EFFECTS OF ENFENAMIC ACID AND ITS ZINC SALT ON WOUND-HEALING

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Summary : Dose-matched comparison between zinc-salt of enfenamic acid and the parent compound was carried out in experimentally wounded male albino rats bearing either sutured incision, dead-space or excision wounds. Result showed that enfenamic-acid significantly decreased breaking-strength of incision wounds and this effects was totally reversed by zinc present in enfenamic acid-zinc salt. Both the parent compound and the salt had significant granulation suppressant effect in dead-space wounds. In excision wounds epithelization was enhanced by enfenamic acid only. The wound contraction was not affected by either test compound.

A salutary effect of zinc salt of enfenamic acid on wound strength was considered an important advantage

Key words : breaking strength enfenamic acid enfenamic acid-zinc epithelization graunlation wound contraction wound-heating

INTRODUCTION

In recent years non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen (17) and enfenamic acid (10) are recommended to relieve post-operative pain and edema. Since inflammation is a prerequisite to initiate healing (15) it is not surprising that NSAIDs modify healing process (3, 8). Diwan and Kulkarni (3) found that enfenamic acid (Enf) promotes epithelization and wound contraction marginally but retards gain in breaking-strength (tensile-strength) and granulation tissue formation. Further, it has been reported that zinc promotes healing (14) and reverses suppressant effect of steroids on wound-healing (4). It was therefore, hypothesised that if zinc is incorporated into Enf, it would reverse the suppressant effect of Enf on breaking-strength. Accordingly, locally developed and spectrally confirmed

enfenamic acid-zinc (Enf-Zn) was compared with the Enf at three matched doses, on three different models of experimentally inflicted wounds in male albino rats. Wound-healing involve phases like granulation, collagenation, collagen-maturation, contraction and epithelization. Almost every wound has these phases to varying degee. However, there is no one single wound model to monitor all phases, hence different wound models are commonly employed.

MATERIAL AND METHODS

1. *General :* Wounding was done on overnight starved male albino rats weighing 150-200 g, under ether anaesthesia. Full aseptic measures were found unnecessary and animals did not receive either local or systemic chemotherapeutics. All animals were weighed before, during and after the study period. Each animal was housed separately and had free access to water and food. The vehicle (4% gum acacia solution) and the drug suspensions were administered once a day in the morning by gavage taking care that the total volume fed was about 0.5 *ml/100 g*. The drug treatment was, 7, 10 and 21 days for incision, dead space and excision wounds respectively.

2. *Wounding techniques :*

a) Incision wounds : Employing Hount's (6) method, two 6 cm long paravertebral incisions were made through full thickness of the skin on either side of vertebral column. Wounds were closed with interrupted sutures 1 cm apart. The sutures were removed on 7th day and the breaking-strength was measured on 10th day by the method of Lee (8).

b) Dead space wounds : Four sterilized cotton discs of uniform surface and weight, cut from dental rolls were implanted subcutaneously, one in each groin and axilla by slightly modified method of D'Arcy *et al.* (1). The granuloma harvested over cotton discs were removed on 10th day, dried at 60°C overnight and weighed. From these weights, the granuloma weight was calculated as *mg* percent of body weight (2).

c) Excision wounds : A circular piece of full-thickness skin (500 mm^2) was cut off from a pre-determined area on the back of the chest (11). To monitor changes in wound area and wound-shape, the wounds ware traced on 1 mm^2 graph paper on the day of wounding and on alternate days till the healing completed. By plotting percent wound contraction against log-days, wound closure 50 (WC50) was estimated (11). The wounds were also inspected for complete epithelization as indicated by falling of eschar without any raw wound left behind; days required for this was taken as period of epithelization.

3. *Drugs, doses and groups :*

All animals bearing a given wound were divided into seven groups 8-10 animals each. One group received vehicle, three groups received three selected doses (44, 88 and 176

mg/kg) of Enf and remaining three groups received 50, 100 and 200 *mg/kg* of Enf-Zn matched for Enf content. The highest dose of Enf used in this study was computed for rats (12) from highest clinical recommended dose (16); further the highest dose of Enf-Zn was 8th fraction of oral LD_{50}.

Results were analysed by ANOVA in case of incision, excision and dead space wound studies. Unpaired 't' test was applied for results of epithelization study.

RESULTS

Incision wounds : Analysis of variance showed that in case of Enf, there was drug related but not dose related difference between Enf and control values of breaking-strength ($P<0.01$). Similar analysis between Enf and Enf-Zn clearly showed that there is not only drug related but also dose related differences : Enf-Zn was found to reverse healing suppressant effect of Enf at all dose levels and further there was progressive promotion of healing with increasing doses of Enf-Zn. Thus. with higher doses of Enf-Zn used, the breaking-strength was not significantly different from control (Table I).

TABLE I : Effect of Enf and Enf-Zn on breaking strength and granuloma weight in incision and dead space wounds respectively.

Drug	*dose (mg/kg)*	*Bereaking strength (Grams) (Mean±S.E.M.)*	*Granuloma weight as mg% body weight (Mean±S.E.M.)*
Control	—	298±14.6	19.8±0.65
Enf	44	200±3.5*	13.7±0.67**
Enf-Zn	50	234±13.3	14.9±0.71**
Enf	88	190±8.1*	13.8±0.48**
Enf-Zn	100	240±9.7	15.4±1.3**
Enf	176	199±9.5*	12.0±1.14**
Enf-Zn	200	301±18.0	14.5±0.88**

Note : ANOVA showed that * were signficantly ($P<0.01$) less than control and Enf-Zn values and ** were significantly ($P<0.01$) less than control only.

Dead space wound : Dry weight of 10-day old granulomas expressed as *mg%* body weight was 19.8±0.65 in control animals. Analysis of variance clearly showed that both Enf and

its salt reduced the granuloma weights at all dose levels significantly ($P<0.01$) to about 13 *mg*% irrespective of dose (Table I). The results indicate that granulation suppressant effect of Enf has low ceiling effect and presence of zinc does not modify it.

Excision wounds : As can be seen from the Table II, when WC50 were compared there was no real difference between control, Enf, and Enf-Zn. The wounds of control animals were fully epithelized by 21±0.44 days. In lower doses neither the parent compound nor the salt affected this. However, the highest dose of Enf (176 *mg/kg*) hastened the epithelization by 3 days ($P<0.001$) but the salt failed to do so.

TABLE II : Effect of Enf and Enf-Zn on wound contraction and epithelization in excision wound.

Drug	*Dose (mg/kg)*	*Wound contraction: % original size (500 mm²) Mean±S.E.M.*				*Epithelization period (days) Mean±S.E.M.*
		7th day	*14th day*	*21st day*	*28th day*	
Control	—	54±3.9	88±0.99	93±1.0	94±0.7	21±0.44
Enf	44	64±0.96	86±0.77	93±0.42	93±0.32	21±1.2
Enf-Zn	50	64±1.0	86±0.99	93±0.83	94±0.6	21±1.33
Enf	88	63±2.0	88±1.0	93±0.16	94±0.36	22±0.63
Enf-Zn	100	63±2.2	86±0.72	93±0.91	94±0.92	22±0.73
Enf	176	68±2.6	91±1.0	96±0.36	95±0.55	18±0.42 ($P<0.001$)
Enf-Zn	200	66±2.9	91±0.72	95±0.4	94±0.57	21±0.98

Note : WC50 in control - 7.1 (4.7-10.6) days; values for Enf and Enf-zn fall within the range of control.

Weight changes in the animals receiving either the parent compound or the salt were not significantly different from the changes seen in controls.

DISCUSSION

Our results with Enf corroborates the finding of Diwan and Kulkarni (3) in that there is significant ($P<0.01$) decrease in breaking-strength and granulation; further Enf in highest dose used promoted eqithelization.

Enf-Zn reversed the unfavourable effect of Enf on breaking strength and brought it to almost control value particularly at its highest dose. This finding vindicates the assumption

that incorporation of zinc into Enf would reverse breaking-strength suppressant effect of Enf. However, both Enf and Enf-Zn significantly suppressed granulation at all dose levels and they were almost equipotent. Suppression of granulation and resultant decrese in collagen can be the cause of decrease in breaking-strength. This probably is the case with Enf. But with the zinc salt there is actually gain in breaking-strength despite observed decrease in granulation (e.g., dead space wound results). The possible reason is that zinc is known to be involved (13) in the synthesis of lysyloxidase an enzyme that promotes collagen maturation which determines the breaking-strength (18) and this may exert a salutary effect on the available granulation tissue.

In case of excision wound neither the parent compound nor its salt significantly affected wound contraction. Epithelization however, was promoted by Enf. The possible mechanism involved may include an increase in epithelial migration and multiplication and perhaps inhibition of chalone which is known to inhibit mitosis (19). Unlike the parent compound the salt did not hasten epithelization.

Can an agent modify some aspects of healing without affecting other aspects? Hunt *et al.* (7) found that vitamin A could reverse suppressant effect of corticosteroids on epithelization only but not on other phases of healing. Similarly, Tridax has been reported (5) to reverse suppressant effect of steroids on breaking-strength, wound contraction etc. but not on granulation phase. Even corticosteroid can differentially affect the healing process (9). Hence, it is not surprising that Enf-zn, that suppress granulation, could still promote a gain in breaking-strength. Granulation depends on fibroblast proleferation, capillary bud formation and collagen laying, while the breaking-strength is determined by cross-linking and maturation of collagen (18). Indeed, it is in a way better if a drug has differential action as said above, so that one could use it more discretely to influence some but not all phases of healing.

Enf has recently been advocated (10) for post operative (e.g., episiotomy) pain and edema. Our finding that Enf decreases gain breaking-strength however, suggests that it would lead to formation of a weak scar. Enf-Zn does not suppress breaking-strength and would seem to be a better drug. Enf-Zn was also observed to have better antiedema and analgesic effects (authors' unpublished data), prognosticating its usefulness in post-operative period.

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