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Studies on the Mechanism of Action of Salicylates VI: Effect of Topical Application of Retinoic Acid on Wound-Healing Retardation Action of Salicylic Acid

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Abstract □ Oral administration of sodium salicylate or prednisone and topical application of salicylic acid or hydrocortisone in NIB retards healing and the inhibitory action of either one of these drugs can be reversed by local application of retinoic acid. Topical application of retinol, retinyl esters, or retinoic acid alone also promotes skin wound healing in the rat.

Keyphrases □ Salicylic acid effect—wound healing □ Hydrocortisone, predisone effect—wound healing □ Retinoic acid effect—drug-retarded wound healing □ Wound healing—tensile strength

In a recent report it was shown that intraperitoneal injection of retinol (vitamin A) promotes skin wound healing in rats (1). In the present study, it is shown that local application of retinol, retinyl acetate, or retinoic acid, dissolved in a nonionic base (NIB, aqueous) promotes skin wound healing. It was also shown previously that aspirin retards wound healing and this inhibitory effect can be reversed by the injection of retinol intraperitoneally (1, 2). In the present study, it was found that local application of salicylic acid or oral administration of sodium salicylate and local application of hydrocortisone or oral administration of prednisone, a dehydrogenated analog of cortisone, also retards wound healing and retinoic acid can reverse the retardation action of these anti-inflammatory agents.

EXPERIMENTAL[1]

Materials and Drugs—Retinol and retinyl esters were dissolved in NIB so that each 30 g. of NIB contained 1,000,000 USP units. The strength of retinoic acid used was 1% in NIB.

Application of NIB Preparations—NIB preparations were applied, with gentle rubbing, directly on the sutured wound immediately after wounding. The application was repeated, once a day, on the first and second days after wounding. For the control, only NIB was applied.

Administration of Drugs—Sodium salicylate, dissolved in a small amount of water, and prednisone, suspended in corn syrup, was fed to rats daily for 4 days through a short stomach tube (PE 160) connected to a blunt hypodermic needle (No. 17) attached to a 50-ml. syringe, starting 1 day before operation. The dosage levels for sodium salicylate and prednisone were 50 and 2.5 mg. per rat per day, respectively.

Wound Procedure—Sprague-Dawley male rats, weighing 230 to 240 g., were anesthetized with ethyl ether in an open mask. The hair on the back was depilated with an electric clipper. One incision, 6 cm. in length, was made through the skin and cutaneous muscles, at a distance about 1.5 cm. from the midline on each side. No ligatures were used. Bleeding usually ceased after a few minutes. The incisions were closed with continuous through-and-through sutures with stitches 0.5 cm. apart. Black silk surgical thread (No. 3-0) and curved needle (No. 19) were used. The continuous suture was pulled tight enough to secure good adaptation of the wound edges. The wounds were left undressed.

Measurement of Healing—Tensile strength, the force required to open a healing skin wound, was used to measure healing. On the seventh day after wounding the tensile strength of the wound was measured with a simple laboratory-made tensiometer as described previously (1).

RESULTS AND DISCUSSION

The results of the effect of retinol, retinyl acetate, and retinoic acid on skin wound healing in rats are summarized in Table I. The mean tensile strength of the control animals from Group I was 451 ± 12 g. These animals received only topical application of NIB. Results not shown here indicated that NIB does not have any effect on wound healing. The mean tensile strength of Group II animals receiving retinyl acetate was 522 ± 9 g., or 16% higher than that of the control. The mean tensile strength of Group III animals receiving local application of retinol was 515 ± 8 g. The increase in the mean tensile strength of Group IV animals receiving retinoic

Table I—Effect of Topical Application of Retinol, Retinyl Esters, and Retinoic Acid on Wound Healing

Group	No. of Animals	Drugs Applied	Mean Tensile Strength, g.	Percent Control
I	11	NIB	451 ± 12	100
II	9	Retinyl acetate	522 ± 9	116
III	8	Retinol	515 ± 8	114
IV	8	Retinoic acid	573 ± 17	127

[1] Retinol, all *trans*, Sigma grade, Type X; retinyl acetate, all *trans*, Sigma grade, Type I; and retinoic acid, all *trans*, Sigma grade, Type XX, were crystalline synthetic compounds obtained from Sigma Chemical Co., St. Louis, Mo. Retinyl palmitate (Myvax) was obtained from Distillation Products Industries, Division of Eastman Kodak Co., Rochester, N. Y. Sodium salicylate, reagent grade, was obtained from J. T. Baker Chemical Co., Phillipsburg, N. J. Prednisone is a product of Upjohn Co., Kalamazoo, Mich. Nonionic base (NIB, aqueous) and 1% hydrocortisone in NIB were prepared by the Pharmaceutical Technology Laboratory, San Francisco Medical Center, San Francisco, Calif.

Table II—Retinoic Acid and Healing Retardation Action of Hydrocortisone, Prednisone, and Salicylate

Group	No. of Animals	Drugs Given[a] Orally	Drugs Given[a] Topically	Mean Tensile[b] Strength, g.	Percent[c] Control
I	11	—	NIB	451 ± 12	100
II	8	Na-SA	—	358 ± 10	79
III	7	Na-SA	1% RA in NIB	448 ± 12	99
IV	11	—	3% SA in NIB	400 ± 6	88
V	12	—	3% SA and 1% RA in NIB	492 ± 10	109
VI	7	Prednisone	—	346 ± 11	77
VII	8	Prednisone	1% RA in NIB	439 ± 13	98
VIII	16	—	1% HC in NIB	414 ± 8	92
IX	12	—	1% HC and 1% RA in NIB	476 ± 9	106

[a] SA = salicylic acid; RA = retinoic acid; HC = hydrocortisone. [b] The differences of mean tensile strengths between the groups receiving RA and corresponding groups not receiving RA are highly significant ($p < 0.001$ Student t test). [c] The differences in tensile strengths between experimental animals not receiving RA (Groups II, IV, VI, and VIII) and the control (Group I) are highly significant ($p < 0.001$, Student t test).

acid was 573 ± 17 g. In all cases, the differences in mean tensile strength between the experimental and control animals are highly significant ($P < 0.001$, Student t test). Synthetic crystalline all *trans* retinoic acid has no biologic unit; a 1% preparation which contains about the same amount of retinoic acid as retinyl acetate in its NIB preparation. Retinoic acid is significantly more active than retinol or retinyl acetate. In the subsequent studies, we chose to use 1% retinoic acid.

It is interesting to know that there is no difference existing between the growth activities of all *trans* retinoic acid and all *trans* retinol. The activity of all *trans* retinoic acid proved to be of the same magnitude as retinol in both chicks and rats (3).

Table II shows the retardation of wound healing action of salicylic acid, hydrocortisone, and prednisone. It also shows that the inhibitory effect of these drugs can be reversed by applying retinoic acid topically to the wound. In a previous report it was shown that oral administration of aspirin retards skin wound healing in rats and this inhibitory effect can be reversed by injecting retinol intraperitoneally. In Table II, Group II animals received sodium salicylate orally and the mean tensile strength was reduced to 358 ± 10 g., which is 79% of the control. Group III animals were treated the same way as Group II except that retinoic acid in NIB was applied to the wound once a day during the first three days of wounding. The mean tensile strength of Group III animals was increased to 448 ± 12 g., which is a 25% increase as compared with that of Group II animals and is about the same as the control. Group IV animals received daily topical application of salicylic acid, the mean tensile strength was reduced to 400 ± 6 g., which is 88% of the control. Group V animals received the same treatment as those of Group IV except that 1% of retinoic acid was added to the salicylic acid NIB preparation. The mean tensile strength of Group V animals was increased to 492 ± 10 g. The mean tensile strength of Group V animals is 23% higher than that of Group IV animals and is 10% higher than the control. Group VI animals received prednisone orally, the mean tensile strength of these animals was reduced to 346 ± 11 g., which is 76% of the control. Group VII animals received topical application of 1% retinoic acid in addition to oral administration of prednisone. The mean tensile strength of Group VII animals was increased to 439 ± 13 g., which is close to the control. Hydrocortisone in NIB was applied to the wounds of Group VIII animals and hydrocortisone in NIB, with an addition of 1% retinoic acid, was applied on Group IX animals. The mean tensile strength of the healing wound of Group VIII animals was 414 ± 5 g., which is 92% of the control. The tensile strength of Group IX animals was 476 ± 9 g., which is 15% higher than that of Group VIII. The healing wounds of animals receiving prednisone or salicylate had noticeably less edema than those of the control. On the seventh day after operating, the wounds looked better healed than those of the animal receiving local application of retinoic acid in addition to oral administration of prednisone or salicylate; however, the tensile strengths of the healing wounds of the former were much weaker. Also noticeable were more granulation tissue and vesiculations in the healing wounds of those animals receiving retinoic acid.

Clinically, hydrocortisone, prednisone, and salicylic acid are very commonly used. Hydrocortisone and salicylic acid preparations for topical uses are quite popular. Whitfield ointment, USP, contains 3% salicylic acid. Prednisone is a potent synthetic analog of cortisone which is only used orally. It has been used in a large variety of diseases and it is not uncommonly used on surgical patients to reduce edema or inflammation. Sodium salicylate has been employed in the symptomatic therapy of acute rheumatic fever for many decades. Oftentimes it is still the drug of choice in many incidences. It has been known for a long time that cortisone retards healing in humans and laboratory animals. The present study provides evidence that salicylates, hydrocortisone, and prednisone also retard healing. The retardation action of these drugs can be reversed by applying retinoic acid on the wound. These findings illustrate the principle that one can use a second drug (vitamin A or retinoic acid) to modify the untoward effect (wound-healing retardation) of a useful drug (salicylates or anti-inflammatory steroids).

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