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Effect of Locally Applied Anti-Inflammatory Substances on Rat Skin Wounds. (31751)

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The underlying property common to all anti-inflammatory substances is the ability to interfere with one or more of the biochemical responses of tissue to injury. Thus, these drugs may variously interfere with: (a) energy production in connective tissue by inhibiting generation of ATP, anaerobic glycolysis and oxidation of reduced DPN(1,2), (b) increased capillary permeability and edema formation by inhibiting formation and release of biogenic amines or bradykinin(3-5), (c) formation of granulation tissue by inhibiting synthesis of collagen and acid mucopolysaccharides(6-17). Tests for anti-inflammatory compounds have been based on each of these aspects of the inflammatory response. However, the metabolic actions of drugs may either be exerted directly on the inflamed tissue or indirectly by disrupting homeostasis. Thus drug-induced anorexia, toxicity, analgesia, counterirritation or activation of the hypothalamo-hypophyseal-adrenal axis may in turn modify the course of an inflammatory response(18-20), making it frequently difficult to distinguish between direct and indirect drug actions *in vivo*.

The responses of the skin to wounding are typical of those observed in any inflammatory process and include increased capillary permeability, edema formation, pain, leukocytic infiltration, fibroplasia and granulation(21, 22). The degree of healing of the wound may be easily measured by the determination of its tensile strength(23). Furthermore, the healing process may be divided into 3 consecutive phases(21) each of which may be studied separately. These are: (a) the lag or substrate phase which is characterized by an "acute inflammation" (1-5 days); (b) the fibroplastic or collagen phase (5-14 days); (c) the maturation of scar phase (15 days).

We have now studied the effects of local application of several anti-inflammatory substances on each of these phases of healing skin wounds in rats. Such studies may provide an insight into the stage in the inflammatory process at which various types of anti-inflammatory substances exert their action.

Materials and methods. Male Wistar rats weighing 170 $\pm$ 10 g were used in these studies. On day 1, the rats were anesthetized with ether, the skin of the dorso-lumbar region was shaved with electrical clippers and a wound approximately 1 inch in length was incised. A weighed amount of the test material was sprinkled along the incised wound and the wound was closed with 3 Michel wound clips. Eight to ten rats from each group were killed and the skin wound tensile strength was measured on day 3, 9 and 15. The tensile strength of the wounds was measured *in situ* according to the method previously described(23).

Briefly, the apparatus used to measure the wound tensile strength consisted of 2 alligator clips, a ball-bearing pulley system, plastic volumetric cylinders and a constant rate of flow water source. After the rat was killed, one clip was attached to one side of the wound and to a stationary post; the other clip was attached to the opposite side of the wound and to the volumetric cylinder *via* a ball-bearing pulley system. A constant flow of water was allowed to fill the cylinder until the wound was disrupted. The volume (ml) and/or weight (g) of water was recorded and

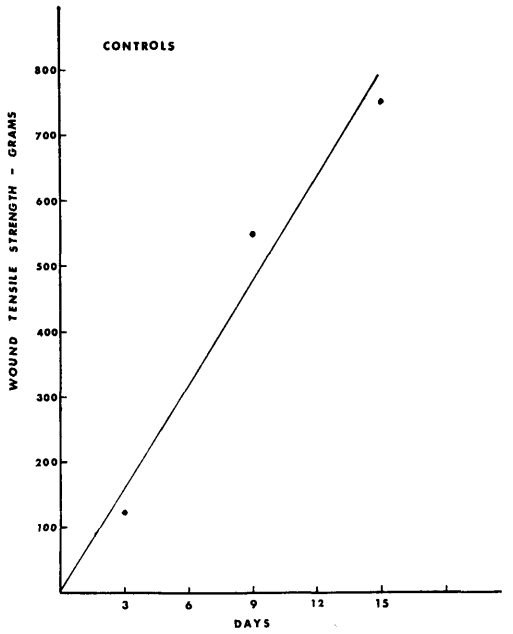

FIG. 1. Mean wound tensile strength of untreated control animals (20 assays).

was considered proportional to the tensile strength of the wound. A decrease in wound tensile strength (WTS) in treated groups in comparison with controls was taken as an indication of interference with wound healing.

Results. The course of wound healing in untreated control animals is shown in Fig. 1. The data represent the mean wound tensile strength (WTS) of approximately 20 assays.

A single application of 25 mg of cortisone acetate to skin wounds resulted in a significant decrease in WTS when measured at 3, 9 or 15 days after wounding (Table I). This dose was also associated with a reduced body weight gain on the 3rd and 9th days. Lower doses of locally applied cortisone acetate did not significantly alter WTS although body weight gain was moderately suppressed (Table I). Hydrocortisone acetate applied in doses of 5 or 25 mg reduced WTS on days 3, 9 and 15 and even a dose of 1 mg was associated with significantly retarded healing on days 9 and 15 (Table I). Body weight gain was moderately reduced by all 3 doses. Methylprednisolone depressed WTS on days 3 and 9 when applied in doses of 1, 5 or 25 mg, but only the 25 mg dose significantly retarded healing on day 15 (Table I). All dose levels of methylprednisolone severely reduced body weight gain.

Acetylsalicylic acid in local doses of 50-200 mg effectively depressed WTS on days 9 and 15 but not on day 3. On the other hand, indomethacin and phenylbutazone were ineffective at the doses used (Table II) with the exception that an obviously toxic dose of indomethacin (5 mg/rat) significantly reduced WTS at 3 days. The rats so treated did not survive until day 9. All the doses of indomethacin used caused a consistent but statistically insignificant reduction in WTS at 3 days.

Discussion. Local application of the steroids, hydrocortisone acetate and methylprednisolone, reduced WTS when measured in the substrate, collagen or scar phases of wound healing. Hydrocortisone did so at doses which did not greatly impair body weight gain whereas methylprednisolone was most effective at a dose which caused an actual body weight loss. These body weight changes indicate that significant amounts of methylprednisolone were absorbed into the systemic circulation and the results cannot therefore be attributed solely to a local action. However, at low doses (1 mg) it was evident that methylprednisolone exerted its greatest effect during the substrate phase whereas hydrocortisone was more effective than methylprednisolone during the maturation or scar phase. The two compounds were equipotent during the collagen phase. From these results it may be inferred that methylprednisolone is more potent than hydrocortisone acetate in suppressing edema, leukocytic infiltration and the synthesis of collagen, whereas hydrocortisone acetate may be more effective than methylprednisolone in preventing maturation and contraction of the scar. These inferences are supported by the known effects of these steroids(6-17,24). The selective effect of these compounds on a particular phase of wound healing is probably due to the difference in absorption rates. The depression of WTS by cortisone acetate applied in high doses could be due to systemic absorption and conversion to hydrocortisone(25). Some local activity is also possible inasmuch as subcutaneous injections of approximately the same total dose

TABLE I. Effect of Locally Applied Anti-Inflammatory Steroids on Tensile Strength of Healing Wounds.

Compound	No. of animals	Dose, mg/rat	Body wt change 3D	Body wt change 9D	Body wt change 15D	%Change as compared to control (WTS) 3D	%Change as compared to control (WTS) 9D	%Change as compared to control (WTS) 15D
Cortisone acetate	24	1	+17	+64	+ 64	— 2	+ 1	—11
	24	5	+12	+61	+ 67	—15	— 2	—16
	24	25	+ 2	+48	+ 85	—52*	—35*	—32*
Hydrocortisone acetate	24	1	+17	+59	+ 85	—14	—29*	—34*
	24	5	+18	+58	+113	—42*	—42*	—47*
	24	25	+15	+46	+ 66	—39*	—69*	—54*
Methylprednisolone	24	1	+ 1	+35	+ 85	—44*	—31*	— 5
	24	5	—10	+26	+ 66	—69*	—54*	— 8
	24	25	—32	—22	+ 20	—76*	—67*	—55*

* Significant at $P < 0.05$.

TABLE II. Effect of Locally Applied Non-Steroidal Anti-Flammatory Compounds on Tensile Strength of Healing Wounds.

Compound	No. of animals	Dose, mg/rat	Body wt change 3D	Body wt change 9D	Body wt change 15D	%Change as compared to control 3D	%Change as compared to control 9D	%Change as compared to control 15D
Acetylsalicylic acid	24	50	+24	+63	+104	+ 7	—19*	—21*
	24	100	+32	+64	+ 99	— 6	—29*	—24*
	24	200	+26	+58	+ 95	— 2	—42*	—31*
Indomethacin	24	.2	+38	+55	+118	—27	+ 4	+ 5
	24	.6	+33	+61	+113	—21	—18	+ 8
	24	1.8	+26	+44	+121	—30	—11	+ 4
	24	5.0	—22	—†	—†	—37*	—†	—†
Phenylbutazone	24	25	+33	+62	+119	+ 9	— 3	—11
	24	50	+35	+56	+109	—13	—10	— 2
	24	100	+25	+50	+120	—11	—12	—12

* Significant at $P < 0.05$. † All rats died.

(24 mg) failed to influence the substrate phase of healing in a previous study(23).

Of the non-steroidal anti-inflammatory substances tested, only acetylsalicylic acid depressed WTS although its effect was delayed beyond the substrate phase possibly due to the lack of absorption. It is inferred therefore that acetylsalicylic acid inhibits the synthesis of collagen and mucopolysaccharides in agreement with reports which indicate that salicylates inhibit cotton pellet granulomas (26), uptake of radio-labeled proline into bone matrix(27), S^{35} incorporation into cartilage slices(28) and the synthesis of glucosamine 6-phosphate(29).

Phenylbutazone and indomethacin were without effect on wound healing when applied locally. These substances are known to inhibit other inflammatory processes when administered systemically, *e.g.*, carrageenin-induced rat paw edema and the formation of cotton pellet-induced granulomas(3,26,30). These differences are not due to a failure of local absorption since locally applied phenylbutazone significantly inhibited an experimentally induced polyarthritis(31).

Although measurement of effects on WTS would not itself serve as an assay method for anti-inflammatory substances, we believe it may be of definite value in (a) evaluating the mode of action of anti-inflammatory compounds and (b) in helping to select compounds which may have minimal adverse effects on normal body defense mechanisms while providing adequate anti-inflammatory activity.

Summary. We have studied the influence of local application of anti-inflammatory substances on the tensile strength of skin wounds (WTS) in rats at 3, 9 and 15 days after

wounding. These time intervals permitted observation of interference with any or all of the 3 phases of healing: The substrate phase (acute inflammation), the collagen phase and the scar phase. Methylprednisolone was more inhibitory than hydrocortisone acetate during the substrate phase while the reverse was true during the scar phase. The 2 steroids were equipotent during the collagen phase. Cortisone acetate reduced WTS only at a high dose where systemic absorption and transformation to hydrocortisone might have occurred. Of the non-steroidal substances tested only acetylsalicylic acid interfered with healing and this effect was restricted to the collagen and scar phases. Indomethacin and phenylbutazone were without effect on wound healing in non-toxic doses. Thus, although wounding the skin elicits a sequence of responses typical of those observed in many types of inflammation, these responses may either be totally uninfluenced or selectively suppressed by local application of various anti-inflammatory substances. Tests of this type may be useful in selecting anti-inflammatory compounds which have minimal adverse effects on the healing process.

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