

A Method for Differentiating Nonspecific Irritants from Anti-Inflammatory Agents Using the Carrageenin Abscess Test. (31584)

S. GOLDSTEIN, R. DEMEO, I. SHEMANO, AND J. M. BEILER
National Drug Company, Research Laboratories, Philadelphia, Pa.

Several nonspecific irritants were shown to have anti-inflammatory properties in 3 models of inflammation(1). Their properties, in these experimental models, were similar to those of known anti-inflammatory agents. The anti-inflammatory activity, in general, appeared to be dose responsive, and the substances were active in adrenalectomized as well as intact animals.

Many clinically effective anti-inflammatory agents have been reported to produce signs of irritation. Anderson(2) demonstrated that single oral doses of cinchophen, aspirin, phenylbutazone, chloroquine, indomethacin, mefenamic acid and ibufenac produce gastric erosion and hemorrhage in fasted guinea pigs. He also demonstrated that cortisone produces similar effects after repeated doses. Winder *et al*(3) reported that mefenamic acid when administered subcutaneously to guinea pigs produced erythema, hemorrhage and necrosis at the injection site.

The investigator is therefore faced with the problem of interpreting his results, and deciding whether anti-inflammatory activity is due solely to irritation or whether there is another component. A test is proposed which may differentiate between the two types of compounds.

Methods. A carrageenin abscess was produced in female Sprague-Dawley CD rats by the method described previously(4). These animals, weighing 60 to 80 g, were obtained from the Charles River Breeding Laboratories. The anti-inflammatory drugs (acetylsalicylic acid, phenylbutazone, indomethacin, mefenamic acid and hydrocortisone) and the irritants (croton oil*, formalin and kaolin) were administered subcutaneously either at a site (scapula area) distant from the abscess or mixed with the carrageenin.

The vehicles used for the subcutaneous injections (shown in the Tables) were either

distilled water or saline with or without Tween 80 (0.09% w/v). Eight rats per group were injected (0.5 ml/rat) subcutaneously in the scapula area with the drug solution or vehicle immediately following the subcutaneous injection of a 2% sterile carrageenin solution (0.5 ml/rat) at the base of the tail.

Anti-inflammatory agents and irritants administered with the carrageenin were triturated in the sterile 2% carrageenin solution. Equal volumes of distilled water, representing the volume of drug used, were mixed with the carrageenin solution for the control groups. These rats were also injected subcutaneously with 0.5 ml of the carrageenin mixture at the base of the tail. Twenty-four hours following the carrageenin injection, the rats were killed with ether and the carrageenin-induced abscess was removed and weighed.

Concentrations of the irritants and anti-inflammatory agents used in this study are given in the Tables. The statistical significance of the drug changes indicated in the Tables was determined by comparing the absolute values using Student's "t" test. Results were considered significant when the P value was equal to or less than 0.05.

Results. The effects of kaolin, formalin and croton oil on the carrageenin abscess following local administration with the carrageenin or subcutaneous administration in the scapula area are summarized in Table I. All 3 irritants produced significant decreases in abscess weight when administered subcutaneously at the various concentrations studied. When these irritants were mixed with the carrageenin, a significant increase in abscess weight was obtained at all the concentrations of kaolin and at the higher concentrations of formalin and croton oil. The 2 and 4% concentrations of formalin administered subcutaneously were lethal, whereas these same concentrations administered with the carrageenin had no toxic effect. The apparent anti-inflammatory effect following subcutaneous

* Croton oil N.F. VII, Magnus, Mabee & Reynard Inc., New York.

TABLE I. Effect of Irritants on the Carrageenin Abscess when Administered Locally with the Carrageenin or Subcutaneously.

Treatment	% Concentration of irritant	Irritant injected subcutaneously†			Irritant mixed with the carrageenin		
		No. of rats	Abscess wt (mg) ± S.D.	% Change from control	No. of rats	Abscess wt (mg) ± S.D.	% Change from control
Control		8	2720 ± 434		8	2626 ± 496	
Kaolin	32	8	1777 ± 289*	-35	8	3699 ± 750*	+41
	16	8	1816 ± 448*	-33	8	3437 ± 567*	+31
	8	8	1989 ± 255*	-27	8	3319 ± 548*	+26
	4	8	1998 ± 373*	-27	8	3099 ± 378*	+18
Control		8	3413 ± 345		8	3394 ± 535	
Formalin	4	2†	1109 ± 230*	-68	8	4418 ± 639*	+30
	2	7†	1500 ± 418*	-56	8	4060 ± 820	+20
	1	8	2120 ± 232*	-38	8	3002 ± 362	-12
	0.5	8	2176 ± 299*	-36	8	3038 ± 372	-10
Control		8	2930 ± 308		8	2899 ± 670	
Croton oil	0.5	8	1368 ± 256*	-53	8	3829 ± 639*	+32
	0.125	8	1650 ± 231*	-44	8	3832 ± 347*	+32
	0.031	8	2287 ± 269*	-22	8	3470 ± 311*	+20
	0.008	8	2442 ± 136*	-17	8	3226 ± 348	+11

* Significant difference from control ($P \leq 0.05$).

† Irritant was toxic at this concentration.

‡ Control groups received distilled water and in the croton oil group Tween 80 was also added.

administration and the inflammatory effect following administration with the carrageenin appear to be dose respondent.

In Table II are summarized the anti-inflammatory effects obtained with acetylsalicylic acid, phenylbutazone, mefenamic acid, indomethacin and hydrocortisone. These compounds, mixed with the carrageenin, produced significant decreases in abscess weight at concentrations equal to or less than those effective subcutaneously. This effect is diametrically opposite to that obtained with irritants. Mefenamic acid, indomethacin and hydrocortisone when mixed with the carrageenin produced anti-inflammatory effects at concentrations several times lower than the concentrations effective subcutaneously. Acetylsalicylic acid and phenylbutazone differed from the above compounds. The effective concentrations of phenylbutazone in the abscess were only slightly less than the concentrations effective subcutaneously. Acetylsalicylic acid was equally effective whether administered subcutaneously in the scapula area or mixed with the carrageenin.

Discussion. There have been few experimental attempts to differentiate nonspecific irritants from "useful" agents. Jori(5) pro-

posed a dye extravasation test based on the distribution of Evans Blue to detect what he termed "aspecific effects." Indomethacin, phenylbutazone and acetylsalicylic acid when administered intraperitoneally did not increase extravasation of Evans Blue in the peritoneal cavity whereas irritants did. However, when the nonsteroid agents were administered orally, acetylsalicylic acid behaved similarly to the irritants. The disadvantage of this method is the necessity of dye determinations on the extracts of the peritoneal fluid.

The method described here enables an investigator to differentiate very simply between the nonspecific irritants and the anti-inflammatory agents studied without the necessity of dye determinations. The irritants administered with the carrageenin significantly increased abscess weight, whereas similar concentrations administered at a site distant from the abscess significantly decreased abscess weight. The anti-inflammatory compounds decreased abscess weight when administered at either site.

Hydrocortisone, indomethacin and mefenamic acid were effective locally at concentrations several times less than those required subcutaneously to produce similar anti-inflam-

TABLE II. Effect of Anti-Inflammatory Agents on the Carrageenin Abscess when Administered Locally with the Carrageenin or Subcutaneously.

Treatment	% Concentration of compound	Compound injected subcutaneously†			Compound mixed with the carrageenin		
		No. of rats	Abscess wt (mg) ± S.D.	% Change from control	No. of rats	Abscess wt (mg) ± S.D.	% Change from control
Control		8	3076 ± 394		8	3076 ± 564	
Acetylsalicylic acid	5	8	1865 ± 422*	-39	8	2010 ± 372*	-35
	2.5	8	2211 ± 347*	-28	8	2355 ± 298*	-23
	1.25	8	2295 ± 380*	-25	8	2659 ± 166*	-14
	.625	8	2702 ± 362	-12	8	3018 ± 364	-2
Control		8	2738 ± 361		8	2944 ± 314	
Phenylbutazone	.2	8	2045 ± 304*	-25	8	2058 ± 229*	-30
	.1	8	2114 ± 273*	-23	8	2279 ± 421*	-23
	.05	8	2386 ± 355	-13	8	2066 ± 361*	-30
	.025	8	2684 ± 358	-2	8	2568 ± 515	-13
Control		8	2745 ± 451		8	3133 ± 628	
Mefenamic acid	.27	8	2121 ± 334*	-23	8	1971 ± 294*	-37
	.09	8	2805 ± 256	+ 2	8	1919 ± 177*	-39
	.03	8	2706 ± 428	- 1	8	2048 ± 367*	-35
	.01	8	2809 ± 246	+ 2	8	2065 ± 294*	-34
	.0033	8	2869 ± 293	+ 5	8	2385 ± 471*	-24
Control		8	2543 ± 378		8	2795 ± 508	
Indomethacin	.004	8	2187 ± 289*	-14	8	1564 ± 209*	-44
	.002	8	2257 ± 380	-11	8	1728 ± 166*	-38
	.001	8	2459 ± 316	- 3	8	1881 ± 192*	-33
	.0005	8	2455 ± 377	- 3	8	2113 ± 244*	-24
	.00025	8	2593 ± 530	+ 2	8	2283 ± 287*	-18
Control		8	2740 ± 376		8	2850 ± 157	
Hydrocortisone	.0032	8	2645 ± 427	- 3	8	2205 ± 286*	-23
	.0016	8	2514 ± 391	- 8	8	2305 ± 368*	-19
	.0008	8	2500 ± 478	- 9	8	2443 ± 293*	-14
	.0004	8	2934 ± 382	+ 7	8	2924 ± 638	+ 3

* Significant difference from control ($P \leq .05$).

† Control groups received saline plus Tween 80.

matory effects. This may indicate that mefenamic acid and indomethacin, like hydrocortisone, have more of a local effect than phenylbutazone and acetylsalicylic acid. The fact that phenylbutazone and acetylsalicylic acid were active locally only at or just below systemically effective concentrations, may indicate that these drugs must be absorbed from the abscess area before they can exert their anti-inflammatory activity.

Summary. A method is described for differentiating between non-specific irritants and clinically effective anti-inflammatory agents using the carrageenin abscess test. Irritants mixed with the carrageenin increased abscess weight at concentrations which reduced abscess weight when injected subcutaneously at

a distant site (scapula area). The anti-inflammatory compounds reduced the abscess weight when mixed with the carrageenin at concentrations below or equal to those concentrations effective systemically.

The authors wish to thank Geigy Pharmaceuticals for phenylbutazone, Merck Sharp and Dohme for indomethacin and Parke, Davis and Co. for mefenamic acid. We are also grateful to Mrs. C. Intrieri for help in preparation of this manuscript.

1. Goldstein, S., DeMeo, R., Shemano, I., Beiler, J. M., Arch. int. Pharmacodyn., in press.
2. Anderson, K. W., Proc. Int. Symp., Excerpta med. Found., Milan, 1964, p245.
3. Winder, C. V., Wax, J., Scotti, L., Scherrer, R. A., Jones, E. M., Short, F. W., J. Pharmacol.

Exp. Therap., 1962, v138, 405.

4. Goldstein, S., Schnall, M., Arch. int. Pharmacodyn., 1963, v144, 269.

5. Jori, A., Bernardi, D., Med. Pharmacol. exp., 1966, v14, 500.

Received September 6, 1966. P.S.E.B.M., 1966, v123.

A Unique Hyperfine ESR Spectrum in Mouse Neoplasms Analyzed By Computer Simulation.* (31585)

M. J. BRENNAN, T. COLE, AND J. A. SINGLEY

Michigan Cancer Foundation, and Ford Motor Company Scientific Laboratories, Detroit and Dearborn, Mich.

Electron spin resonance (ESR) spectra have been demonstrated in many intact plant and animal tissues. We have recently undertaken a detailed mapping of the ESR spectra occurring in various organs of genetically pure inbred strains of laboratory mice(1). This study parallels that of Nebert and Mason(2) but has concentrated on the region of $g = 2.1 - 1.9$, the region of organic free radical lines, where they recorded broader spectra. Our objective was to determine the characteristics and replicability of ESR spectra in normal adult tissues and to compare these with homologous tissues in various states of active cell-multiplication including the neonatal, regenerating, and neoplastic conditions.

We here report observations in neoplastic spleen and in the JAX C 1300 neuroblastoma. A unique spectrum showing nitrogen hyperfine splitting was found to occur in these tissues. This spectrum has been seen only in neoplastic tissues. Because of its unusual appearance and its association with the neoplastic state we have studied the phenomenon in detail.

Experimental procedures. BALB C+ mice and C₃H+ mice, bred and carried in our own laboratories, maintained on Purina lab chow and housed in fiberglass cages, were used. Cedar shavings were used for bedding. Males and females 6 months old were used in the normal series and as transplant recipients for the 2 neoplasms. No strain differences in tissue signals were noted.

Animals were killed by CO₂ asphyxiation. As rapidly as possible, the tissue to be studied

was excised, minced into a Petri dish supported over ice chips, packed into signal-free fused-silica tubes of 3 mm inside diameter, and immersed in a liquid nitrogen Dewar. We estimate that time from excision to cooling to 1-5°C was in the order of 2 to 3 minutes and that samples had been taken to 77°K within 5 minutes of excision. Throughout these studies standard methods for cleaning glassware and surgical instruments were employed and the same set of surgical tools was used. Experiments with Ehrlich ascites suspensions showed little or no change in transplantation behavior of cells handled in this way and subsequently thawed and transplanted from that of control non-frozen suspensions of the same tumor.

The ESR spectrometer used in these experiments was a homodyne type(3) employing 100 kc/sec magnetic field modulation and operating at 9.6 Gc/sec. A cylindrical sample cavity operating in the TE₀₁₁ mode was used. The cavity and associated waveguide were enclosed in a Mylar bag which was immersed in a Dewar of liquid nitrogen. Sample temperature was determined by thermocouple to be within 3°C of the temperature of the liquid nitrogen during the recording of the spectra. Higher frequency spectra were observed by changing the microwave assembly, sample cavity, and klystron to components operating at 14 Gc/sec. The sensitivity of the apparatus was sufficient to provide a signal to noise ratio of 200 to 1 for the 0.00033% pitch in KCl standard sample (Sample type 904450, Varian Associates, Palo Alto, Calif.), microwave power 1 milliwatt and time constant 1 sec. ESR spectra were simulated on a GE-235 computer.

* This work was supported in part by the Jimmy Morrison Fund.