Studies on the Mechanism of Action of Salicylate II. Retardation of Wound Healing by Aspirin

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Aspirin, like cortisone, reduces the tensile strength of the healing skin wound in rats. The possible mechanism of action of these anti-inflammatory agents on wound healing has been discussed.

It is well known that cortisone reduces the rate of wound healing in experimental animals (1–3). It is also known that there is a time relationship between administration of cortisone and wound healing in rats (4). When cortisone is given during the entire healing period after infliction of the wounds, the healing processes are retarded. However, when cortisone is given 2 days or later after wound infliction, it exerts no effect on healing. Dunphy and Jackson (5) attribute the effect of cortisone on wound healing to its anti-inflammatory activity. Inflammation is a necessary process involved in the early stage of wound healing (6, 7). In the present study, it was found that aspirin, another anti-inflammatory agent, behaved exactly like cortisone in retardation of wound healing in rats.

METHODS

Wound Procedure—Sprague-Dawley rats of either sex weighing 450-500 Gm., were anesthetized with ethyl ether in an open mask. The hair on the back was depilatated with an electric clipper. One 6-cm. long incision 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 spontaneously after a few min. The incisions were closed with continuous through-and-through sutures with stitches 0.5 cm. apart. Black silk surgical thread No. 000 or No. 30 and curved needles No. 19 were used. The continuous suture was pulled tight enough to secure good adaptation of the wound edges. The wounds were left undressed.

Administration of Drugs—Aspirin, neutralized with calculated amount of sodium carbonate solution before use, was fed to the rat daily through a short stomach tube (P.E. 160) attached to a blunted hypodermic needle (No. 17) connected to a 5-ml. syringe, starting 1 day before operation. Two dosage levels, 150 mg. and 75 mg./Kg. of body weight, were given to different groups of rats.

Measurement of Healing—Tensile strength, the force required to open a healing skin wound, was used to measure healing. The instrument for this measurement is called tensiometer which is usually designed on the same principle as the thread tester used in the textile industry (4). Since there was no commercially made tensiometer available, a simple instrument for the skin wound healing measurement was made. It consisted of a 6 × 12 in. board with one post of 4 in. long fixed on each side of the longer ends. The board was placed at the end of a table. A pulley (with bearing) was mounted on the top of one of the posts. An alligator clamp, with 1-cm. clamp width, was tied on the tip of the post without pulley by a piece of fishing line (20-lb. test monofilament) so that the clamp could reach the middle of the board. Another alligator clamp was tied on a longer piece of fishing line with a 1-L. polyethylene bottle tied on the other end. Before testing, the animal was anesthetized with ethyl ether in an open mask. The sutures of the wound were cut out with a pair of scissors. The animal was then placed on a stack of paper towels on the middle of the board. The amount of the towels could be adjusted so that the wound was on the same level of the tips of the posts. The clamps were then carefully clamped on the skin of the opposite sides of the wound at a distance of 0.5 cm. away from the wound. The longer piece of fishing line was placed on the pulley, and the position of the board was adjusted so that the polyethylene bottle was freely hanging in the air. Water was added to the polyethylene bottle at a rapid but constant rate by siphon from a large reservoir (20-L. bottle) until the wound began to open up. The amount of water in the polyethylene bottle was weighed and considered as the tensile strength of the wound. Two to three determinations were made on each wound. The mean of the determinations made on wounds on both sides of the animal was taken as the tensile strength of the wound. The tensile strengths reported in the paper were all measured on the seventh day after wounding.

RESULTS

Controls—The tensile strength of skin wound of untreated rats was determined after a healing period of 7 days. Usually three readings were taken on each 6-cm. long wound whenever possible. The tensile strength of wound on one side of the back did not differ greatly from that of the wound inflicted on the other side of the back. The averaged value for 21 animals tested was 438 ± 17.7 Gm. (Table I, Group I).

Treated Animals—Rats receiving an oral dose of 150 mg. of aspirin per kilogram of body weight daily, starting 1 day before wounding, have an averaged tensile strength of 262 ± 8.7 Gm. on the seventh day after wounding, which is about 40% inhibition (Table I, Group II). The inhibition is significant ($p < 0.001$).

When the dose of aspirin given was reduced to one half or 75 mg. (Group III), the averaged tensile strength of the wound was 343 ± 91 Gm. or 22% inhibition.

However, when the feeding of even the larger dose of aspirin (150 mg.) was started 2 days after the wounding, the averaged tensile strength was 350 ± 10.9 Gm. or 80% of the control. These results are in good agreement with those obtained by Sandberg (4) when cortisone was given under similar conditions.

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TABLE I—TENSILE STRENGTH OF HEALING WOUNDS

Group[a]	No. of Animals	Aspirin Fed Daily: Amt., mg./Kg.	Aspirin Fed Daily: Time Started	Averaged Tensile Strength, Gm.	% of Control
I	21	—	—	438 ± 17.7	100
II	13	150	1 day before wounding	262 ± 8.7	60
III	12	75	1 day before wounding	343 ± 21.0	78
IV	7	150	2 days after wounding	350 ± 10.9	80

[a] Differences between I and II, I and III, I and IV, II and IV are significant ($p < 0.001$).

The body weight of every rat was recorded at the beginning and the end of the experiment. The averaged change in body weight did not, in most of the cases, vary more than 10 Gm.

DISCUSSION

The importance of inflammation in wound healing has long been recognized by surgeons (5). Experimentally, Selye (7) has shown that the connective tissue response to a granuloma pouch made by injecting air subcutaneously can be varied directly with the degree of inflammation created in the pouch. The injection of croton oil or other irritants into the pouch sets up an acute inflammatory reaction followed by a rapid growth in connective tissue. The injection of cortisone inhibits the inflammation and reduces the subsequent production of connective tissue. Other studies on the effect of cortisone on wound healing (4) have shown that if cortisone is given during the entire healing period, it inhibits the inflammatory response and subsequent healing processes. However, if cortisone is given some time after the wounding, so that the initial inflammatory response has already taken place, it has no effect upon the healing.

It has been suggested that lysosomal enzymes are responsible for inflammation (8). Lysosomes are cytoplasmic organells which contain the bulk of acid hydrolases (9). The proteolytic enzymes in lysosomes can break down plasma proteins into polypeptides. Some of these polypeptides may increase capillary permeability and thus cause inflammation. Anti-inflammatory steroids: cortisone, hydrocortisone, prednisone, prednisolone, betamethasone can protect lysosomal membrane (10, 11). The protective action on lysosomal membrane is one of the current theories on the mechanism of action of anti-inflammatory steroids (10).

Aspirin is another anti-inflammatory agent which also protects lysosomal membrane (12). In the present study, it has been shown that aspirin also retards wound healing in the same manner as cortisone. It is very likely that aspirin shares the same mechanism of action in retarding wound healing as cortisone. This belief is further strengthened by the fact that when aspirin was given 2 days after the wounding the inhibitory effect was greatly reduced. This followed the same pattern when cortisone was applied this way (4).

SUMMARY

It has been demonstrated that aspirin retards wound healing in the rat and the mechanism of this action has been discussed.

A simple laboratory-made tensiometer has been described.

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Keyphrases

Salicylates—action mechanism
Aspirin—wound healing effect
Tensile strength—healing incision
Apparatus—tensile strength measurement