Effect of Acetylsalicylic Acid and Hydrocortisone on the Pleural Response to Evans Blue- Carrageenin (32751)

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The effusion seen in the pleural cavity following the intrapleural injection of Evans blue or Evans blue–carrageenin provides a means of evaluating compounds for anti-inflammatory activity (1–4). Active compounds reduce the volume of pleural fluid when measurement of effect is made at 6 or 17 hours following the administration of the irritant.

Little attention has been given to the other phases of the response to pleural irritation and the effect of anti-inflammatory drugs on them. This study describes the fluid changes seen in the three phases and shows the effect of steroidal, hydrocortisone, and monsteroidal, acetylsalicylic acid, anti-inflammatory compounds on them.

Method. The method used was similar to that described previously (1). Male Carworth Farm and Charles River CD rats weighing between 260 and 400 gm were randomized into groups of six. Compounds were administered by intragastric intubation (1 ml/100 gm of body wt.) in an aqueous suspension with a few drops of Aquet,[1] a synthetic detergent, as the suspending agent. The control group received only the vehicle. To determine the effect of acetylsalicylic acid and hydrocortisone on the initial two phases, these compounds were administered by intubation 1 hour before the intrapleural injection of 5 ml of 0.075% Evans blue–0.025% carrageenin type 7 in sterile pyrogen free saline at 37°C to etherized animals. At various intervals, the animals were sacrificed with chloroform. Pleural fluid was removed by mild suction, collected in a calibrated centrifuge tube and measured. In the last phase, the animals were dosed orally with water 1 hour before, and compounds and vehicle were given orally 30 hours following the injection of the irritant. At the latter time, a control group was sacrificed, and the average volume of pleural fluid was determined. Subsequently, animals in the control and experimental groups were sacrificed at 48, 72, and 96 hours, and the volume of pleural fluid in each animal was measured.

The oral anti-inflammatory potency of hydrocortisone compared to that of acetylsalicylic acid was determined in a 6-point assay in which the volume of pleural fluid was measured 6 hours following the administration of the irritant. This aided in selecting equivalent doses for studying their effect on the three phases.

Activity on each phase was indicated by a significant change from the control slope.

Statistical procedures used were those described by Bliss (5) and Emmens (6).

The following compounds were used: Evans blue,[2], carrageenin type 7,[3] hydrocortisone alcohol,[4] and acetylsalicylic acid.[5] The particle size of the acetylsalicylic acid ranged between 7 and 12 μ.

Results. Fig. 1 shows the pleural response to the injection of 5 ml of 0.075% Evans blue–0.025% carrageenin type 7. As indicated by changes in the volume of pleural fluid, three phases are evident and are henceforth designated as: *absorptive, effusive* or *inflammatory,* and *resorptive.* In the absorptive phase, fluid leaves the cavity until a minimum volume of pleural fluid is reached at approximately 90 min. The volume of fluid absorbed represents approximately 20% of the initial volume administered. This is followed by the effusive or inflammatory phase where there is a pouring of fluid into the cavity with a maximum pleural volume at about 24 hours. At this point, the animals appear blue, indicating substantial absorption of the Evans blue into the systemic circulation. In the resorptive phase, which begins at approximately 30 hours, the pleural

[1] Emil Greiner Co., New York, New York.

[2] Matheson Coleman and Bell, Chicago, Illinois.

[3] Marine Colloids, Inc., New York, New York.

[4] Upjohn Co., Kalamazoo, Michigan.

[5] Dow Chemical Co., Midland, Michigan.

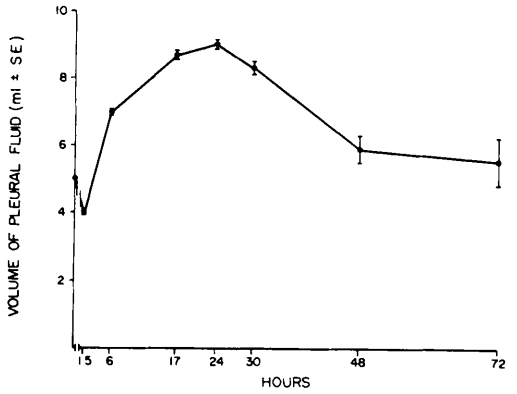

FIG. 1. Pleural response to the injection of 5 ml of 0.075% Evans blue–0.025% carrageenin type 7.

fluid begins to gradually leave the cavity and at 72 hours folllowing the injection of the Evans blue–carrageenin, it was still present in a measurable quantity.

To determine equipotent doses of acetylsalicylic acid and hydrocortisone, the anti-inflammatory potency of the steroid relative to the acid was determined by measuring the volume of pleural fluid at 6 hours following the administration of the irritant. Figure 2 shows that hydrocortisone was 0.92 (.54–1.55) times as potent as acetylsalicylic acid, indicating similar oral antieffusive activity. The index of precision was low ($\lambda_c = -.33$), and factorial analysis of the data showed that the dose graded effect for the two compounds was highly significant ($p < .01$) and parallel (Table I). A dose of 150 mg/kg orally of acetylsalicylic acid or hydrocortisone

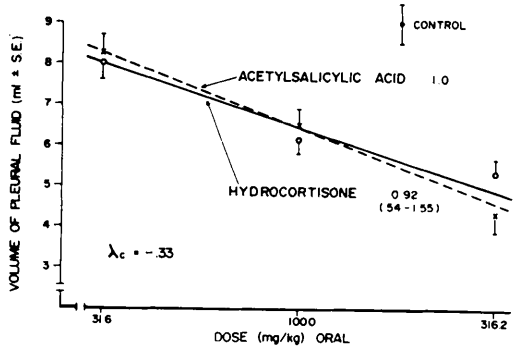

FIG. 2. Relative oral anti-inflammatory potency of acetylsalicylic acid and hydrocortisone in the 6-hour Evans blue–carrageenin pleural effusion assay.

was selected for studying their effect on the three phases.

Figure 1 showed the absorptive phase briefly as indicated by the volume of pleural fluid at 90 min following the injection of the irritant. This phase was further investigated by measuring the pleural volume at 30-min intervals following the administration of the Evans blue–carrageenin. Figure 3 shows that the maximum decrease in pleural volume occurred at 90 min, and that the volume of

TABLE I. Factorial Analysis of 2 × 3 Assay Comparing the Anti-inflammatory Activity of Acetylsalicylic Acid with Hydrocortisone.

Source of variation	Variance	F
Between drugs	.12	.11
Regression	60.80	53.30[a]
Departure from parallelism	.27	.24
Curvature of combined curve	.06	.05
Opposed curvature of separate curves	.13	.11
Error	1.14	

[a] $p < .01$.

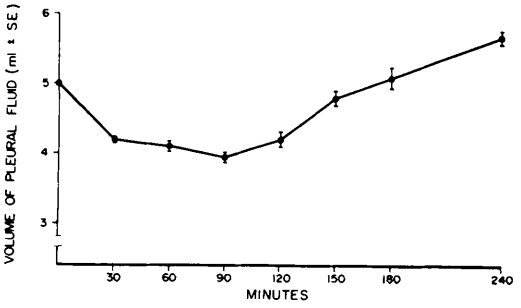

FIG. 3. Fluid changes in the absorptive and effusive phases.

fluid absorbed was again approximately 20% of the initial volume. In studying the effect of the two anti-inflammatory compounds on the absorptive phase, the pleural volumes were measured at 15, 45, and 90 min. Figure 4 shows that the two compounds were ineffective on this phase as the rates of decrease of the pleural volumes were not significantly different from the control (hydrocortisone vs control, —.74 vs —.47, $F = 2.32$, $p > .05$; acetylsalicylic acid vs control, —.59 vs —.47, $F = .7$, $p > .05$

To determine the effect of the anti-inflammatory compounds on the effusive phase, a

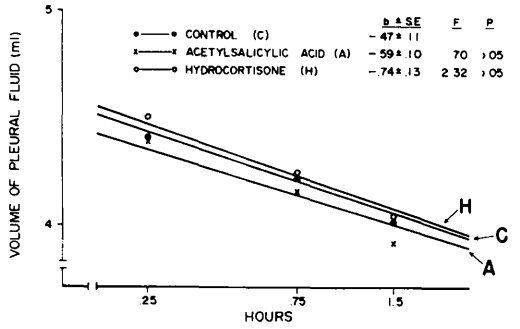

FIG. 4. Effect of 150 mg/kg/ orally of acetylsalicylic acid and hydrocortisone on the absorptive phase.

control group was sacrificed at 90 min when the rate of effusion first exceeds the rate of absorption (Fig. 3). Figure 5 shows that at 6 and 17 hours, acetylsalicylic acid and hydrocortisone suppressed the inflammatory response to the same degree. Their slopes were significantly different from the rate of effusion of fluid in the control animals (hydrocortisone vs control, $+2.0$ vs $+4.7$, $F = 13.21$, $p < .01$; acetylsalicylic acid vs control, $+2.3$ vs $+4.7$, $F = 13.47$, $p < .01$).

Figure 6 shows that hydrocortisone at 150 mg/kg orally was active on the resorptive phase while acetylsalicylic acid at a similar dose was unable to increase the outflow of fluid from the pleural cavity. The slope for hydrocortisone was significantly higher than the control (-12.74 vs -8.06, $F = 12.76$, $p < .05$) while those for acetylsalicylic acid and control animals were similar (-8.06 vs -7.89, $F = .29$, $p > .05$).

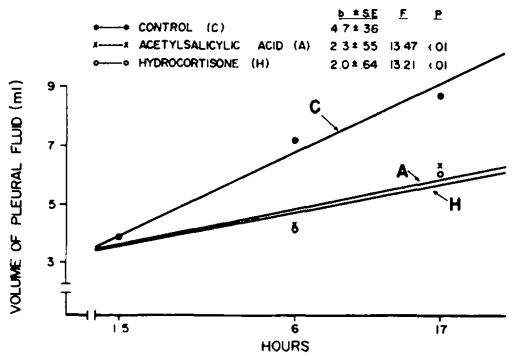

FIG. 5. Effect of 150 mg/kg orally of acetylsalicylic acid and hydrocortisone on the effusive phase.

Discussion. The response to the intrapleural injection of Evans blue–carrageenin is characterized by three sequential phases: absorptive, effusive or inflammatory, and resorptive (Fig. 1). In the absorptive phase, there was a net outflow of fluid from the cavity which results in a minimum pleural volume at 90 min. This phase occurred irrespective of the volume of Evans blue–carrageenin administered (unpublished data) and was unaltered by treatment with hydrocortisone or acetylsalicylic acid (Fig. 3). The initial absorption may be due to the movement of water and electrolytes through the capillary wall (7) prior to the onset of the inflammatory phase. Other investigators observed the absorptive phase, although not specifically pointing it out, when using either acacia alone or in combination

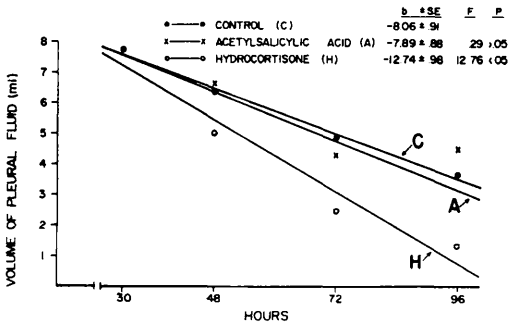

FIG. 6. Effect of 150 mg/kg orally of acetylsalicylic acid and hydrocortisone on the resorptive phase. Compounds were administered 30 hours following the injection of the Evans blue–carrageenin.

with Evans blue as the pleural irritant (8,9). However, this initial outflow was apparently not seen when arginine, dimethyl sulfoxide, or turpentine were employed as the phlogistic agent (10–12). Since these agents were apparently more irritating to the pleural cavity than the Evans blue–carrageenin, as evidenced by the rapid onset and degree of effusion following their administration, the absorptive phase was either masked or precluded.

In the effusive or inflammatory phase, the exudation of fluid was due to increased vascular permeability as a consequence of the pleural irritation (8). Hydrocortisone and acetylsalicylic acid suppressed this phase to

the same degree and their effect persisted longer than 17 hours following the injection of the irritant (Fig. 5). Their effectiveness was apparently due to their ability to prevent increased capillary permeability (13, 14) rather than to increase the rate of outflow of lymph from the pleural cavity, the mechanism proposed for chymotrypsin (9).

The relative insensitivity of this model to hydrocortisone is difficult to explain on the basis of the data presented. In the carrageenin induced foot edema in the rat, hydrocortisone was clearly more potent than acetylsalicylic acid (15, 16). However, in the turpentine induced pleural effusion, Spector and Willoughby (12) used 200 mg/kg ip of the steroid to produce minimal activity. One possible explanation is the inability of the steroid to penetrate the inflammatory site in sufficient concentration unless high doses are administered.

In the resorptive phase, there was a gradual decrease in the volume of pleural fluid in the control animals which was still measurable at 96 hours following pleural irritation (Fig. 6). Acetylsalicylic acid was ineffective while hydrocortisone facilitated the outflow of fluid from the cavity (Fig. 6). In what may be a similar clinical situation, the intrapleural injection of hydrocortisone to patients with pleurisy of tuberculosis and rheumatic origin caused a decrease in the protein content of the pleural fluid accompanied by general systemic improvement (17). Hydrocortisone may act on the resorptive phase by increasing the flow of fluid out of the cavity via the lymphatics since Courtice and Simmons (18) showed that absorption from the pleural cavity of plasma labeled with Evans blue occurred via the lymphatic system.

Summary. The pleural response to the intrapleural injection of 5 ml of 0.075% Evans blue–0.025% carrageenin type 7 is characterized by three sequential phases: absorptive effusive or inflammatory, and resorptive. Hydrocortisone and acetylsalicylic acid did not affect the absorptive phase, were equipotent in suppressing the effusive phase, and only the steroid facilitated the outflow of fluid from the pleural cavity in the resorptive phase.

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