Efficacy, Safety, and Costs of 0.25% Timolol Gel in Healing Surgical Open Wounds: A Randomized Trial

Background and Significance

Healing of a cutaneous defect by second intention is a complex process. Migration of fibroblasts, keratinocytes, and other cell types to the site of defect and their proliferation under stimulation by cytokines and growth factors occur during this process. The role of topical beta-blockers in promoting wound healing is currently emerging in the international literature (1-3). β2-Adrenergic receptors (B2AR) are the only subtype of beta-adrenoceptors expressed on skin (4-6). They can be found in secretory coil of apocrine glands, keratinocytes, fibroblasts and melanocytes. The distribution of these receptors provides insight on dermatological disorders that may be affected by β-blockers. Keratinocyte migration occurs by the facilitation of chemotaxis, the polarization of cells, and activation of extracellular signal–related kinases essential in the signaling of promigratory pathways. The B2AR activation inhibits keratinocyte migration by activating the serine/threonine phosphatase 2A, which downregulates phosphorylation of extracellular signal–related kinases necessary for migration. Therefore, B2AR antagonists prevent the phosphorylation of phosphatase 2A and have the downstream effect of extracellular signal–related kinase promotion, inducing a promigratory pathway in keratinocytes (4-6). Keratinocyte migration also occurs by galvanotaxis, a phenomenon in which cells migrate in response to electric stimuli. Keratinocytes can be stimulated to migrate with the formation of electrical poles and the application of electrical fields. The B2AR antagonists improve the ability of keratinocytes to respond to such migratory cues, whereas the B2AR agonists decrease keratinocytes' ability to respond, further implicating the use of topical timolol for recalcitrant wounds (4-6). Angiogenesis and dermal fibroblast proliferation are also regulated by B2ARs. The B2AR antagonists have been found to promote angiogenesis in chick chorioallantoic membrane assays and in vivo murine wound models. Dermal fibroblast migration is also increased (by 27%) when exposed to B2AR antagonists, and epidermal differentiation is improved with B2AR antagonists and β1- and β2-receptor antagonists (5-10).

Topical beta-blockers have been gaining increasing popularity and evidence over the last few years as enhancers of wound healing in acute and chronic open wounds. In particular, timolol, which is generally prescribed in the clinical setting to treat ocular and systemic hypertension, in the 0.25% gel formulation may represent a commercially available, safe and simple, painless–though perhaps moderately expensive–treatment for improving both acute and chronic open wounds, as well as for improving long-term cosmetic outcomes.

Specific Aims

To assess in a randomized fashion the efficacy and safety of topically applied 0.25% timolol gel in promoting wound healing and improve scar cosmesis of surgical open wounds <1.5cm when compared to the standard of care (SOC) by:

1. Evaluating healing in response to treatment with 0.25% topical timolol gel versus SOC in terms of wound surface area reduction of open surgical wound;
2. Evaluating cosmetic outcomes of surgical wounds in terms of blinded physician (4D multidimensional VAS) and patient (Visual Analogue Scale, VAS) assessment at 3 and 6 months follow up;
3. Evaluating patient discomfort during the healing process by means of a patient pain VAS;
4. Determining the side effects associated to 0.25% topical timolol versus SOC; and
5. Determining costs associated to the use of 0.25% topical timolol versus SOC.

Subject Selection

Any subjects who are interested in participating in the study will be initially approached by either a treating physician other than the PI or co-investigator to discuss treatment. All patients ≥18 years of age of either gender and any racial or ethnic background evaluated at the Mohs and Dermatologic Surgery Center who need to undergo a surgical intervention including a reconstructive phase will be eligible for participation in this study. The treating physician (which could include the PI if she is treating the patient) will approach the potential subject and will inform patients about the study during their initial consultation at the Mohs and Dermatologic Surgery Center, Dana-Farber/Brigham and Women's Cancer Center at Faulkner Hospital. If a subject indicates they would possibly be interested or would like more information, then another member of the research team will go in to privately discuss the possibility of participating. The team member will assure patients they will receive the same quality of care either way, and that their decision not to participate will in no way affect the care they receive. The patients will be encouraged to make further inquiries about the study if they are interested. Informed consent will be obtained prior to initiating the treatment. If patients are interested in taking part in the study, the investigator will approach them in private to review the consent form and address any study-related questions.

Subjects will be included for the followings reasons:

1. Age greater than 18 years
2. Open surgical wound ≤1.5cm
3. No hypersensitivity with use of 0.25% timolol gel

Subjects will be excluded for the following reasons:

1. Age less than 18 years of age
2. Open surgical wound >1.5cm
3. Pregnant women
4. Known bradycardia
5. Use of systemic retinoids within 1 month
6. Any hypersensitivity with use of 0.25% timolol gel

Subject Enrollment

The principal investigator or a co-investigator will inform patients about the study during their initial consultation at the Mohs and Dermatologic Surgery Center, Dana-Farber/Brigham and Women's Cancer Center at Faulkner Hospital. The patients will be encouraged to make further inquiries about the study if they are interested. Informed consent will be obtained prior to initiating the treatment. If a subject indicates they would possibly be interested or would like more information, then another member of the research team will go in to privately discuss the possibility of participating. The team member will assure patients they will receive the same quality of care either way, and that their decision not to participate will in no way affect the care they receive.

Study Procedures

The study protocol will begin immediately post-surgery. Eligible subjects will be assigned by computer-based randomization to case (0.25% timolol gel) or control (SOC) group and treated as follows:

Case group:
1) Timolol 0.25% gel will be applied to wound bed immediately after surgery before dressing is applied
2) Starting the day after surgery: each day, the patient will cleanse the surgical site, apply 0.25% topical timolol gel (1 drop = 0.1ml for each cm^2 of wound area), and re-cover wound with clean dressing
3) This daily routine continues for 12 weeks' post-surgery (even if the surgical defect has completely healed in the interim)

SOC group:
1) Vaseline will be applied to wound bed immediately after surgery before dressing is applied
2) Starting the day after surgery: each day, the patient will cleanse the surgical site, apply Vaseline, and re-cover wound with clean dressing
3) This daily routine continues for 12 weeks' post-surgery (even if the surgical defect has completely healed in the interim)

Patients will be followed up at 7, 15, and 30 days' post-surgery and then at 3 and 6 months. Standardized pictures will be taken at each time point. Wound surface area assessed by histogram planimetry (11), as well as wound infection rate will be assessed by a blinded

physician at each follow up visit. Wound area tracing by histogram planimetry will be conducted exclusively on the pictures. No tracings will be put in contact with the patients' wounds. 4D multidimensional VAS by a blinded physician and patient "scar satisfaction VAS" will be recorded at the 3 and 6 months. Patient "pain perception VAS" will be recorded at each follow up visit. Patient and physician will record any side effects occurring during the study period. Total costs in USD will also be assessed in both groups. Patients with any missing data will be asked to if they have been applying the Timolol or the Vaseline regularly, the duration of their compliance, their overall satisfaction with the wound healing process, the worst pain that they can recall and when was it after the surgery, and any other comments or concerns, including any side-effects or difficulties during the study period that they may want to share with us.

Biostatistical Analysis

To detect a 25% difference in time to improvement in each treatment arm with an 80% power, a total of 38 subjects will be enrolled in both the treatment and SOC groups (alpha level=0.05). Total subject enrollment will be 88 to adjust for subjects lost to attrition. This was calculated by considering the percentage of patients that would be healed by 30 days. In the timolol group, we estimate 85% and in the control group we estimate 60%. The Mohs and Dermatologic Surgery Center has 3 surgeons who use second intention healing on approximately 505 cases per year (~42 cases per month). If 50% of subjects agree to participate, enrollment will be complete within approximately 5 months.

Risks and Discomforts

The risks and discomforts of the topical application of 0.25% timolol gel include erythema, itching, irritant or allergic contact dermatitis in case of patient sensitization. Other discomforts and risks, including pain and infection, are implied in the surgical procedure patients have undergone (in both groups).

Potential Benefits

The expected benefits of topical 0.25% timolol gel dressings in open surgical wounds when compared to SOC include: quicker healing of wound sites, better long term scarring and thus cosmetic outcome, improved patient satisfaction, and decreased patient pain during the post-surgical healing phase.

Monitoring and Quality Assurance

The study coordinator and the principal investigator will review the study data on an ongoing basis to ensure data quality and compliance with IRB approved protocol. Any deviations from the IRB approved protocol will be reported to the Partners IRB Office in a timely manner. Data will be reviewed regularly by the principal investigator and study coordinator to identify and resolve discrepancies. Patient identifiers will only be accessible to study staff and all data will be

de-identified before review and analysis. Any deviations from the IRB approved protocol will be reported to the Partners IRB Office in a timely manner.

Risks to confidentiality will be minimized by using coded data and controlling access to the study database. Only study staff will have access to patient identifying information.

Unanticipated problems involving risks to subjects or others will be submitted through Insight within 5 working days/7 calendar days of the date the investigator first becomes aware of the problem. For adverse events (AEs), any unanticipated untoward or unfavorable medical occurrence, including abnormal sign, symptom, or disease, that indicates that the research places subjects at increased risk of physical or psychological harm than previously known/recognized will be submitted through Insight/eIRB. For non-AEs, unanticipated incidents, experiences, information, outcomes, or other problems that indicate that the research places subjects at an increased risk of physical, psychological, economic, legal, or social harm than was previously known or recognized will also be submitted through Insight.

References

1. Braun LR, Lamel SA, Richmond NA, Kirsner RS. Topical timolol for recalcitrant wounds. *JAMA Dermatol*. 2013; 149: 1400-1402.

2. Ali A, Herndon DN, Mamachen A, et al. Propranolol attenuates hemorrhage and accelerates wound healing in severely burned adults. *Crit Care*. 2015; 19: 217.

3. Vestita M, Bonamonte D, Filoni A. Topical propranolol for a chronic recalcitrant wound. *Dermatol Ther*. 2016; 29: 148-149.

4. Thomas B, Kurien JS, Jose T, Ulahannan SE, Varghese SA. Topical timolol promotes healing of chronic leg ulcer. *J Vasc Surg Venous Lymphat Disord*. 2017 Nov;5(6):844-850.

5. Manahan MN, Peters P, Scuderi S, Surjana D, Beardmore GL. Topical timolol for a chronic ulcer--a case with its own control. *Med J Aust*. 2014 Jan 20;200(1):49-50.

6. Vestita M, Filoni A, Bonamonte D, Annoscia P, Giudice G. Topical 0.5% Timolol for Chronic Refractory Wounds. An Observational Prospective Study. *Plast Reconstr Surg Glob Open*. 2017 Sep; 5(9 Suppl): 21.

7. Lev-Tov H, Dahle S, Moss J, Isseroff RR. Successful treatment of a chronic venous leg ulcer using a topical beta-blocker. *J Am Acad Dermatol*. 2013 Oct;69(4):e204-5.

8. Braun LR, Lamel SA, Richmond NA, Kirsner RS. Topical timolol for recalcitrant wounds. *JAMA Dermatol*. 2013 Dec;149(12):1400-2.

9. Tang JC, Dosal J, Kirsner RS. Topical timolol for a refractory wound. *Dermatol Surg*. 2012 Jan;38(1):135-8.

10. Zheng Z, Liu Y, Yang Y, Tang J, Cheng B. Topical 1% propranolol cream promotes cutaneous wound healing in spontaneously diabetic mice. *Wound Repair Regen*. 2017 May;25(3):389-397.

11. Yesiloglu N, Yildiz K, Cem Akpinar A, et al. Histogram Planimetry Method for the Measurement of Irregular Wounds. *Wounds*. 2016; 28: 328-333.