Comparative Effects of Some Immunosuppressive and Anti-Inflammatory Drugs on Allergic Encephalomyelitis and Adjuvant Arthritis. (32035)

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Allergic encephalomyelitis (AE) and adjuvant-induced polyarthritis (AA) are two experimental models of interest because of their presumed immunological etiology and histopathologic features which grossly resemble various human allergic and inflammatory (auto-immune) diseases(1-3). The histological and clinical pathologies of both AE and AA can be prevented in various species in the laboratory by immunosuppressive and anti-inflammatory drugs that have been used with some success in human autoimmune disease(4-6). The present study compares the effectiveness of 4 clinically useful compounds on various parameters of AE and AA in an isohistogenic strain of rats and considers the value of these pathological models in studying immnosuppressive and anti-inflammatory compounds.

Methods. Male Lewis strain rats (Microbiological Associates, Bethesda, Md.) weighing 185-210 g were used. Twelve animals were assigned randomly to each experimental drug or control group. AE and AA were induced in separate groups of rats and studied concurrently. The experiment was repeated in a second group of animals and the results combined, resulting in 24 rats per compound studied in both AE and AA. Body weights were recorded at intervals. The effect of drugs on injected paw volume measurements in AE was determined in 2 additional experiments.

AE was induced by the subplantar injection of 0.05 ml of an encephalitogenic emulsion consisting of equal parts of a 40% W/V homogenate of isologous spinal cord in 0.5% aqueous phenol and Freund's complete adjuvant (4 mg/ml killed tubercle bacilli) into the right hind paw(7,8). Using this method hindquarter paralysis of 90 to 100% of the animals is routinely achieved.

AA was induced by the subplantar injection of 0.05 ml of a suspension of heat-killed tubercle bacilli in mineral oil (5 mg/ml) into the right hind paw(9). Paw volume was measured with a mercury plethysmograph coupled to a pressure transducer(10), and recorded on a Varian Model G-10 graphic recorder. The right hind paw volume of each rat was measured immediately before antigen injection (control) and again 14 days later (test); the difference was the degree of swelling. Blood counts were done immediately before autopsy according to standard hematological techniques.

All compounds, except cortisone acetate, were suspended in one part aluminum hydroxide gel* to one part water, and were administered orally in a volume of 1 ml. Fresh suspensions were prepared daily. Cortisone acetate suspension† was injected subcutaneously. Controls received aluminum hydroxide gel vehicle only. Drug treatment was begun on the day of challenge with antigen and autopsies were performed 14 days later. All drugs except 6- mercaptopurine (6-MP) were given 5 days a week for 2 weeks (10 doses); 6-MP was administered on alternate days for 2 weeks (6 doses).

Results. The physiological responses to AE and AA were somewhat similar (AE and AA controls, Table I). AA animals exhibited the classical signs of an alarm reaction to stress (11): adrenal and splenic hypertrophy, thymic involution and leucocytosis as a result of neutrophilia associated with a relative lymphopenia. Similar results occurred in AE, however, in contrast AE animals exhibited splenic involution and no increase in total leucocytes although a relative neutrophilia and lymphopenia occurred as in AA. There was considerable difference in the body weight pattern between AE and AA animals (con-

* Aludrox, Wyeth Laboratories.

† Cortone, Merck Sharp and Dohme.

TABLE I. Comparison of Effects of Drugs on Experimental Allergic Encephalomyelitis (AE) and Adjuvant Arthritis (AA) in Lewis Rats.*†

Drugs	Dose & route, mg/day	No. rats at autopsy	No. dead/ total rats	% Protection‡	Organ weights, mg			Hematology		
					Thymus	Spleen	Adrenals	WBC $\times 10^3$/mm^3	Polys, %	Lymphs, %
Experimental allergic encephalomyelitis										
AE control	—	23	1/24	—	169 ± 13	321 ± 11	39 ± 1	13 ± 0.9	53 ± 3	43 ± 2
Normal animals	—	48	0/48	—	440 ± 8§	489 ± 10§	35 ± 1§	14 ± 0.4	24 ± 1§	71 ± 1§
Cortisone acetate	8.5 s.c.	24	0/24	100	28 ± 1§	267 ± 9§	15 ± 1§	12.8 ± 0.6	65 ± 2§	31 ± 2§
6-Mercaptopurine	20 p.o.	23	1/24	100	50 ± 3§	354 ± 15	34 ± 1§	9 ± 0.5	34 ± 3§	63 ± 3§
Phenylbutazone	30 p.o.	22	2/24	0	180 ± 9	342 ± 15	42 ± 1	16 ± 1.0§	61 ± 2§	36 ± 2§
Aspirin	36 p.o.	24	0/24	16	211 ± 20	349 ± 12	34 ± 2§	13 ± 0.7	60 ± 2	37 ± 2
Adjuvant arthritis				Increase in paw vol (ml)						
AA control	—	31	5/36	.99 ± .07	151 ± 13	605 ± 35	48 ± 2	30.8 ± 1.8	61 ± 1	28 ± 1
Normal animals	—	48	0/48	.10 ± .01	440 ± 8§	489 ± 10§	35 ± 1§	14.0 ± 0.4§	24 ± 1§	71 ± 1§
Cortisone acetate	8.5 s.c.	22	2/24	.13 ± .04§	18 ± 4§	313 ± 18§	20 ± 1§	10.2 ± 0.4§	63 ± 3	32 ± 2
6-Mercaptopurine	20 p.o.	15	9/24	.05 ± .05§	44 ± 6§	389 ± 17§	36 ± 1§	8.1 ± 0.5§	25 ± 3§	70 ± 3§
Phenylbutazone	30 p.o.	23	1/24	.53 ± .05§	247 ± 17§	539 ± 49	44 ± 1§	29.8 ± 1.6	64 ± 1	26 ± 2
Aspirin	36 p.o.	23	1/24	.62 ± .07§	177 ± 23	591 ± 49	49 ± 3	28 ±20	53 ± 3§	38 ± 3§

* All drugs were administered on the day of antigen (day 0). 6-mercaptopurine was administered on alternate days for 2 wk (total of 6 doses); the remaining drugs were given 5 days a week for 2 wk (total of 10 doses).

† All results are expressed as mean ± S.E. and were obtained 14 days after antigen administration. For comparative purposes, results for normal rats are combined and shown with both AE and AA.

‡ Indicates complete protection from flaccid paralysis of the hindlimbs, fecal impactation and urinary incontinence observed in AE.

§ $P < .05$ compared with control.

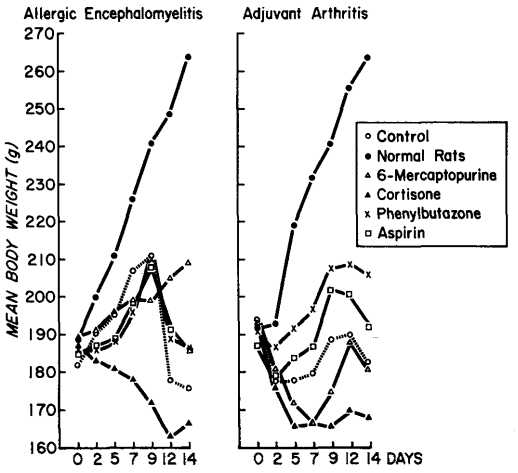

FIG. 1. Effect of drugs on growth of rats with allergic encephalomyelitis and adjuvant arthritis. Each point represents the mean of the group. Group size on day 0 is 24 rats per group except for adjuvant arthritis controls which represent 36 rats. Group size on autopsy day 14 is indicated in Table I. Doses and schedules of drug administration as in Table I.

trols, Fig. 1). Encephalitic rats gained weight at the same rate as normal animals for 9 days after antigen administration, but then lost weight rapidly, the mean loss from day 9 to scoring day 14 being 45 g. In contrast, arthritic rats showed an initial loss of weight which continued to day 7, after which some increase occurred.

In AE rats cortisone or 6-MP therapy afforded complete protection from hindlimb paralysis, whereas phenylbutazone and aspirin were ineffective (Table I). Spleen weight was significantly reduced by cortisone but unaffected by the other drugs. Thymic involution occurred in the cortisone and 6-MP groups only. In contrast to the other drugs 6-MP tended to normalize the polymorphonuclear leucocyte to lymphocyte ratio, and caused a slight leucopenia. In addition to the gross hindlimb paralysis seen in AE, the paw receiving the encephalitogenic antigen became inflamed and swollen approximately 5 days after subplantar injection. When examined in a subsidiary experiment, only cortisone and 6-MP significantly reduced this local inflammatory response (Table II).

AA induced swelling of the injected foot was inhibited by all compounds with cortisone or 6-MP having the greatest effect (Table I). Both cortisone and 6-MP prevented the absolute leucocytosis and splenic enlargement observed in AA but, as in AE, they also enhanced thymolymphatic involution. Again only 6-MP modified towards normal the ratio of polymorphonuclear leucocytes to lymphocytes. Involution of the adrenals of the cortisone treated rats occurred in both the AE and AA groups doubtlessly reflecting the ACTH blocking action of the steroid, whereas 6-MP appeared to prevent hypertrophy of the adrenals seen in AE and AA controls. 6-MP was considerably more toxic in arthritic than encephalitic rats.

The body growth curves for encephalitic rats show that cortisone caused loss of weight from the beginning of treatment while the patterns for phenylbutazone and aspirin were not much different from positive controls (Fig. 1). 6-MP treated rats, however, did not undergo the characteristic weight loss after day 9 but actually gained weight from days 9 to 14. In arthritic rats cortisone also caused weight loss while phenylbutazone and aspirin, in contrast to their effect in AE rats, tended to prevent the weight loss seen in positive controls. 6-MP had little or no effect on weight change in arthritic animals.

Discussion. Allergic encephalomyelitis and adjuvant arthritis have been extensively studied and evidence has accumulated suggesting that both are immunological diseases manifested in a delayed type of reaction through essentially identical mechanisms (12-14). Adjuvant arthritis, however, differs from AE and other experimental autoimmune diseases in that tissue antigen is not neces-

TABLE II. Effects of Drugs on Paw Swelling in Experimental Allergic Encephalomyelitis.*†

Drugs	No. rats	Increase in paw vol (ml)
AE control	28	.61 ± .03
Normal animals	20	.12 ± .01‡
Cortisone acetate	24	.35 ± .02‡
6-Mercaptopurine	24	.48 ± .03‡
Phenylbutazone	24	.53 ± .04
Aspirin	24	.55 ± .04

* All drugs were administered at same doses and schedules as in Table I.

† Results are expressed as mean ± S.E. and were obtained 14 days after antigen administration.

‡ $P < .05$ compared with positive controls.

sary for its induction; thus the responsible antigen remains unknown. Furthermore, adjuvant arthritis occurs with normal intensity in thymectomized rats, in whom other apparently similar immunological phenomena are suppressed, implying that this syndrome may not depend upon a delayed-type immunological response(15). Moreover, Ward *et al*(4) on the basis of studies on immunosuppressive agents, have hypothesized that adjuvant arthritis is not the result of hypersensitivity.

In the present study the immunosuppressive agents, 6-MP and cortisone were prophylactic for the paralysis seen in encephalomyelitis and the swollen paws of both the paralyzed and arthritic rats. At doses similar to those previously reported active in AA(5,16) the anti-inflammatory drugs phenylbutazone and aspirin inhibited the arthritic but not the encephalitic response. Sodium salicylate in combination with para-aminobenzoic acid has been shown to have some inhibitory effect on AE in guinea pigs when administered subcutaneously(17). Under conditions of these experiments a stressor effect of sodium salicylate could not be excluded(18). A comparison of these studies with the lack of oral activity of acetylsalicylic acid in rats reported here is not possible. 6-MP not only blocked the paralytic and hematologic symptoms of AE, but also was able to correct the characteristic body weight decline of AE and enabled the animals to continue to gain weight. Aspirin and phenylbutazone, on the other hand, were somewhat effective on growth of arthritic rats, while 6-MP was ineffective actually causing a greater weight loss than controls. The well known growth inhibiting properties of cortisone obscured its effect on the growth curves. 6-MP was the only compound able to reverse the abnormal shift of polymorphonuclear leukocytes and lymphocytes which occurred both in AE and AA.

The dissociation of effect of these drugs on various manifestations of AE and AA indicates that the pathological basis of these two disorders in Lewis rats may be fundamentally dissimilar. Another possibility, however, is that both models have similar immunological mechanisms but that the acute inflammatory response of AA is more susceptible to anti-inflammatory agents, as is suggested by the absence of response of the inflamed paws of encephalitic rats to the anti-inflammatory drugs phenylbutazone and aspirin. Anti-inflammatory activity has been described for the immunosuppressive agent 6-MP(19,4,6). If adjuvant arthritis is not the result of a delayed hypersensitivity reaction, this anti-inflammatory activity could explain the apparent paradoxical efficacy of 6-MP in arthritis. In view of the pronounced anti-immune, anti-inflammatory activity of cortisone(1) it is not surprising that this compound was active in both AE and AA.

The atypical response of the AE animals to a systemic stressor (Freund's adjuvant) is interesting. We can offer no definite explanation for the absence of splenic hypertrophy and absolute leucocytosis, changes which would be anticipated in response to a general stressor. However, the fulminating paralysis may have altered the nature of this response.

Nuss and Winter(20) obtained inhibition of arthritic foot swelling with the anti-inflammatory agents indomethacin and flufenamic and mefenamic acids which according to preliminary data‡ are orally inactive in AE. Pending the detailed study of additional drugs it can tentatively be stated that allergic encephalomyelitis appears suitable for detection of immunosuppressive agents and adjuvant arthritis for immunosuppressive and "pure" anti-inflammatory compounds. Simultaneous use of both models in the Lewis rat should permit the separation of the two pharmacological entities.

Summary. Experimental allergic encephalomyelitis (AE) and adjuvant arthritis (AA) were induced in male Lewis rats. 6-Mercaptopurine and cortisone inhibited both AE and AA, but phenylbutazone and aspirin were effective only in AA. The different effects of these immunosuppressive and anti-inflammatory drugs on clinical signs, organ and body weights, and hematologic changes in AE and AA suggest either that the pathogenesis of the two models is dissimilar or that the

‡ Unpublished observations from our laboratories.

acute inflammatory response is of greater etiological importance in arthritis. AE and AA should be useful for differentiating the immunosuppressive and anti-inflammatory activities of drugs.

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Influence of Age and Dietary Stress on Hexobarbital Activity in Mice.* (32036)

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The activity of the liver enzyme systems concerned with drug detoxication can be influenced by numerous factors including age and dietary intake of the animal. In the newborn mouse the activity of these drug metabolizing enzymes is very low(1). The ability of the liver enzymes to detoxify drugs increases rapidly during the growth period of the animal and reaches a relatively steady state in the well-nourished adult. However, when the adult animal is subjected to starvation or is maintained on a low level of dietary protein, the activity of drug metabolizing enzymes is significantly decreased (2,3). In the present study, using mice from weaning to young adulthood, we have compared the effect of two isocaloric diets, differing only in protein and carbohydrate content, on the development and stability of the liver enzyme system concerned with hexobarbital detoxication.

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Materials and methods. Diet. The two semisynthetic, isocalroic diets used in this study were similar to those we used previously with rats(4) but differed in that starch was substituted for a portion of the carbohydrate in the diet (Table I). The addition of starch allowed for pelleting of the diet. The use of such a pelleted diet considerably minimized urinary and fecal contamination of the food by the mice.

Animals and their care. The CF-1 male