Effects of Topical Hydrocortisone and Acetylsalicylic Acid on the Primary Lesion of Adjuvant-Induced Arthritis (35811)

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Symptoms of arthritic disease and local inflammatory conditions are amelioriated by systemic doses of aspirin and corticosteroids; however, severe side-effects sometimes force discontinuance of therapy (1).

Adjuvant arthritis in rats exhibits many similarities to human arthritis (2) and is a suitable model for measuring activity of systemically administered antiarthritic/anti-inflammatory agents (3). It is produced in rats by a single intradermal injection of Freund's complete adjuvant into a hindpaw footpad. The injected leg becomes inflamed (increase in temperature and volume) and reaches maxmal size within 3 to 5 days. Secondary lesions which occur after a delay of approximately 10 days are characterized by inflammation of noninjected sites (hindleg, forepaws, ears, nose, and tail) and are considered immune responses (4).

Recent studies by Pathak *et al.* (5) and Yankell *et al.* (6) demonstrated that hydroalcoholic vehicles improve the efficacy of topical sunscreen agents. These effects are attributed to enhanced skin penetration and binding in the presence of the hydroalcoholic vehicle (7).

The purpose of this investigation was to determine whether local application of aspirin or hydrocortisone in hydroalcoholic solutions would alter the inflammatory parameters (edema, hyperpyrexia, and hyperalgesia) associated with the primary lesion of adjuvant arthritis.

A. Methods and Materials. 1. Adjuvant arthritis—primary lesion. The primary lesion of adjuvant arthritis was produced by a single intradermal injection of 0.75 mg of *M. butyricum* (code 513026 Difco Labs, Detroit, Michigan) suspended in 0.1 ml of white paraffin oil (N.F. Atlantic Richfield Co., Philadelphia, Pa.) into a footpad of the left hindpaw of male Lewis inbred rats (Microbiological Associates, Baltimore, Md.), weighing between 180 to 200 g. The rats were anesthetized with ether inhalation prior to and during adjuvant injection. Edema volume, local hyperpyrexia, and pain threshold of the injected leg were measured 3 days following adjuvant injection.

2. Drugs and solutions. All drug solutions and suspensions were prepared daily prior to use.

Topical Preparations

Six percent acetylsalicylic acid (aspirin, USP, Merck & Co., Rahway, N.J.) solutions were prepared by first dissolving the drug in 95% ethanol USP (U.S. Industrial Chemicals Co., N.Y., N.Y.) and then diluting with distilled water to obtain a 75% alcohol concentration. Two percent hydrocortisone alcohol (Nutritional Biochemicals Co., Cleveland, Ohio) was prepared by solubilizing the drug in 70% ethanol. Topical application was accomplished by immersing the rat hindleg up to the anatomical hairline in drug or vehicle solutions for 30 sec prior to and during the development of the primary lesion.

Oral preparations. Acetylsalicylic acid and hydrocortisone were prepared as homogenized suspensions in aqueous 0.5% gum tragacanth. Drugs and vehicles were administered orally in a volume of 10 ml/kg of body weight by intubation with a blunted 20-gauge needle.

3. Experimental protocol. All rats received both oral and topical treatments using appropriate drugs and control vehicles. Drug sus-

pensions or control vehicle (0.5% gum tragacanth) were administered orally once daily for 4 days. Beginning 1 hr after the oral administration, drug solutions or control vehicle (ethanol solution) were applied topically four times daily at 2.5-hr intervals on days 1, 2, 3, and twice daily on day 4. Adjuvant (*M. butyricum* suspended in paraffin oil) was injected 1 hr after the third topical application on day 1. The parameters of the primary lesion (*i.e.*, edema volume, local hyperpyrexia and pain threshold) were measured 2.5-hr after the final topical application on day 4.

4. Paw temperature. Temperature (°) of the plantar surface of the left hindpaw was measured by means of a Tele-thermometer (Model 43A, Yellow Springs Corp., Colorado) and "banjo" thermistor probe.

5. Hindleg (edema) volume. Hindleg volume was determined by a modified method of Van Arman *et al.* (8). On the day of assay (3 to 4 hr postdrug administration), the inflamed hindleg was immersed to the anatomical hairline in a mercury reservoir. The mercury column was connected to a pressure transducer (Model P23BB, Statham, Hato Rey, Puerto Rico). The output from the transducer was led through an amplifier to a digital voltmeter (Model HP-3440A), Hewlett-Packard, King of Prussia, Pa.) and to a digital recorder (Model J74562A, Hewlett-Packard, King of Prussia, Pa.). The digital recordings were calibrated, and a linear relationship between millivolts and milliliters was obtained by placing cylinders of known volumes into the mercury reservoir.

6. Pain threshold. Pain threshold on the rat paw was measured by a modification of the methods of Gilfoil *et al.* (9) and Winter and Flataker (10). The animal rested in a prone position on a molded plastic block with hindpaws placed in a vertical position. The horizontal pressure apparatus consisted of a Teflon-ringed plunger in a brass tube fitted with a Teflon tip measuring 2 mm in diameter.

Air pressure was maintained at 6.5 psi and driven at 20 mm Hg/sec. Solenoid electric valves permitted an even pressure flow without distortion as contact was made with the inflamed paw. Pain threshold was defined as that pressure required to evoke a tugging of the left hindfoot or a jumping or squeaking response.

To permanently record pain threshold, a mercury manometer was fixed in parallel circuit with a transducer (P23AA, Statham Transducers, Inc., Hato Rey, Puerto Rico). The pressure (mm Hg) was converted to millivolts by feeding the signal into a digital voltmeter (Model 3440-A, Hewlett-Packard Co., King of Prussia, Pa.). The digital recorder was calibrated with a mercury manometer by manually inducing various pressures so that linear response was obtained. A two-position switch was used. Position one activated the air flow through the system to drive the piston; and position two, a freeze-hold switch, activated solenoid valves and stopped air flow to maintain an airtight pressure system. Simultaneously, the input signal was locked in the digital voltmeter and the pressure reading was printed in millivolts by a digital recorder (Model J74562A, Hewlett-Packard Co., King of Prussia, Pa.).

7. Body weight change. The body weight of each rat was measured initially prior to the Freund's complete adjuvant injection and on each subsequent day of the experiment. The final weight changes were calculated from the initial weight of the animal and reported as weight gain or loss.

8. Statistics. All data were statistically analyzed for significant differences between drug treated and control (vehicle treated) animals using Student's *t* test. Pooled standard errors were calculated from the analysis of variance.

B. Results. 1. Acetylsalicylic acid. The effect of topically and orally administered acetylsalicylic acid on the primary lesion of adjuvant arthritis is summarized in Table I. The topical application of 6% acetylsalicylic acid in hydroalcoholic solution (2 to 4 times/day) prior to and during the development of the primary lesion significantly inhibited edema formation, local hyperpyrexia, and adjuvant-induced weight loss. The topically applied acetylsalicylic acid solution produced an antiedema effect equivalent to that produced by the daily oral administration of the same drug at a dose of 200 mg/kg. In

TABLE I. Effect of Topical and Oral Administration of Acetylsalicylic Acid on the Primary Lesion (injected hindleg, day 4) of Adjuvant Arthritis.[a]

Test material[b]	Route	Dose[c]	Pain threshold (mm Hg)	Hindleg vol (ml)	Paw temp (°)	Body wt change from day 1 (g)
Acetylsalicylic acid	Oral	200 mg/kg	63.4[d]	2.47[e]	31.06[e]	—16.8
	Topical	6% in 75% ethanol	43.9	2.59[e]	30.02[e]	—13.2[d]
Control			38.3	3.34	31.82	—17.0
Pooled SE			±8.1	±0.05	±0.21	±1.0

[a] All values represent the average of 12 animals.

[b] All groups received identical dosing regimen by administering control vehicles orally and/or topically.

[c] Doses administered daily for 4 days; refer to Methods for detailed description.

[d] Indicates significant difference from control: $p < 0.05$; [e] $p < 0.01$.

contrast to results obtained following the topical application, however, the oral administration of acetylsalicylic acid (200 mg/kg) elevated pain threshold, produced less effect on local hyperpyrexia, and failed to inhibit adjuvant-induced body weight loss.

2. *Hydrocortisone.* The effect of topically and orally administered hydrocortisone on the primary lesion of adjuvant arthritis is summarized in Table II. The topical application of 2% hydrocortisone in hydroalcoholic solution (2 to 4 times/day) prior to and during the development of the primary lesion significantly inhibited edema formation and local hyperpyrexia but aggravated the adjuvant-induced body weight loss. The oral administration of hydrocortisone at a daily dose of 10 mg/kg did not significantly alter edema formation, local hyperpyrexia, or adjuvant-induced body weight loss. However, preliminary data (unpublished data from our laboratories) demonstrated that higher daily oral doses of hydrocortisone (20 mg/kg) significantly inhibited edema formation and aggravated adjuvant-induced body weight loss but did not significantly effect local hyperpyrexia.

C. Discussion. Acetylsalicylic acid and hydrocortisone in hydroalcoholic solutions applied topically to the primary lesion of adjuvant arthritic rats are absorbed in pharmacological concentrations as evidenced by their local anti-inflammatory activity and systemic alterations in body weight. Although topically applied acetylsalicylic acid produced equivalent antiedema activity, as well as greater effects on local hyperpyrexia and adjuvant-induced body weight loss than the or-

TABLE II. Effect of Topical and Oral Administration of Hydrocortisone on the Primary Lesion (injected leg, day 4) of Adjuvant Arthritis.

Test material[b]	Route	Dose[c]	Hindleg vol (ml)	Paw temp (°)	Body wt change from day 1 (g)
Hydrocortisone	Oral	10 mg/kg	3.04	31.43	—16
	Topical	2% in 70% ethanol	1.89[e]	30.77[d]	—29[e]
Control			3.39	31.64	—16
Pooled SE			±0.35	±0.27	±1.8

[a] All values represent the average of 5–6 animals.

[b] All groups received identical dosing regimen by administering control vehicles orally and/or topically.

[c] Dose administered daily for 4 days; refer to Methods for detailed description.

[d] Indicates significant difference from control: $p < 0.05$; [e] $p < 0.01$.

ally applied compound, only the orally administered acetylsalicylic acid significantly elevated pain threshold. These results support the thesis that the anti-inflammatory activity of acetylsalicylic acid occurs locally; whereas analgesia induced by this drug is due to effects on the central nervous system (11). These results further suggest that a higher local concentration of acetylsalicylic acid was achieved following topical application, and that local hyperpyrexia and adjuvant-induced body weight loss are produced by factors released at the site of inflammation.

Topically applied hydrocortisone appeared to exert greater antiedema activity and less antipyretic effect than topically applied acetylsalicylic acid. Further experimentation, however, is required to substantiate this impression. The aggravation of adjuvant-induced body weight loss produced by hydrocortisone is similar to the effects produced by systemic doses of cortisone (12) and is believed to be due to growth inhibiting properties. The effect on body weight produced by topically applied hydrocortisone demonstrate the high degree of absorption with this preparation. The efficient absorption of topically applied steroidal and nonsteroidal antiinflammatory agents, solubilized in hydroalcoholic solutions, suggests an advantageous therapeutic approach to the treatment of local inflammatory conditions, and the adjuvant arthritic rat appears to be an appropriate model to detect and elucidate these topically effective anti-inflammatory preparations.

Summary. Topical application of aspirin (6%) or hydrocortisone (2%) in hydroalcoholic solutions significantly reduced edema formation and local hyperpyrexia associated with the primary lesion of adjuvant arthritis in rats. Topical application of aspirin prior to and during the development of the primary lesion also significantly reduced adjuvant-induced body weight loss; whereas topically applied hydrocortisone aggravated body weight loss. Oral doses of aspirin (200 mg/kg) produced antiedema effects equal to those of topically applied aspirin (6%). In contrast to the topically applied aspirin, orally administered aspirin elevated pain threshold but failed to reduce adjuvant-induced body weight loss, and had significantly less effect on local hyperpyrexia. These results support the concept that the elevation in pain threshold (analgesia) produced by aspirin is due to a central effect rather than local anti-inflammatory activity; they also demonstrate that the adjuvant arthritic rat is a suitable model to detect and distinguish characteristics of topically effective anti-inflammatory preparations.

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