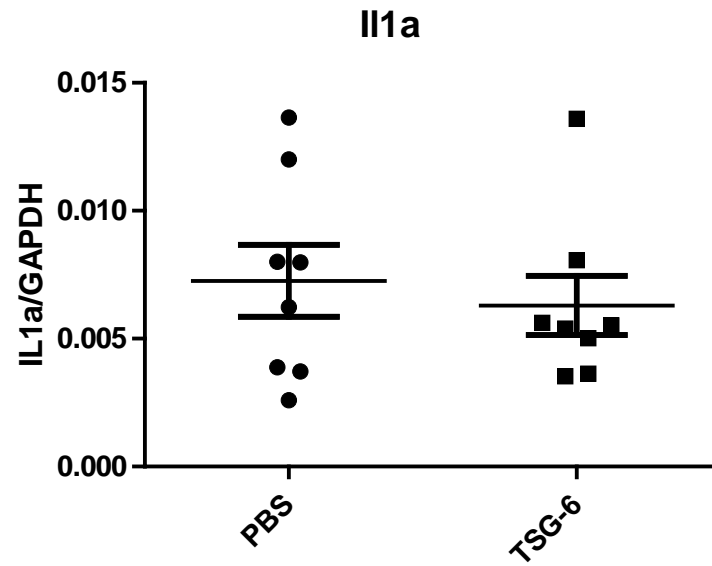
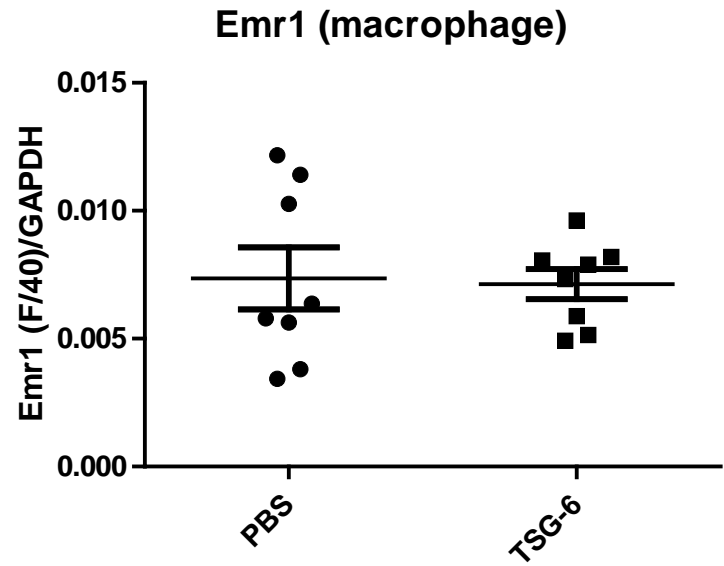


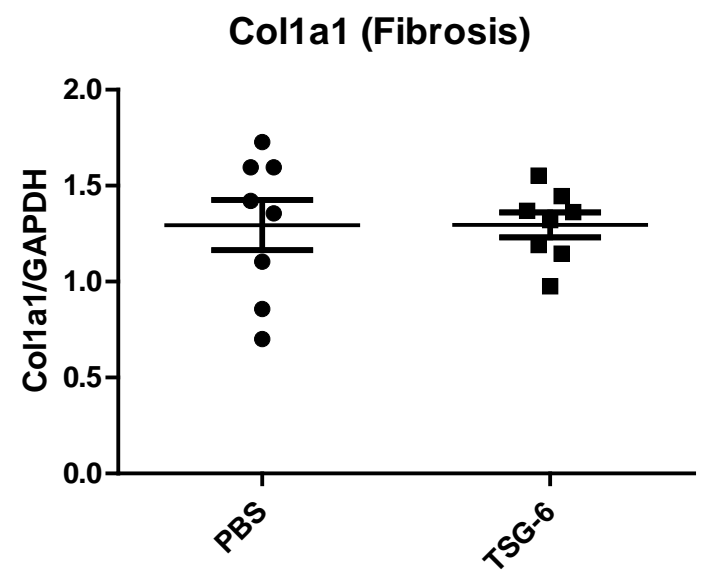
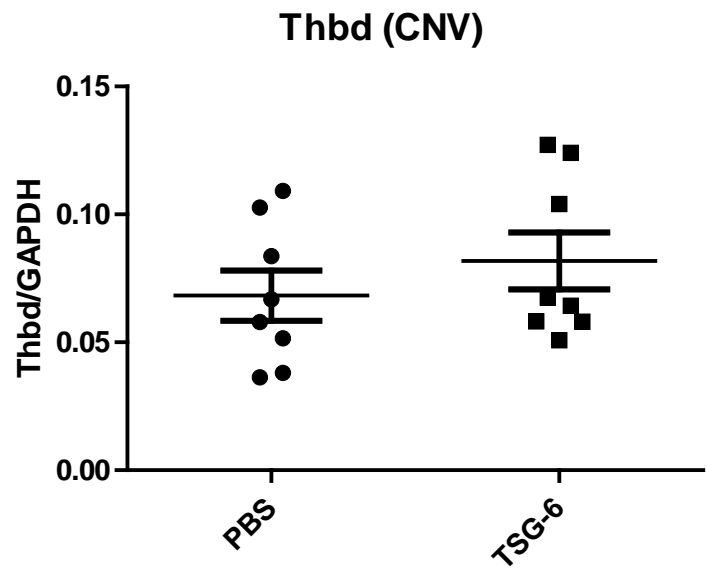
Opacity (Dr. Oh)



Microscope grade

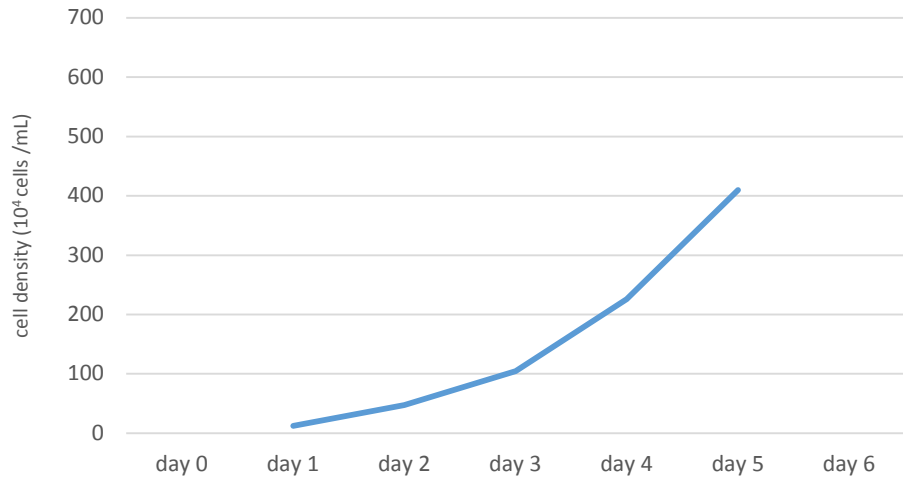




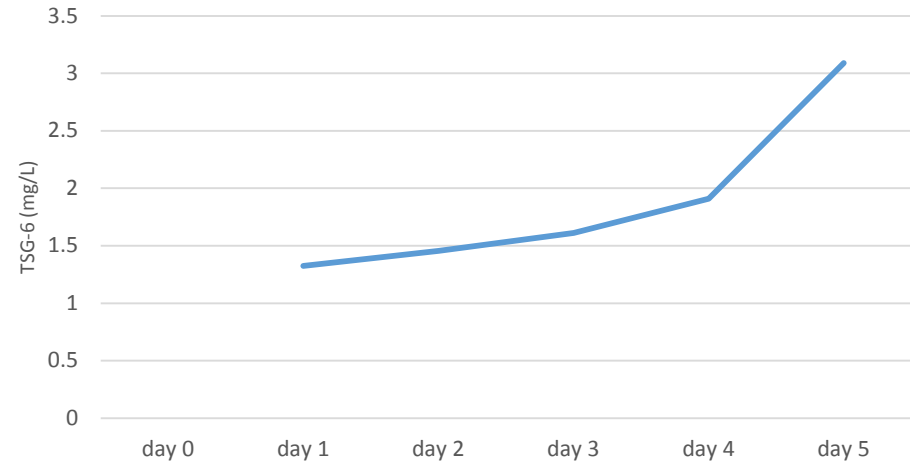


Appendix 2

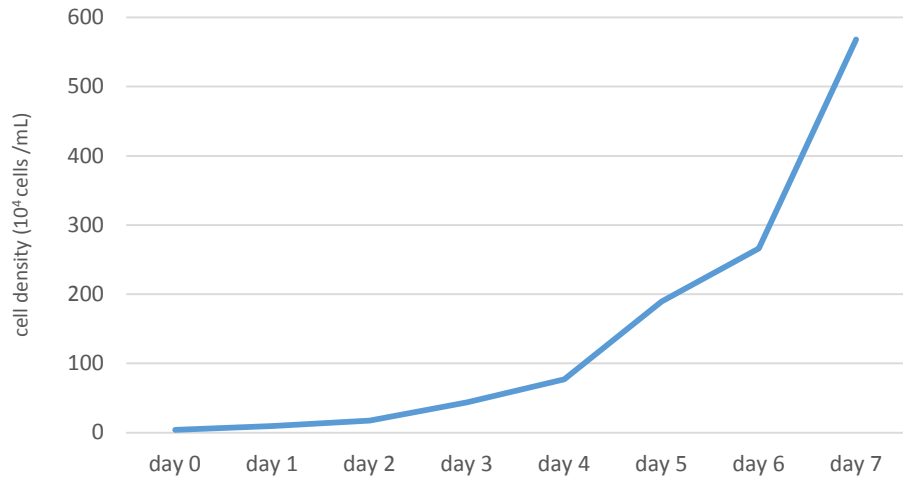
Bio Reactor_7



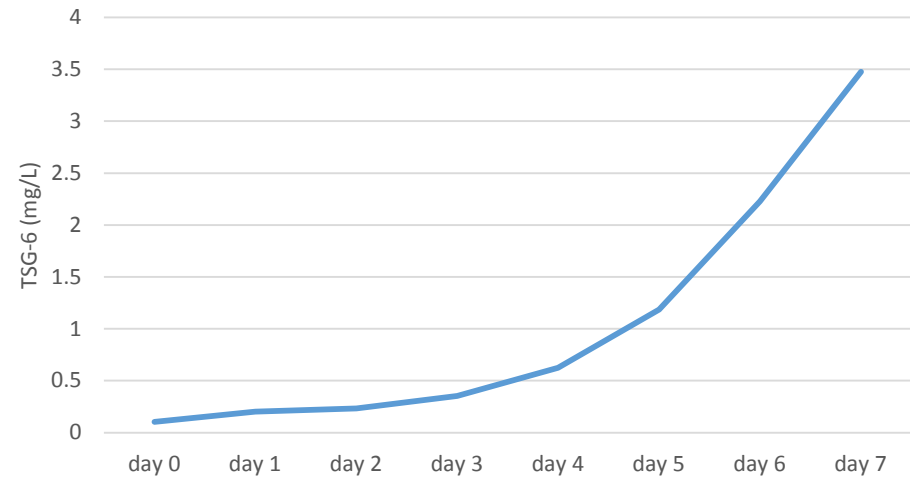
TSG-6 (BioReactor_7)



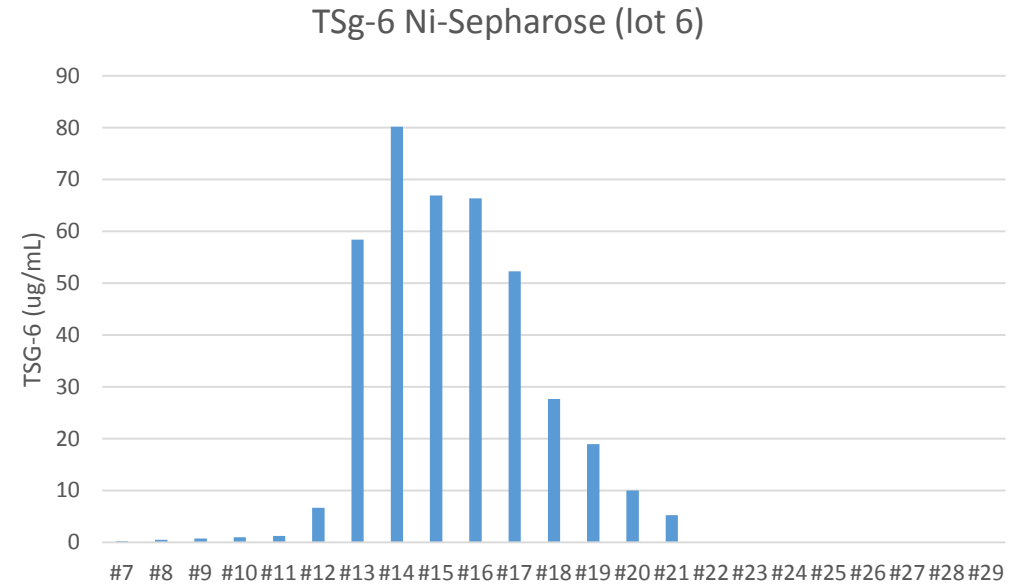
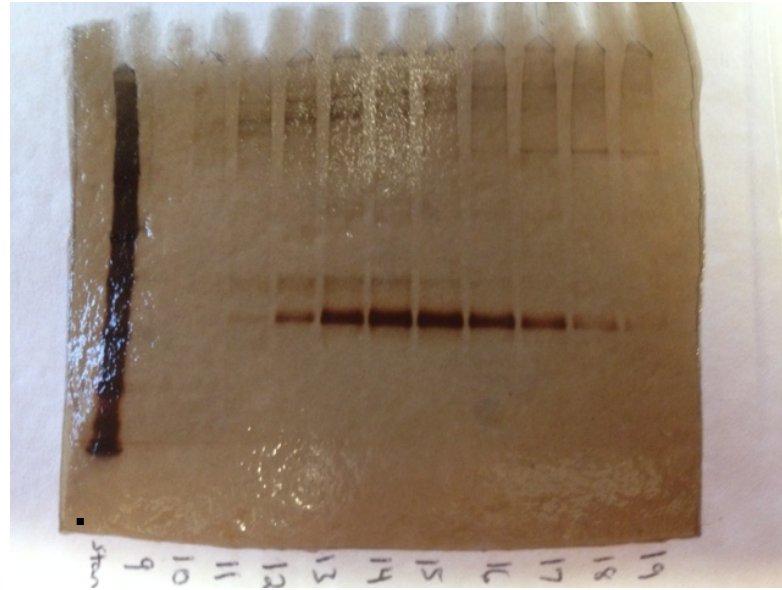
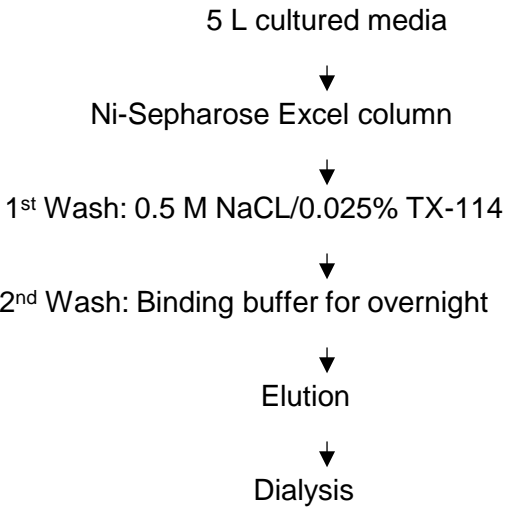
Bio-Reactor_8



TSG-6 (BioReactor_8)

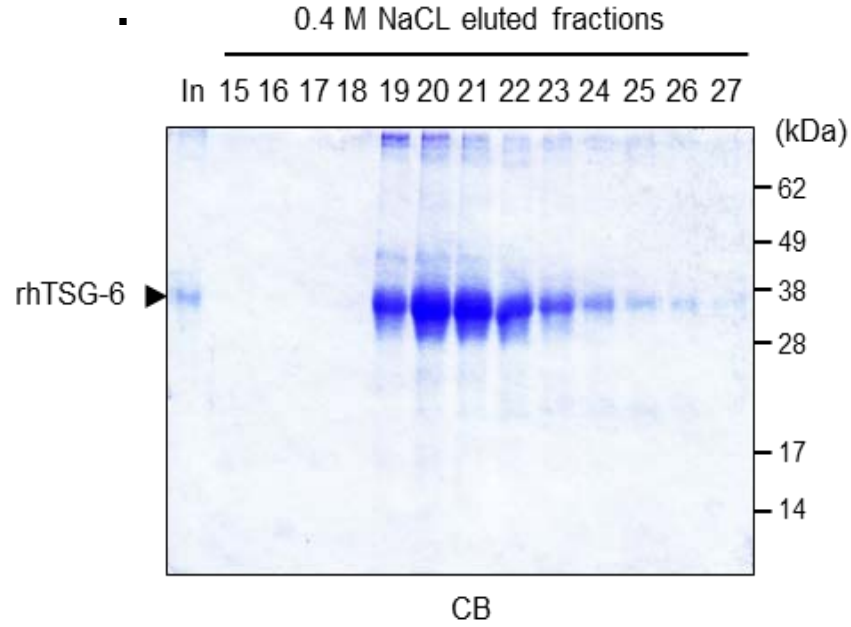
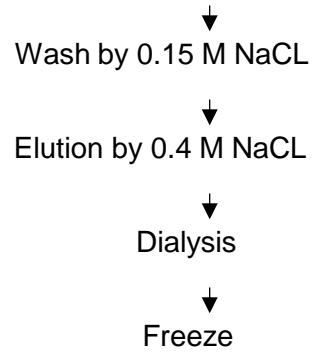


Ni-Sepharose column (1st Column)



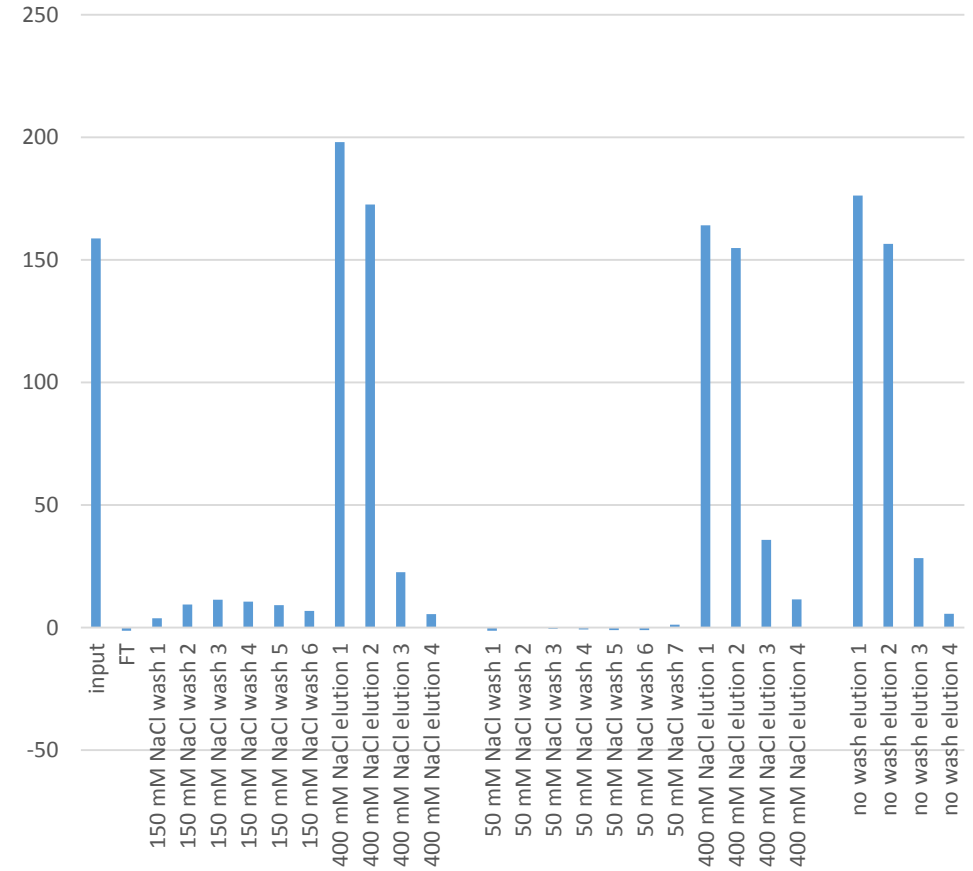
Q-Sepharose FF column (2nd Column)

Q Sepharose FF column

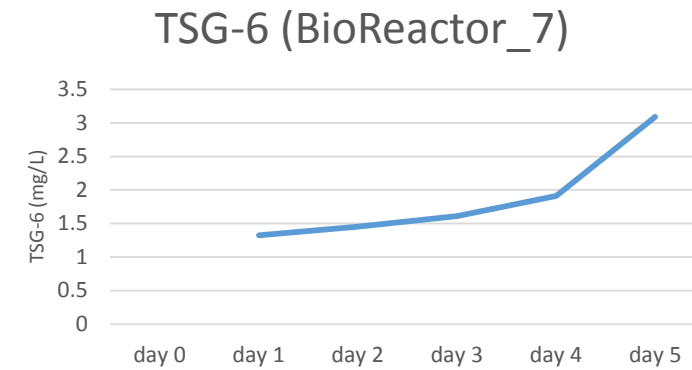
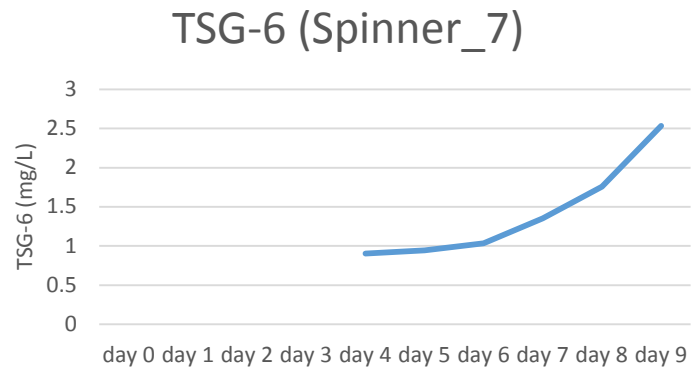
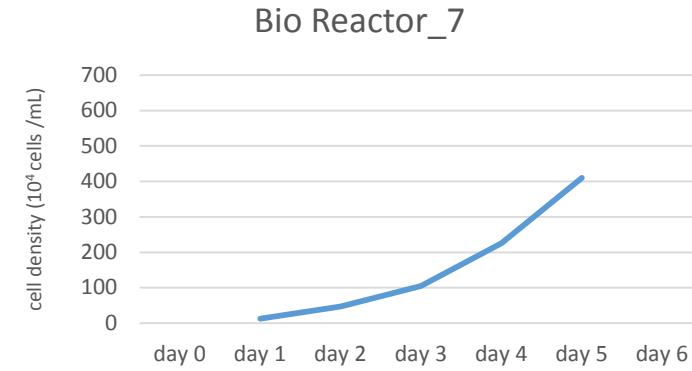
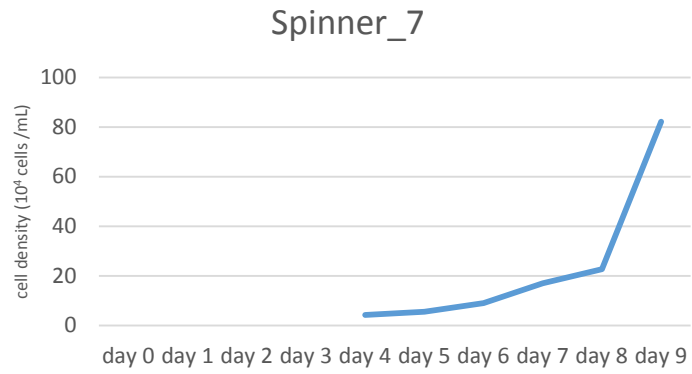


	Bioreactor	Ni-Column	Q-sepharose
TSG-6 Yield	47.751 mg	26.794 mg	4.771 mg
recovery		56%	17.80%

TSG-6 Q Sepharose FF



TSG-6 production summary; Lot 7



	volume (mL)	total protein concentration (ug/mL)	total protein (ug)	TSG-6 concentration (ug/mL)	TSG-6 (ug)	recovery of TSG-6 %	TSG6/total protein %
Bioreactor	5500			3.09	16995		
Ni-sepharose column	150	96.7	14505	38.9	5835	34.3	40.2
Q Sepharose column	26	213.4	5548.4	210.75	5479.5	32.2	98.8

Appendix 3

Comprehensive Profiling of Alkali Injuries to the Cornea

Hosoon Choi^{1,2}, Casie Phillips^{1,2}, Darwin Prockop², Joo Youn Oh⁴, Roxanne Reger², Eileen Stock³, Samuel Fulcher¹

¹Central Texas Veterans Research Foundation Temple, TX

²Institute for Regenerative Medicine, Texas A&M Health Science Center College of Medicine at Scott & White, Temple, TX

³Baylor Scott and White Health Care Temple, TX

⁴Department of Ophthalmology, Seoul National University Hospital, Seoul, Korea

Objective/Purpose;

Corneal chemical injuries may cause extensive tissue damage which can result in permanent visual impairment. These injuries often result from accidents occurring in the home or work place, however no effective therapy exists for severe chemical injuries of the cornea. Numerous cellular interactions and alterations occur after corneal chemical injury which are mediated by leukocytes, fibroblasts and endothelial cells, and are influenced by the combined actions of proteinases, growth factors, and cytokines which are directed to corneal regeneration and healing. This study comprehensively examined the effect of alkali injury to the cornea over time in order to further research in the development of a novel therapy with the anti-inflammatory protein TNF-stimulating gene 6 (TSG-6). TSG-6 may modulate the excessive inflammatory response that exacerbates the injury to the cornea caused by chemical exposure to the eye.

Keywords; cornea, alkali injury, rat, inflammation, chemical burn

Materials and Methods;

A corneal alkali injury was produced with a 3-mm diameter circular piece of filter paper soaked in 1 N NaOH, and applied to the central cornea on the right eye for 30 seconds. Immediately after alkali exposure, the ocular surface was rinsed with 50 mL of PBS for 2 minutes. Gross examination for cornea opacity was performed under a dissecting microscope. The injured corneas were fixed in 10% buffered formalin and embedded in paraffin. Tissues were stained with hematoxylin and eosin (H&E) for histopathological examination, and were examined with immunohistochemistry. Neutrophil infiltration was examined by assays for myeloperoxidase (MPO) which is contained within neutrophil granules. Real-time PCR (RT-PCR) was used to evaluate mRNA expression levels of cytokines, chemokines, and genes involved in neovascularization, lymphangiogenesis and fibrosis.

Results and Conclusion

Corneal opacity rapidly developed after injury by day 1, and persisted throughout the study period (21 days). Corneal neovascularization developed as early as day 1, and increased over the entire study period. The inflammatory response as measured by biochemical markers correlated with concentration of NaOH applied and began within 2 hours of injury, and persisted throughout the study period. Neovascularization, lymphangiogenesis and fibrosis progressed throughout the time course of the study period.

Presentation Type; Free papers **Topic Categories;** Cornea, External Eye Diseases, Eye Trauma

Appendix 4

Title:

The Efficacy of TSG-6 for Acute Corneal Alkali Injuries in a Rat Model

Submitter's E-mail Address:

sfulcher@sw.org

Interested in chairing a panel?

No

Keywords:

Alkaline burn, Cornea and Inflammation

Purpose:

To test the efficacy of the promising inflammation modulatory protein TSG-6 for ocular alkali injury in a rat model with topical drops, intracameral anterior chamber application, and intravenous injection.

Methods:

Rat corneas were exposed to 0.5N or 1N NaOH soaked filter paper and thoroughly rinsed. The inflammation modulatory protein TSG-6 was applied once either topically, via anterior chamber (AC) injection, or intravenous (IV) application immediately after injury in the treatment groups, and were matched with control groups treated with saline. The corneas were assayed for inflammatory markers, and were clinically graded for opacification over a 7 day time course in a blinded manner by two corneal specialists. Each group consisted of 8 rats treated with TSG-6 or saline following a 0.5N or 1N NaOH injury. Results from treatment and control groups were analyzed and compared.

Results:

No statistically significant difference ($p > .05$) was found for levels of inflammatory markers or degree of corneal opacification between the treatment and control groups for eyes treated with either topical or anterior chamber TSG-6 performed once at the time of injury. Initial results for one time treatment with single low dose intravenous TSG-6 show no significant treatment benefit for TSG-6, and further tests with increased dosing and multiple intravenous injections with and without topical application are underway and will be reported upon completion.

Conclusion:

Topical or AC application of the promising inflammation modulatory protein TSG-6 applied once at the time of injury is not effective in a rat model of corneal alkali injury. Further testing will determine if high dose multiple intravenous applications of the promising inflammation modulatory protein will be effective in this devastating injury.

Samuel F.A. Fulcher, MD (Presenting Author); Hosoon Choi, PhD; Casie Phillips; Joo Youn Oh, MD, PhD; Roxanne L. Reger, MS; Darwin J. Prockop, MD, PhD

Appendix 5

Inflammation Modulatory Protein TSG-6 for Chemical Injuries to the Cornea

MR130174

W81XWH-14-1-0495



PI: Samuel F.A. Fulcher MD

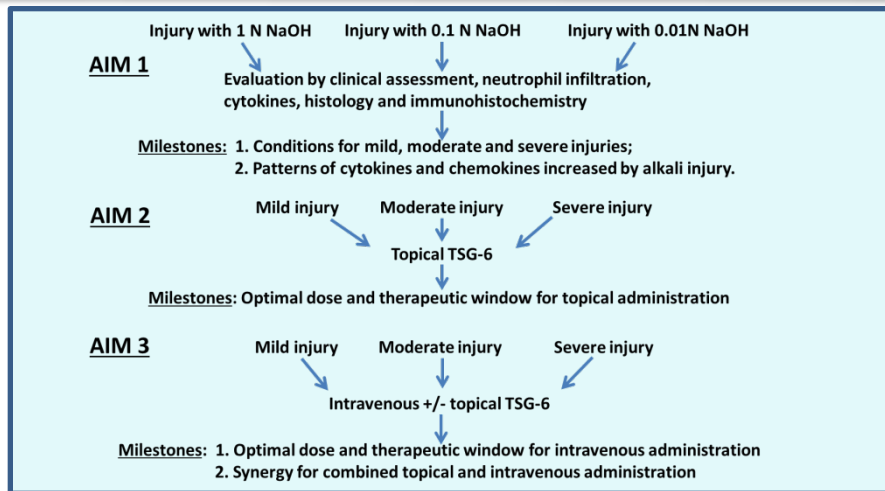
Org: Central Texas Veterans Foundation Award Amount: \$985,149

Study/Product Aim(s)

- **Aim 1.** Determine the timing and patterns of inflammation and other cellular and molecular changes in response to the severity of alkali injury. The data will allow us to select the optimal conditions for evaluating the effectiveness of TSG-6 therapy in Aims 2 and 3.
- **Aim 2.** Establish the optimal dose and the time window for effective topical and anterior chamber administration of TSG-6 therapy as a function of the severity of the alkali injury.
- **Aim 3.** Establish the optimal dose and the time window for effective intravenous administration of TSG-6 as a function of the severity of the alkali injury as well as combined topical and IV administration.

Approach

Expose the corneas of rats to varying concentrations of alkali. Assays of inflammatory markers and clinical grading of injury and healing will be used to assess effectiveness of treatment. The results will establish the limits under which the limbal epithelial stem cells can still be rescued by modulating inflammation with TSG-6.



Outline of Proposed Project

Timeline and Cost

Activities	CY	14	15	16	17
Aim 1. Establish appropriate conditions for testing TSG-6.		█			
Aim 2. Optimal dose and time window for topical, AC TSG-6.			█		
Aim 3. Optimal dose and time window for intravenous and topical + IV TSG-6.				█	
Estimated Budget (\$K)		\$298	\$356	\$331	\$985

Goals/Milestones (Example)

CY14 Goal – Establish the appropriate conditions for testing TSG-6

- Determine timing and patterns of cellular and cytokine inflammatory responses as a function of alkali injury severity.

CY15 Goals – Optimize treatment parameters for topical TSG-6.

- Optimize topical dose of TSG-6
- Determine time window for topical therapy

CY16 Goal – Optimize treatment parameters for intravenous TSG-6.

- Optimize intravenous dose of TSG-6
- Determine time window for intravenous therapy
- Determine synergistic effects of combined topical and intravenous TSG-6.

Comments/Challenges/Issues/Concerns

- Aims 1 and 2 are completed, Aim 3 is underway.

Budget Expenditure to Date

Projected Expenditure: \$654,036

Actual Expenditure: \$597,452 (as of 9/8/2016)