# INFLUENCE OF CALCIUM CHANNEL BLOCKERS ON WOUND HEALING - AN EXPERIMENTAL STUDY

[1]Patil PA [2]Agnihotri RL [3]Singh KR

[1]Dept of Pharmacology, J.N.Medical College, Belgaum -590010.India
[2] Agnihotri RLK, Semler Research Center Private Limited, Bangalore, India.
[3]Dept of Pharmacology, T.N.Medical College & B.Y.L Nair Ch.Hospital, Mumbai-400008.India.

**Summary**

Calcium channel blockers are known to interfere with the excitation contraction coupling, a key event in muscle contraction by affecting the calcium influx. Since the contraction of myofibroblasts (specialized cells) contribute to excision wound healing, calcium channel blockers viz nifedipine, verapamil & diltiazem could retard the healing by interfering with their contraction. Due to paucity of such information, the present study was planned & the above mentioned drugs were investigated for their action in excision, resutured incision & dead space wounds in male Wistar rats. Differential action was observed with the above three drugs in the present study, wherein nifedipine, verapamil & diltiazem significantly hampered resutured incision & dead space wound healing whereas diltiazem & verapamil enhanced excision wound healing. The scar area was significantly decreased by all the three drugs in the excision wound model.

**Key words** Nifedipine, Diltiazem, Verapamil, Wound Healing

*Corresponding author: Patil P.A., Professor,
Dept of Pharmacology & Pharmacotherapeutics,
J N Medical College, Belgaum-590010,Karnataka, India. Phone: 0831- 24091828, Fax: 08312470759.
Email: drpapatil@yahoo.co.in

## Introduction

Calcium is one of the vital elements present in the body essential for numerous physiological processes viz release of neurotransmitters [1] & autocoids [2], contraction of smooth & skeletal muscles [1], etc. Calcium required for the release of inflammogens [2] is obtained from extracellular sources through slow calcium channels present in the plasma membrane.

Calcium channel blockers (CCBs) impede the influx of calcium through these channels & are expected to affect the cytosolic calcium & hence inflammation [3,4].
Thus drugs that interfere with inflammation, an obligatory step in wound repair are expected to influence wound healing.

CCBs also interfere with the excitation contraction coupling, a key event in muscle contraction by affecting calcium influx. Since the contraction of myofibroblasts (specialized cells) contributes for excision wound healing, CCBs could retard the healing by interfering with their contraction.
An attempt has thus been made in the present study to investigate the effects of CCBs viz nifedipine, verapamil & diltiazem on wound healing.

## Materials & Methods.

### *Animals & drug treatment*

Healthy male Wistar rats weighing 175±25g were housed individually & acclimatized to the laboratory for a week under 12:12 light dark cycle. The animals were fed on standard pellet diet (Amrut brand) & water ad lib, where as they were starved overnight the day prior to experimentation. The study was approved by the institutional animal ethics committee constituted as per CPCSEA guidelines. Depilation at the wounding site was done a day before wounding.

Wound Models: Resutured incision wounds were inflicted with two 6 cm long parallel para vertebral incisions under light ether anesthesia as described earlier[5].Sutures were removed on the 7th day; breaking strength was measured on the 10th post wounding day, by the continuous water flow technique of Lee[6].

Excision wounds were inflicted as described by the method of Morton & Malone[7], by excising the full thickness (approximately 500 $mm^2$) from the nape of the neck under light ether anesthesia. Wound closure rate & epithelization time were assessed by tracing the wound on polythene paper from the wounding day, followed by 4, 8, 12, 16 & 18th day & subsequently on alternate days till complete epithelization (fall of scab without any raw area).Similarly scars were traced on complete epithelization to assess wound contraction by noting the scar shape & size.

Dead space wounds were inflicted by implanting sterile cotton pellets (10mg) & cylindrical grass piths (2.5 cm X 0.3 cm) subcutaneously in the groin & axilla alternatively by the technique of D'Arcy et al. as described by Turner[8].On the 10th post wounding day, all the granulation tissues were removed under light ether anesthesia. Cotton pellet granulomas were dried overnight at $60^0$C to record the dry weight which was expressed as mg/ 100g body weight as suggested by Dipasquale & Meli [9]. One of the granulation tissue over the grass pith was opened & trimmed to a rectangular piece for estimation of breaking strength, whereas the other piece was preserved in 10% formalin for histological studies.

All the wounding procedures were carried out aseptically & none of the animals received any local or systemic antimicrobials.

After wounding, the animals were divided into control & treatment groups (n=6, in each) for each of the wound models to receive treatments. The drugs were administered

in their therapeutically equivalent doses as calculated with the help of conversion table devised by Paget & Barnes[10]. Verapamil (10mg/kg) & diltiazem(10mg/kg) were administered intraperitoneally suspended in normal saline & nifidepine( 5mg/kg) orally suspended in 2% gum acacia once a day in the volume of 5ml/kg.

Control groups received equal volumes of the vehicle. The duration of treatment was 10 days for animals inflicted with incision & dead space wounds, whereas it was continued till complete epithelization in animals bearing excision wounds.

***Statistical analysis***

The results were analysed by student 't' test expressed as mean ± S.E. $p<0.05$ was considered as significant.

## Results

Resutured incision wounds: Nifedipine, verapamil & diltiazem significantly ($p< 0.001$) decreased the wound breaking strength compared to that of control.(Table I)

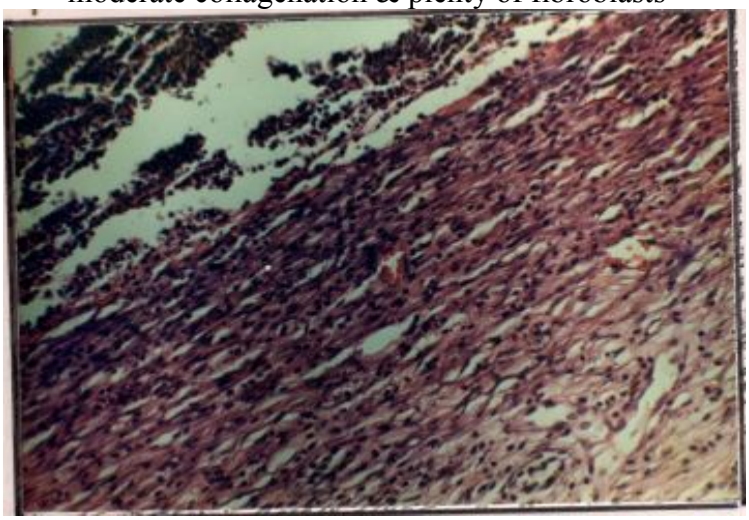
Dead Space wounds: Nifedipine, verapamil & diltiazem significantly ($p<0.001$) decreased the breaking strength of the granulation tissue similar to its effect on resutured incision wound(Table I).Cotton pellet granuloma weight was decreased significantly ($p<0.001$) in the nifedipine(17±1.20g), verapamil (14.7±0.62g) & diltiazem(13.30±2.44g) treated groups as compared to control(23±1.08g).(Table I). Histopathological studies revealed scanty granulation tissue in all the three treatment groups in contrast to control. However there were plenty of fibroblasts & moderate collagenation in the three treatment groups similar to control.(Figure I,II,III,IV).

Table I : Effect of calcium channel blockers on resutured incision & dead space wounds

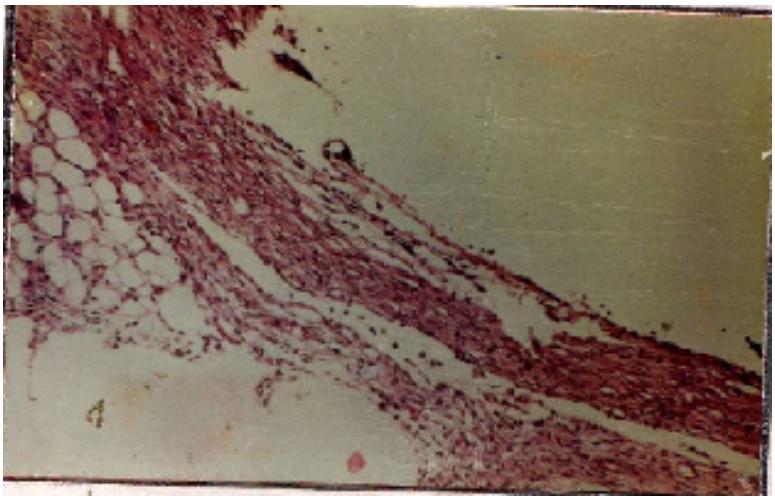
| Group n= 6 in each | Dose mg/kg orally | Resutured incision wound breaking strength (g) | Granulation tissue | |
|---|---|---|---|---|
| | | | Breaking Strength (g) | Dry weight (mg% of body wt) |
| Control | 2% gum acacia (5ml/kg) | 283.5 ± 8.16 | 286.16 ± 16.19 | 23 ± 1.08 |
| Nifedipine | | 197.7 ± 6.76 ** | 211.6 ± 4.01 ** | 17 ± 1.20 ** |
| Verapamil | | 204.33 ± 4.86 ** | 215 ± 5.32 ** | 14.7 ± 0.62 ** |
| Diltiazem | | 200.5 ± 11.22 ** | 200.83 ± 5.54 ** | 13.30 ± 2.44 * |

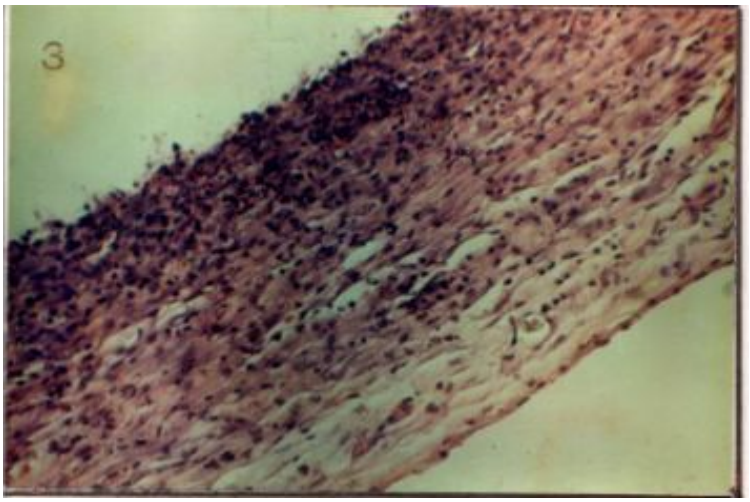
* $p \leq 0.01$ & ** $p \leq 0.001$

( I ) Nifedipine group : Shows scanty granulation tissue, moderate collagenation & plenty of fibroblasts

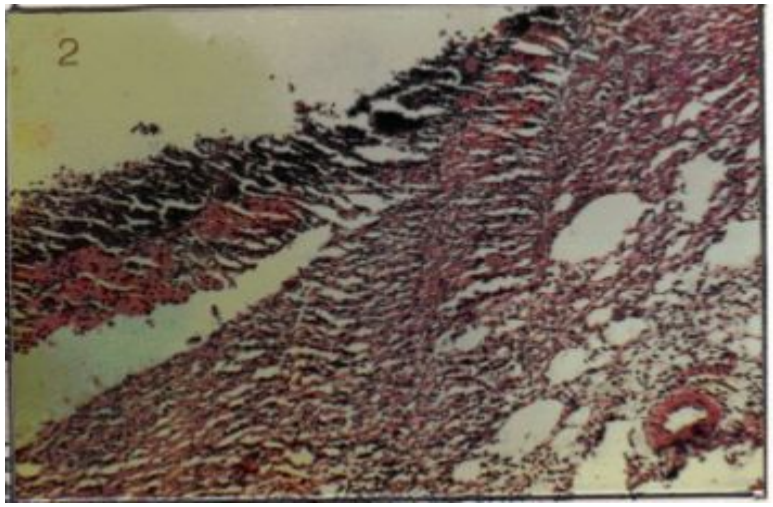


( II ) Diltiazem group : Shows scanty granulation tissue, moderate collagenation & plenty of fibroblasts




( III ) Verapamil group : Shows scanty granulation tissue, moderate collagenation & plenty of fibroblasts




( IV ) Control group : Shows plenty of granulation tissue, moderate collagenation & plenty of fibroblasts

Excision wounds: The rate of wound closure in the verapamil & diltiazem groups were significantly more on the $12^{th}$ & $16^{th}$ day as compared to that of control. However no significant change in the rate of wound closure in all the three treatment groups was observed on the $4^{th}$, $8^{th}$ & $18^{th}$ days as compared to control (Table II). The time taken for epithelization was 20.83±0.9 days in the control group, while it was significantly ($p<0.02$) decreased in the verapamil ( 16.5±1.43 days) & diltiazem (15.83±1.53 days) groups respectively. Nifedipine group showed insignificant change as compared to control (Table II). The scar areas were significantly ($p<0.0001$) decreased in all the three groups as compared to control denoting enhanced wound epithelization (Table II).

Table II : Effect of calcium channel blockers on excision wounds

| Group n=6 in each | Dose (mg/kg) orally | Wound closure (% of original area in mm2 on day ( Mean +- SE) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 4 | 8 | 12 | 16 | 18 | Days for complete closure | Scar area |
| Control | 2% gum acacia (5ml/kg ) | 26.91 ± 4.88 | 61.86 ± 5.11 | 86.65 ± 2.33 | 98.17 ± 0.52 | 99.60 ± 0.05 | 20 .83 ± 0.9 | 44.33 ± 5.15**** |
| Nifedipine | | 35.72 ±5.23 | 63.72 ± 4.26 | 92.15 ± 2.45 | 99.03 ± 0.45 | 99.78 ± 0.10 | 17 ± 1.47 | 16.16 ± 2.41**** |
| Verapamil | | 31.56 ± 3.45 | 62.75 ± 1.49 | 91.32 ± 2.37 | 99.51 ± 0.30 ** | 99.73 ± 0.16 | 16.5 ± 1.43* | 17.16 ± 2.82**** |
| Diltiazem | | 39.25 ± 3.97 | 65.45 ± 5.75 | 95.29 ± 1.40 *** | 99.79 ± 0.20 * | 99.80 ± 0.20 | 15.83 ± 1.53 * | 15.50 ± 3.23 **** |

* $p \leq 0.02$, ** $p \leq 0.05$ & *** $p \leq 0.001$, $p \leq 0.0001$

## Discussion

The complex process of wound healing involves various phenomenon like wound contraction, granuloma formation, etc. The results of the present study clearly indicate a differential action of calcium channel blockers on all the three wound models employed. Nifedipine, diltiazem & verapamil significantly promoted scar contraction whereas only verapamil & diltiazem hastened excision wound closure on the 12th & 16th day. This observation was contrary to our expectation since CCBs have a relaxant effect on various smooth muscles [11,12] ,thus indirectly hampering the contraction of myofibroblasts at the wound edge. It could be hypothesized on the present observation that myofibroblasts at the wound edge possess a different variety of calcium channel, other than the "L" type (probably the "T" type)which are not sensitive to the CCBs used in the present study.

As a consequence these CCBs by blocking the "L" channels in the other tissues might increase availability of calcium ions at the wound edge leading to increased contraction of myofibroblasts which explains the hastened contraction of excision wounds in all the CCB treated groups.

Nifedipine failed to enhance epithelization probably since it is poorly distributed[13] in the body & lacks active metabolites in contrast to verapamil & diltiazem.

In the incision wound model all the three CCBs decreased the wound breaking strength significantly. There is no previous study showing the influence of nifedipine, verapamil & diltiazem on this wound model but literature survey does suggest that CCBs have significant anti-inflammatory activity. [14].Salicylate like anti inflammatory drugs are known to suppress the healing as indicated by decreased wound breaking strength in animals [15] by suppressing fibroblasts & thereby decreasing collagen content. Thus it can be suggested that anti inflammatory activity of CCBs could be due to their interference with the secretion of inflammatory mediators viz histamine, kinins, 5-HT, & prostaglandins since calcium is involved in their release.

Granuloma dry weight & granuloma breaking strength were decreased by all the three CCBs in the present study. It is logical to attribute the following observations to the anti inflammatory activity of the drugs since the breaking strength of the granuloma is also dependent on the fibroblast population, a source of collagen. NSAIDs like aspirin also decrease granuloma breaking strength.[15]
Histological examination shows scanty granulation tissue, fibroblasts & collagenation in all the three treatment models in contrast to the controls where plenty of granulation tissue was observed.

These findings are in agreement with an earlier report [16] wherein anti inflammatory drugs markedly decreased the

granulation tissue, fibroblasts & collagen content. These observations correlate to the decrease in granuloma dry weight & breaking strength.

## Conclusions

CCBs are widely used in clinical practice & hence the findings of the present study especially with verapamil & diltiazem may have immense clinical relevance. Patients receiving these drugs when subjected to surgery may have delayed wound healing or excessive scar contraction if the wound resembles an excision wound.

The above findings though need to be confirmed by further clinical studies.

## Acknowledgements

The authors are grateful to the Principal, J.N.Medical College, Belgaum for providing facilities & Dr.P.R.Malur, Professor of Pathology for his guidance in histopathological studies. Thanks to Mr. A. V. Karvekar & Mr. M. D. Kankanwadi for their skillful assistance.

## References

1.Hwang OK, & Kaushik MD.The mechanism of inhibitory action of diltiazem on vascular smooth muscle contractility. J Pharmacol Exp Ther, 1981;218:459-463.
2. Doughlas WW. Stimulus-Secretion coupling the concept & clues from chromaffin & other cells. Br.J.Pharmacol, 1968;34:451-74.
3. Hunt TK, Halliday B. Wound healing & wound infection by Hunt TK :281,Appleton century crofts,NewYork,1980

4. Srivastava VK, Saxena KK, Saxena RS & Gupta B. Calcium channel blockers in acute inflammation. Ind. J.Expt.Biol, Jan 1988;26: 70-71.
5. Ehrlich HP, Hunt TK. The effects of cortisone & anabolic steroids on the tensile strength of healing wounds. Ann Surg, 1969;170:203-6.
6. Lee KH. Studies on the mechanism of action of salicylates II.Retardation of wound healing by aspirin. J Pharm Sci, 1968;57: 1942-3.
7. Morton JJ, Malone MH. Evaluation of vulnerary activity by an open procedure in rats. Arch Int Pharmacodyn Ther,1972;196:117-26.
8. Turner RA. 'Anti-inflammatory agent' in screening methods of Pharmacology, 2nd ed. Academic Press; NewYork, 1965.
9. Dipasquale G, Meli A. Effect of body weight changes on the formation of cotton pellet induced granuloma. J Pharm Pharmacol,1965;17:379-82.
10. Paget GE, Barnes JM. Toxicity tests in Lawrence DR & Bacharach AL, Evaluation of drug activities pharmacometrics. London & NewYork; Academic Press, 1964.
11. Patel KR. Calcium antagonists in exercise induced asthma. BMJ, 1981; 282:932.
12. Andersson KE & Ulmsten U. Effects of nifedipine on myometrial activity & lower abdominal pain in women with primary dysmenorrhoea. Br J Obstet Gynecol, 1978; 85: 141-48.
13. Michel T. Treatment of myocardial ischemia. In: Brunton LL, Lazo JS, Parker KL eds. Goodman & Gilman's The Pharmacological basis of Therapeutics.11th ed. McGraw Hill: NewYork, 2006.823-44.
14. Diwan PV, Kulkarni DR. Effects of non steroidal anti-inflammatory agents on wound healing. Ind J Expt Bio; 1986:640-43.
15. Kasi V, Patil PA. Calcium channel blockers potentiate the anti-inflammatory activity of aspirin in albino rats. Dissertation submitted to Karnataka University, Dharwad, 1991(Feb)