



## Original

## Comparison of Different Adrenergic Blockers on Cutaneous Wound Healing in Wistar rats

Veena Nayak, Bharti Chogtu\*, Manohar R Amaresh Parimi, Anil K Mahapatra & Rajdip L Vaishnav

Department of Pharmacology, Kasturba Medical College, Manipal University, Manipal Karnataka-576104

## ARTICLE INFO

Received 22 Apr. 2014  
Received in revised form 01 May. 2014  
Accepted 5 May. 2014

**Keywords:**

Excision wound healing,  
Propranolol,  
Carvedilol,  
Phentolamine.

**Corresponding author:** Department of  
Pharmacology, Kasturba Medical  
College, Manipal University, Manipal  
Karnataka-576104

**E-mail address:**  
bharti\_magazine@gmail.com

## ABSTRACT

**Background:** This study was undertaken to evaluate & compare the effect of nonselective  $\beta$ -blocker,  $\alpha$ -blocker and  $\alpha+\beta$  blocker on excision wounds in animal models. **Methods:** Animals with excision wound models were divided into six groups (n=6) and were administered vehicle, propranolol 1%, propranolol 2%, carvedilol 2%, carvedilol 5% and phentolamine 2% ointments topically respectively daily for 21 days. Contraction was noted every alternate day and wound area was determined. Wound contraction (WC) was also calculated. **Results:** Carvedilol 5%, propranolol 1% and propranolol 2% significantly increased percentage of wound contraction ( $p \leq 0.05$ ) as compared to phentolamine. In comparison to carvedilol 5%, propranolol 1% was superior. **Conclusion:**  $\beta$ -blocker are superior to  $\alpha$ -blockers in the process of wound healing.

© 2014 British Biomedical Bulletin. All rights reserved

### Introduction

Wound healing is the process of tissue repair involving the tissue response to injury. Many factors such as ageing, malnutrition, radiation and diseases like diabetes and hypertension are associated with delayed wound healing.<sup>1</sup>

The wound healing activities of various adrenergic blockers have been studied.  $\beta$ -blockers like timolol, betaxolol and levobunolol have shown to improve wound healing in corneal epithelium in rabbits.<sup>2</sup> A retrospective cohort study showed that beta blockers improved burn

wound healing and reduced mortality in adult patients.<sup>3</sup> Studies have shown that stimulation of  $\beta_2$  adrenergic receptors on keratinocytes can decrease epithelialization.<sup>4</sup>

Since sympathetic innervation to cutaneous tissue is responsible for maintaining homeostasis, pilomotor activities and glandular secretion, blockade of adrenergic receptors may have a role in wound healing.<sup>5</sup> The wound healing activity of beta blockers<sup>6</sup> has been studied however the role of alpha adrenergic blockers on wound healing is controversial. There is



lack of data comparing the effect of adrenergic blockers on cutaneous wound healing. The present study was undertaken to evaluate & compare the effect of propranolol (nonselective  $\beta$ -blocker), phentolamine ( $\alpha$ -blocker) and carvedilol ( $\alpha+\beta$  blocker) on excision wounds in animal models.

## Materials and Methods

### Drugs and chemicals: Animals

Adult Wistar rats weighing 150-200g were used for the study. These rats were bred locally and were housed under controlled conditions in polypropylene cages. All rats were fed with a standard pellet diet and water ad libitum. All animals were kept for overnight fasting and weighed before the experiment. The study was undertaken after obtaining approval from Institutional Animal Ethics Committee vide letter no. IAEC/KMC/46/2012 dated 18<sup>th</sup> May, 2012.

Propranolol, carvedilol and phentolamine were obtained from Sigma Chemicals. Liquid paraffin, hard paraffin (Rankem), ketamine (neon laboratories, Mumbai, India) were also used. All other chemicals were of analytical grade. Ointments of propranolol (1% and 2%), carvedilol (2% and 5%) and phentolamine 2% were prepared by adding preweighed propranolol, carvedilol and phentolamine with liquid paraffin in a mortar while triturating. To this mixture molten hard paraffin was added to obtain the required concentration. They were applied on the excision wounds in single layer thickness.

### Study design and drug administration

The animals were divided into six groups (n=6). The groups I to VI were administered vehicle, propranolol 1%, propranolol 2%, carvedilol 2%, carvedilol 5% and phentolamine 2% ointments topically respectively. The animals were anaesthetized with ketamine 60mg/kg body weight

intraperitoneally prior to and during creation of the wounds. A round seal of 2.5cm in diameter was impressed on the dorsal thoracic region 5 cm away from the ears as described by Morton and Malone.<sup>7</sup>The entire full thickness of skin from the demarcated area was excised. Wounds were cleaned with cotton swab soaked in 70% alcohol. The drugs were administered daily for 21 days or till complete epithelialization whichever was earlier. Contraction, which mainly contributes for wound closure, was studied by tracing the raw wound area on transparent paper every alternate day till wounds were completely covered with epithelium. These wound tracings were retraced on a millimeter scale graph paper, to determine the wound area. Wound contraction (WC) was calculated as a percentage change in the initial wound size i.e,

$$\text{WC (\%)} = \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

Epithelialization period was monitored by noting the number of days required for eschar to fall away, leaving no raw wound behind.

### Statistical Analysis

All results were expressed as mean $\pm$ SEM. The results were analyzed by One Way analysis of Variance (ANOVA) followed by Dunett's Post Hoc test. P<0.05 was taken as significant.

## Results

In the present study, topical administration of 2% and 5% carvedilol and 2% propranolol reduced the duration of epithelialisation significantly (p<0.05) when compared to covehicle treated group (Table 1). The percentage of wound contraction increased significantly in the groups treated with propranolol, carvedilol and

phentolamine when compared to vehicle treated group on days 11 ( $p < 0.05$ ) and 15 ( $p < 0.01$ ) ( Table 2)

### Discussion

Wound healing is a normal biological process achieved through hemostasis, inflammation, proliferation, and remodeling. Many topical agents are being tried to improve wound healing.

Expression of beta adrenergic receptors on human skin has been recognized long back. Cutaneous  $\beta$ - adrenergic receptors maintains epidermal function and integrity. Many studies have shown that adrenergic blockers modulate wound healing. Even impaired cutaneous wound healing secondary to stress in mice is mediated through activation of  $\beta 1$ - adrenoceptors and  $\beta 2$ - adrenoceptors.<sup>8</sup> Recent study has put forth that prolonged systemic exposure of epinephrine leads to continuous trafficking of polymorphonuclear leukocyte to the wound site via IL-6-mediated mechanism, and impairs repair of wounds.<sup>9</sup> In the present study the effect of alpha blocker, beta blockers and alpha + beta blocker on excision wound models in rats has been evaluated and compared. This excision wound model offers the advantages of significant wound volume, involvement of all dermal components, epithelialization only from the wound margins, and the ability to analyze chemistry, histology, and cell populations in the wound.

In this study, the period of epithelialization was significantly decreased in Carvidelol. 2%, Carvidelol 5% and propranolol 2% groups as compared to control. Phentolamine 2%, an  $\alpha$ - blocker did not show any significant difference as compared to control group. Percentage wound contraction was significantly increased in all the groups on day 11 ( $p < 0.05$ ) and day 15 ( $p < 0.01$ ) as compared to control. On intergroup comparison, carvedilol 5%, propranolol 1% and propranolol 2% showed significantly

increased wound contraction percentage ( $p \leq 0.05$ ) as compared to phentolamine on day 15 of study. And propranolol 1% showed significantly superior ( $p \leq 0.05$ ) results as compared to carvedilol 2% on day 15. These results can be attributed to blockade of different receptors. Beta blockers showed better wound contraction than  $\alpha$ - blocker. Propranolol treated group had better wound contraction as compared to carvedilol which is  $\alpha + \beta$  blocker. The duration of epithelialization by  $\alpha$ - blocker was not significantly different from that of control group. So the present study puts forth the superiority of beta blockade as compared to  $\alpha$ - blockade in wound contraction.

A previous study showed that beta blockers improved burn wound healing in mice where mice injected with beta blockers exhibited improved reepithelialization, compared with mice receiving vehicle injection.<sup>10</sup> Another study also showed that low-dose propranolol improved healing in third degree burns in rats and reduced the local inflammatory response.<sup>11</sup> In clinical studies, propranolol improved healing in hospitalized burn patients, because of its ability to decrease energy expenditure and muscle protein catabolism.<sup>12</sup> Authors have further hypothesized that wound healing effects are mediated by  $\beta 1$  receptors as wound healing was delayed less in metoprolol-treated animals as compared to those treated with propranolol.<sup>13</sup>

Some previous studies have shown that  $\alpha$ -adrenergic receptor antagonists improve wound closure and contracture and normalize edema in mice with repeated restraint stress<sup>14</sup> which is in contrary to our results.

The catecholamine receptor autocrine hormonal system contributes to epidermal homeostasis and wound re-epithelialization. Understanding of wound healing process can lead to development of new therapeutic approaches in this area. Beta adrenergic

antagonists by accelerating wound re-epithelialization can support the role of the  $\beta$ -antagonists in the wound repair process. Thus there arises a need to find the exact mechanism and receptor subtypes involved in the wound repair process.

## References

1. Lazarus GS, Cooper DM, Knighton DR, Margolis DJ, Pecoraro RE, Rodeheaver G, Robson MC. Definitions and guidelines for assessment of wounds and evaluation of healing. *Arch Dermatol*. 1994 Apr;130(4):489-93
2. Reidy JJ, Zarzour J, Thompson HW, Beuerman RW. Effect of topical blockers on corneal epithelial wound healing in the rabbit. *British Journal of Ophthalmology* 1994; 78: 377-380
3. Arbabi S, Ahrns KS, Wahl WL, Mark R, Hemmila MR, Wang SC, Mary-Margaret Brandt MM, Taheri PA. Beta-Blocker Use Is Associated with Improved Outcomes in Adult Burn Patients. *The Journal of Trauma, Injury, Infection, and Critical Care* 2004; 56:265–271
4. Pullar CE, Grahn JC, Liu W, Isseroff RR.  $\beta$ 2-Adrenergic receptor activation delays wound healing. *The FASEB Journal* 2006;20:76–86
5. Singer AJ, Clark RA. Cutaneous wound healing. *N Engl J Med*. 1999 Sep 2;341(10):738-46.
6. Souza BR, Santos JS, Costa AM. Blockade of beta1- and beta2-adrenoceptors delays wound contraction and re-epithelialization in rats. *Clin Exp Pharmacol Physiol*. 2006 May-Jun;33(5-6):421-30
7. Mortan, J.J.P. and M.H. Malone, 1972. *Arch. Int. Pharmacodynamics*, 196: 117-126.
8. Souza BR, Porto LC, Costa AM. Cutaneous wound healing of chronically stressed mice is improved through catecholamines blockade. *Exp Dermatol*. 2010 Sep;19(9):821-9.
9. Kim MH, Gorouhi F, Ramirez S, Granick JL, Byrne BA, Soulika AM, Simon SI, Isseroff RR. Catecholamine stress alters neutrophil trafficking and impairs wound healing by  $\beta$ 2-adrenergic receptor-mediated upregulation of IL-6. *J Invest Dermatol*. 2014 Mar;134(3):809-17.
10. Sivamani RK, Pullar CE, Manabat-Hidalgo CG, Rocke DM, Carlsen RC, Greenhalgh DG, Isseroff RR. Stress-Mediated Increases in Systemic and Local Epinephrine Impair Skin Wound Healing: Potential-New Indication for Beta Blockers. *PLoS Medicine* 2009; 6: e1000012
11. Souza BR, Nascimento AP, Costa AM. Low-dose propranolol improves cutaneous wound healing of burn-injured rats. *Plast Reconstr Surg*. 2008;122(6):1690-9.
12. Mohammadi AA, Bakhshaeekia A, Alibeigi P et al. (2009) Efficacy of propranolol in wound healing for hospitalized burn patients. *J Burn Care Res* 30:1013–1017
13. Raut SB, Nerlekar SR, Pawar S, Patil AN. An evaluation of the effects of nonselective and cardioselective  $\beta$ -blockers on wound healing in Sprague Dawley rats. *Indian J Pharmacol* 2012;44:629-33
14. Eijkelkamp N, Engeland CG, Gajendrareddy PK, Marucha PT. Restraint stress impairs early wound healing in mice via alpha – adrenergic but not beta – adrenergic receptors. *Brain Behav Immun* 2007; 21(4): 409-12.

**Table 1.** Period of Epithelialisation in excision wound healing model

Group	Epithelialization period (days) Mean±S.E.M.
Vehicle	20.66±1.11
Carvedilol. 2%	17±0.81*
Carvedilol 5%	16.33±0.42*
Propranolol. 1%	17.5±0.43
Propranolol. 2%	17.16±0.47*
Phentolamine. 2%	19.5±1.23

\*p value ≤ 0.05 vs control

**Table 2.** Wound area (mm<sup>2</sup>) and % wound contraction

Group	% Wound contraction (Mean±S.E.M)		
	Day 5	Day 11	Day 15
Vehicle	13.84±6.46	33.75±4.18	46.47±2.93
Carvedilol 2%	13.20±1.35	48.71±4.18*	73.02±3.54**
Carvedilol 5%	24.86±1.37	60.52±1.15*	83.44±2.62**
Propranolol 1%	23.81±2.60	66.34±3.24*	83.39±3.32**
Propranolol 2%	17.94± 1.63	54.92±2.65*	79.11±2.11**
Phentalamine 2%	18.65±1.82	54.93±3.70*	66.07±3.06**

\*p ≤ 0.05 \*\*p ≤ 0.01 vs control