

**EFFECT OF ANTI LEPROTIC AGENTS ON WOUND HEALING AN
EXPERIMENTAL STUDY**

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Summary

The present study was planned to investigate the effect of antileprotic drugs on resutured incision, excision and dead space wounds in Wister rats.

Resutured incision, excision and dead space wounds were inflicted under light ether anaesthesia aseptically. Control animals received vehicle another groups received dapsone rifampicin and clofazamine orally for a period of 10 days in the incision and dead space wounds, whereas in excision wounds till complete closure. On the 11th day after estimating breaking strength of the resutured incision wounds, animals were sacrificed and granulation tissue removed from dead space wounds to estimate the breaking strength and hydroxyproline content. Quantification of granulation tissue and histological studies were also carried out. Wound closure rate, epithelisation time and scar features were studied in the excision wound models from the day of till complete closure of the wound.

Dapsone significantly promoted the healing process when compared to rifampicin and clofazamine. All the three wound models studied. Histopathological studies revealed increased collagen content and granulation tissue in Dapsone treated group compared to control.

Key words: Dead space wound, excision wounds, healing, incision, dapsone.

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Introduction

Wound being one of the common clinical entities, often challenges the clinicians when associated with infection like leprosy. Leprosy is chronic Diseases caused by mycobacterium leprae .The planter ulceration is probably the Most frequent complication of leprosy these patients receives WHO recommended Multi drug therapy which includes rifampicin clofazamine and dapsone (1). The drug Dapsone do posses anti inflammatory and immune suppressant activity (2) based on Above reported additional pharmacological action they can expected to have Influence on wound healing .the available experiment data provide a conflicting Report regarding the influence dapsone on wound healing therefore the present Study was planned to investigate the effect of anti leprotic agents on three Different wound models viz. Excision, resutured incision and dead space wound in Wistar rats.

Materials and Methods

Animals and drug treatment: Healthy male Wistar rats weighing 175±250 g, were housed individually acclimatized to laboratory for a week under 12; 12 light dark cycle. The animals were fed on standard pellet diet (Amrut brand) and watered *lib*, where as they were starved over night before the day of experimentation with free access to water. The study was approved by the Institutional Animal Ethics Committee constituted as per CPCSEA guidelines. Depilation at wounding site was done a day before wounding.

Wound models: Resutured incision wounds were inflicted with two 6cm long Para vertebral parallel incisions under light ether anaesthesia as described by Erlich and Hunt (3). Sutures were removed on the 7th day; breaking strength was measured on the 10th post wound day, by the continuous water flow technique as described by Lee(4) Excision wounds were inflicted as described by the method of Morton and Malone(5) by excising the full thickness circular skin (approximately 500 mm²) from the nape of neck under ether anaesthesia. Wound closure rate and epithelisation time were assessed by tracing the wound on polythene paper from wounding day, followed by 4, 8, 12, 16, 18, 20th day and subsequently on alternate days till complete epithelisation (fall of scab without only raw area). Similarly scars were traced on complete epithelisation to assess wound contraction by noting scar size and shape. Dead space wounds were inflicted by implanting sterile cotton pellets (10 mg) and cylindrical grass piths (2.5 cm X 0.3 cm) *S.C.* in the groin and axilla alternatively by the technique of D'Arcy *etal as* described by Turner (6). On the 10th post-wounding day, all the granulation tissues were removed under light ether anaesthesia. Cotton pellet granulomas were dried at 60 °C overnight to record the dry weight which was expressed as mg/ 100g body weight as suggested by Dipasuale and Moil (7). One of the granulation tissues over the grass piths was opened and 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 carried out aseptically and none of the animals received local or systemic antimicrobials. After wounding, the animals were divided into control and treatment groups (n=6, in each) for each wound model to receive treatments. The drugs were administered orally in their therapeutic equivalent doses as calculated with the help of conversion table devised by Paget's and Barnes (8). The dose of, clofazamine (50mg), rifampicin 6mg/kg) dapsone (100mg)were suspended in 2% gum acacia and were administered once daily in the volume of 5ml/kg, while control groups received equal volume of the vehicle. The duration of the treatment was 10 days for animals inflicted with incision and dead space wounds, whereas it was continued in animals bearing excision wounds till their complete course.

Statistical analysis: The results were analysed by ANOVA followed by post hoc Dun net's test and expressed as mean \pm SEM. $p < 0.05$ was considered as significant.

Results

Resutured incision wounds:

The mean breaking strength of wounds in control animals were 187.6 ± 8.449 g while dapsone $282 \pm$ ($p < 0.001$) showed significant increase in breaking strength

Resutured incision wounds:

The mean breaking strength of wounds in control animals was $187.1 + 8.670$ g while dapsone showed 242 ± 10.22

Dead space wounds:

Mean dry weight of granulomas in control animals was 32.28 ± 1.27 mg, and 47.72 ± 4.55 mg in dapsone treated groups respectively. There was significant ($p < 0.001$) increase in the granulomas dry weight of dapsone treated group. Breaking strength of the granulomas in the control group was 210.0 ± 11.55 g, while dapsone, treated groups it was 246.7 ± 10.309 g, . Respectively, indicating significant ($p < 0.001$) increase in granulomas breaking of dapsone group. Hydroxyproline content of 300 mg of granulation tissue was estimated and expressed as $\mu\text{g}/300$ gm of granulation tissue. It was significantly increased in the dapsone ($p < 0.001$) and treated group with a mean value of 10.17 ± 0.307 mcg and respectively as compared to the control group with a value of 5.500 ± 0.428 mcg.

Excision wounds:

The rate of wound closure in dapsone treated animals was significantly ($p < 0.01$) respectively more on 4th, 12th, 16th, day as compared to that of control. However, there was no significant change in rate of wound closure in control animals and other drugs. The time for complete epithelisation (days) in control group was 19.8300 ± 0.447 . In comparison to this, dapsone 16.67 ± 0.210 treated group took significantly ($p < 0.001$, $p < 0.05$) less time for complete epithelisation. The mean scar area (mm^2) in the control group was 41.83 ± 4.97 . Dapsone 34.10 ± 1.893 significantly ($p < 0.01$) reduced the scar area (Table 2). Scar was stellate shape in dapsone group while in control treated groups were oval or oblong. Significant reduced scar area in dapsone group indicates maximum contraction of wound as compared to control groups. Hydroxyproline content of 300 mg of granulation tissue was estimated and expressed as $\mu\text{g}/300$ gm of granulation tissue. It was significantly increased in the dapsone ($p < 0.001$) and treated group with a mean value of 10.38 ± 0.307 mcg as compared to the control group with a value of 5.917 ± 0.428 mcg.

Table 1. Effect of various healing agents on resutured incision and dead space wounds

Drugs	Resutured wound breaking strength	Breaking strength	Dry weight	Hydroxyproline(micro gram per 300mg of wet granulation tissue
Control	187.1±8.67 203.3	203.3 ± 12.29	32.28 ±1.01	5.917± 0.41
Dapsone	282 ± 8.3**	246.7± 10.22*	47.74 ±2.29**	10.38 ±0.43**
Rifampicin	202.8 ± 8.4	201.7±8.33	29.92± 1.03	5.458 ± 0.40
Clofazamine	209.5 ± 6.19	233.3 ± 17.26	35.36 ± 1.8	6.833 ± 0.26

Values are mean ± SEM

$P^* < 0.01$; $** < 0.01$ compared to control

Table 2. Effect of various healing agents on excision wounds

Drugs	4	8	12	16	18	Complete closure	Scar area
Controls	19.03± 1.07	53.57 ± 3.04	81.28± 2.96	88.88± 1.63	94.97±2.04	19.83±0.70	41.83±1.57
Dapsone	34.02 ± 1.07	56.42 ± 2.67	94.58± 1.15	99.43± 0.67	100±0.00	16.67±0.42	34.17± 0.70
Rifampicin	21.02± 0.68	57.87 ± 1.73	86.43± 1.83	90.83± 1.38	95.88±0.96	19.50±0.22	40.33±0.95
Clofazamine	18.86 ± 0.86	49.82 ± 1.49	80.40± 1.40	87.92±2.46	94.27±1.75	20±0.52	41±1.75

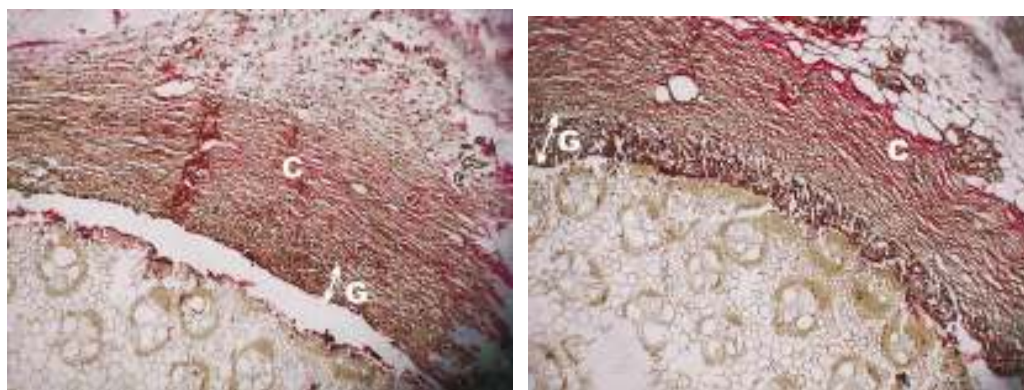
$P^* < 0.05$; $** < 0.01$ compared to controls

Discussion

The main objective of this study is to evaluate the influence anti leprotic agents on healing of excision, resutured incision and dead space wounds in male Wistar rats. The findings of the present study excision wound model clearly indicated that the dapsone drug treated groups significantly enhanced wound healing as assessed by wound closure rate, time taken for complete epithelisation and reduction in scar size. In resutured incision wound model dapsone treated groups significantly increased the strength required to break 10 day old resutured incision wound, compared to control group. In dead space wound studies dapsone treated groups significantly increased the granulomas dry weight, granulomas breaking strength and collagen content as indicated by hydroxyproline estimation. The Histopathological findings of Dapsone treated group showed marked increase in granulation tissue and onset of collagen when compared to control group. Neutrophils that are recruited at sites of inflammation generate superoxide anion which rapidly dismutase's to hydrogen peroxide⁹. H₂O₂ is then transformed in to hypochlorous acid by Neutrophils myeloperoxidase .as consequence of its extremely high reactivity, HOCl represents the most toxic and most potent oxidant generated by Neutrophils, with potentials to cause considerable tissue damage¹⁰. Dapsone reversibly inhibits myeloperoxidase activity by promoting the formation of an inactive intermediate of the enzyme, thus preventing the conversion of hydrogen peroxide to hypochlorous acid¹¹, an extremely potent Neutrophils oxidant.. Generated by Neutrophils, with potential cause considerable tissue damage in many inflammatory diseases. Dapsone stabilizes Neutrophils lysosomes ¹². Significantly decreased area and stellate shape of the scar in the dapsone treated group probably suggest that enhanced healing is due to wound contraction rather than enhanced epithelisation. The prohealing effect of dapsone was confirmed by increased granulomas formation (dry weight) and its rich collagen content (hydroxyproline content) which was further corroborated by Histopathological study of the granulation tissue. The prohealing effect of dapsone in resutured incision wounds and dead space wounds could be explained on the basis of its other reported actions as mentioned earlier. The findings of the present study appear to have clinical relevance, if they could be extrapolated to humans.

Conclusion

The findings of the present experimental study appear to be clinically relevant since such drugs are likely to be used chronically in leprosy patients who are prone for injury. Though rifampicin is commonly used as an anti-leprotic drug, dapsone could be the drug of choice for multi bacillary leprae, to exploit its prohealing activity.



NOTE: MARKED INCREASE IN GRANULATION TISSUE AND COLLAGEN IN DAPSONE TREATED GROUP

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