# EFFECT OF ATORVASTATIN & SIMVASTATIN ON WOUND HEALING IN ALBINO RATS

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**Summary**

To evaluate the effects of atorvastatin & simvastatin on various wound models viz resutured incision, excision & dead space wounds. Atorvastatin & simvastatin in clinically equivalent doses of 7.2 mg/kg were administered orally in different groups of Wistar rats weighing 175±25g to study their effect on various wound models. The animals were administered a single dose of the above drugs once daily for 10 days in the resutured incision & dead space wound model & till complete epithelization in the excision wound model. On the 11$^{th}$ day the wound breaking strength in the resutured incision wound model & the granulation tissue in the dead space wound model was assessed. Histopathological examination & hydroxyproline estimation of the granulation tissue was carried out in the dead space wound model. Both the statins significantly ($p<0.05$) increased the wound & granuloma breaking strength in the respective models.
Time for complete closure & scar area were significantly ($p<0.05$) decreased in the excision wound model with both the statins as compared to controls.

**Keywords**: Atorvastatin, simvastatin, wound healing

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## Introduction

Wound, a common clinical entity as old as mankind, often poses problems in clinical practice. Wound healing is the basic response of living tissue to injury & is influenced by a number of factors including hormones & drugs. Some of the well established factors influencing wound healing are local factors like surgical technique, blood supply, suture material, suture technique, infection, etc & systemic diseases like malnutrition, malignancy, metabolic disorders like diabetes mellitus & variety of drugs like colchicine[1] & 5-fluorouracil[2].

There is a positive correlation between angiogenesis [3-5], endothelial nitric oxide synthase (eNOS)[6,7] and wound healing. Any agent or drug capable of promoting angiogenesis & eNOS activity may promote wound healing. Angiogenesis & fibroblast proliferation result in the formation of granulation tissue, known as healing by first intention.

HMG-CoA (3- Hydroxy , Methyl Glutaryl CoA) reductase inhibitors like statins namely atorvastatin & simvastatin have been reported to promote angiogenesis [8-11] & increase eNOS[12-15]. Hence they could be expected to influence the healing process by virtue of their reported anti-inflammatory action.

Literature survey indicated paucity of information on the influence of atorvastatin & simvastatin on wound healing.The present study was thus planned to investigate the influence of atorvastatin & simvastatin on various wound models *viz* resutured incision, excision & dead space wounds.

## 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 & water ad lib, as well as starved overnight the day prior to experimentation. The study was approved by the institutional animal ethics committee constituted as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments in Animals) 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[16]. Sutures were removed on the 7$^{th}$ day; breaking strength was measured on the 10$^{th}$ post wounding day, by the continuous water flow technique of Lee[17].

Excision wounds were inflicted as described by the method of Morton & Malone[18], 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 & 18$^{th}$ 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[19].On the 10$^{th}$ 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 [20].

One of the granulation tissue over the grass pith was opened & trimmed to a rectangular piece for estimation of breaking strength & hydroxyproline content estimation colorimetrically[21], 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 group) 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[22].

Atorvastatin (7.2mg/kg) & simvastatin (7.2mg/kg) were administered orally suspended in 2% gum acacia once a day in the volume of 5 ml/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 ± SE. $p<0.05$ was considered as significant.

## Results

Resutured incision wounds: Atorvastatin & simvastatin significantly ($p< 0.0001$) increased the wound breaking strength compared to that of control (Table I).

Dead Space wounds: Atorvastatin & simvastatin significantly ($p<0.001$) increased the breaking strength of the granulation tissue similar to its effect on resutured

incision wound(Table I). Cotton pellet granuloma weight was increased significantly ($p<0.001$) in the atorvastatin (59.78±2.44g) & simvastatin (51.51±1.61g) treated groups as compared to control (38.66±1.92g) (Table I). Hydroxyproline content was significantly ($p<0.0001$) increased in the treatment groups as compared to control. (Table I) Histopathological studies revealed proliferation in the atorvastatin & simvastatin groups as compared to control (Figure I,II & III).

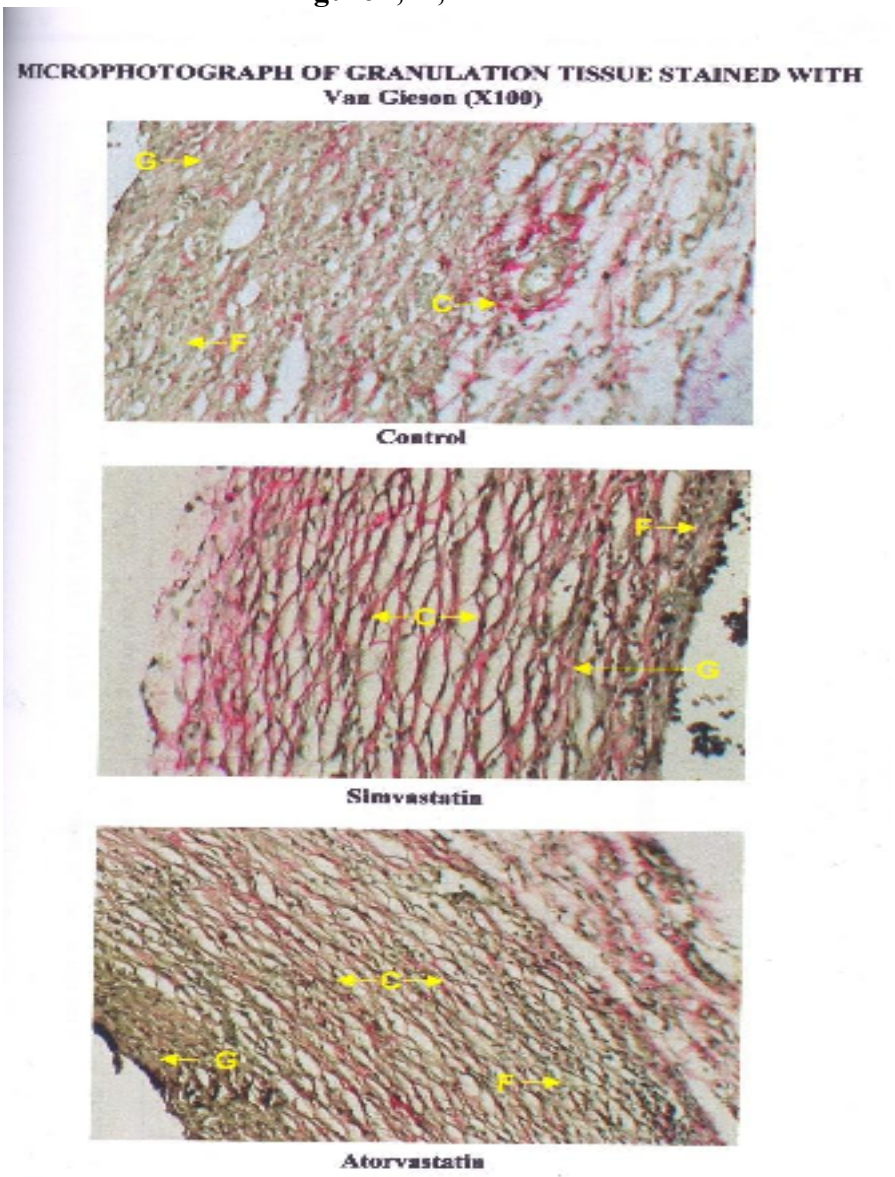
**Table I :**

Effect of atorvastatin & simvastatin 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 weight) | Hydroxyproline (mcg/ 900 mg of wet granulation tissue) |
|---|---|---|---|---|---|
| Control | 2% gum acacia (5ml/kg) | 181.7 ± 13.02 | 165 ± 9.97 | 38.66 ± 1.92 | 5.4 ± 0.08 |
| Atorvastatin | | 328.3 ± 13.52** | 241.7 ± 13.76* | 59.78 ± 2.44* | 9.3 ± 0.11** |
| Simvastatin | | 285± 8.85** | 203.3 ± 4.22* | 51.51 ± 1.61* | 7.6 ± 0.13** |

** $p < 0.0001$ & * $p < 0.001$

**Figure I, II, III**




**Note:** Markedly Increased Granulation Tissue In Treated Groups
F- Fibroblast (Yellow) G - Granulation tissue C - Collagen (Pink)

Excision wounds: The rate of wound closure in the atorvastatin & simvastatin groups were significantly faster on the $4^{th}$, $8^{th}$ ,$12^{th}$, $16^{th}$ & $18^{th}$ day as compared to that of control (Table II).

The time taken for epithelization was 18.67±0.61 days in the control group, while it was significantly ($p<0.0001$) decreased in the atorvastatin (13.17±0.31 days) & simvastatin (14.83± 0.31 days) groups respectively (Table II). The scar areas were significantly ($p<0.0001$) decreased in both the treatment groups as compared to control denoting enhanced wound contraction & epithelization (Table II).

**Table II :**
Effect of atorvastatin & simvastatin on excision wounds

| Group n=6 in each | Dose (mg/kg) Orally | Wound closure (% of original area in $mm^2$ on day ( Mean +- SE) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 4 | 8 | 12 | 16 | 18 | Days for complete closure | Scar area ($mm^2$) |
| Control | 2% gum acacia (5ml/kg) | 24 | 70 | 90 | 96.66 | 98.66 | 18.67 ± 0.61 | 41.67 ± 2.83 |
| Atorvastatin | | 56** | 82* | 98.33** | 100* | 100* | 13.17 ± 0.31** | 22.67 ± 1.77** |
| Simvastatin | | 53** | 80* | 97** | 100* | 100* | 14.83 ± 0.31** | 29 ± 3.98** |

** $p < 0.0001$ & * $p < 0.001$

## Discussion

Wound healing is the basic response of living tissues to injury. The results of the present study indicate that atorvastatin & simvastatin promote healing in all the three wound models employed. The basis of this result is the fact that statins increase the activity of eNOS [12-15] & angiogenesis.[8-11]There is a positive correlation between eNOS & angiogenesis;increase in which favours wound healing.Angiogenesis is required for restoration of blood flow for growing tissue [3-5].This is the basis of wound repair since it is essential for the supply of oxygen & other nutrients required in the cellular & biochemical process of the repair. [3-5]

The other mechanisms involved in the prohealing activity of statins include proper stimulation of the endothelial cell migration,proliferation & differentiation[8]; since i*n vitro* endothelial cell sprouting assays confirmed that eNOS is required for the same.

Inhibition of NAD(P)H oxidase leading to suppression of superoxide formation & oxygen free radical scavenging by statins could also help in promoting the healing by reducing oxidative damage.[23-26]

Healing of excision wound is attributed to phenomenon namely wound contraction & epithelization. The effects of statins on fibroblasts is not well documented. The decrease in scar area indicates that both atorvastatin & simvastatin enhance wound contraction which is attributed to myofibroblasts.

Lovastatin is known to downregulate the myofibroblast function[27] whereas pravastatin increases collagen synthesis[28]. Hence it could be concluded that all statins by virtue of their structure appear to differ in action.

Atorvastatin increased collagen formation & promoted healing of resutured incision wounds in the present study contrary to reports that it inhibits collagen production in human cardiac fibroblasts[29]. This discrepancy could be explained on the basis of species, tissue variation & also since the earlier reports are based on an *in vitro* study.

Cervistatin & atorvastatin have a biphasic action on angiogenesis.They have a pro-angiogenic action in lower concentration & angiostatic activity in higher concentrations [8,14].But recent studies also indicate that the dose dependent actions hold true only in the murine models of angiogenesis[30]

Atorvastatin & simvastatin have promoted healing of resutured incision wounds & dead space wounds by enhancing collagen synthesis as evidenced by increased hydroxyproline synthesis & collagen content via release of vascular endothelial growth factor & enhanced AKT signaling pathway. [9, 30]

## Conclusion

The results of the present study indicate that atorvastatin & simvastatin promote the healing in excision, resutured incision & dead space wounds. The prohealing effects of these drugs may be due to their proliferative activity, enhanced angiogenesis & endothelial nitric oxide release leading to increased blood flow in growing tissue. Its antioxidant property may also contribute to its prohealing effect. However mechanisms of their prohealing effect need to be explored further & extrapolated to clinical studies.

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