THE ANTI-INFLAMMATORY ACTION OF GRISEOFULVIN IN EXPERIMENTAL ANIMALS

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When tested by the cotton-pellet method in rats or the tuberculin-hypersensitivity test in guinea pigs, griseofulvin had a marked anti-inflammatory action. The activity was, weight for weight, less than that of the corticosteroids tested concurrently. The anti-inflammatory action of griseofulvin appears to be independent of any effect upon the pituitary-adrenal axis.

Griseofulvin was first isolated in 1939 from *Penicillium griseofulvum* Dierckx[1], and its chemical structure was established some years later[2]. It was then shown to have antifungal activity *in vitro*, especially against dermatophytes. It is effective orally in the treatment of ringworm in guinea pigs[3], cattle[4] and man[5], producing no untoward effects. It seems that this *in vivo* antifungal action is greater than might have been predicted from *in vitro* tests. In experiments with guinea pigs infected with *Microsporum canis* and treated with griseofulvin, Gentles[3] observed that the "highly inflammatory reaction which developed in all the control animals was prevented". Cochrane and Tullett[6] investigated the griseofulvin treatment of acute inflammatory cattle ringworm in man and reported that the antibiotic caused a rapid disappearance of general malaise, pain and discomfort; they also observed "a rapid disappearance of all inflammatory signs". The work reported here was initiated to enquire whether griseofulvin has an anti-inflammatory action in addition to known antifungal properties.

Experimental

Drugs Tested

In all the experiments on rats and mice, griseofulvin was administered orally as a suspension in 5 per cent gum acacia, each dose in a volume of 0·5 ml./ 100 g. weight. In guinea pigs the antibiotic was administered orally as a suspension in physiological saline with 1:4000 w/v of the wetting agent Triton WR1339, each dose in a volume of 0·5 ml./540 g. weight.

For comparison cortisone acetate was administered orally to rats and mice, except when otherwise stated in the text, in a similar volume per dose; a stock suspension containing 25 mg./ml. was diluted in saline or distilled water to the desired concentration. The guinea pigs received hydrocortisone acetate by subcutaneous injection, each dose of 25 mg./kg. in a volume of 0·5 ml./500 g. weight. Hydrocortisone was used instead of cortisone in guinea pigs, being better tolerated on subcutaneous

injection. In some experiments, rats were each injected daily with a subcutaneous dose of a long-acting corticotrophin preparation (Cortrophin ZN, Organon) of five international units.

Methods

The cotton pellet test in rats[7]. Male albino rats 100–120 g. weight were used. Sterilised cotton wool pellets of known weight (7–10 mg.) were implanted subcutaneously in groups of 5 rats under ether anaesthesia; 4 pellets were placed in each animal, one in each groin and one in each axilla. Griseofulvin was administered orally at doses of 62·5–500 mg./kg. weight on 4 consecutive days, the first dose being given immediately after implantation of the pellets. On the fifth day the rats were killed with chloroform and the pellets dissected; all fat and extraneous tissue was removed, and the pellets dried overnight in an hot air oven at 60°. Groups of control rats received oral doses of tap water in place of the griseofulvin suspension. The weight of tissue in each pellet was calculated, and the mean value for each group was compared with that of the controls. Some additional experiments were done on adrenalectomised rats.

The tuberculin skin sensitivity test. A modification of the methods of Long and Miles[8] was used. Albino guinea pigs of both sexes and weighing 500–600 g. were maintained on a pelleted diet and tap water. They were made sensitive to tuberculin by intramuscular injection of 0·1 mg. (moist weight) of *Mycobacterium BCG* 4–6 weeks before the test and were randomised into groups of 8–10. One group was left untreated and the others were treated with either griseofulvin at doses from 10–250 mg./kg. weight, or with hydrocortisone acetate, 25 mg./kg. The animals were tested by injecting old tuberculin intradermally at two doses, 10 T.U. and 100 T.U. in 0·1 ml. volume, into the depilated flank. Three treatment doses of griseofulvin or hydrocortisone were given at 18 and 2 hours before and 6 hours after the tuberculin test.

The diameters of the zones of oedema caused by the injections were measured with calipers. By plotting the average diameters against the log dose of tuberculin in the control group a standard curve was obtained, from which the apparent potencies of the tuberculin in the treated groups were measured. Thus, for example, if the standard tuberculin when measured on the treated guinea pigs appeared to have 40 per cent of its true potency, this was recorded as a "60 per cent reversal" of the local tuberculin sensitivity in the treated group.

The formalin foot test. The method of Selye[9], as modified by Buttle, D'Arcy, Howard and Kellett[10], was used.

Male albino rats, weighing 100–200 g. were maintained on a pellet diet and tap water. Griseofulvin was administered orally to groups of 8 animals at doses ranging from 62·5–2,500 mg./kg. weight. One and a half hours afterwards, the volume of the left hind-foot of each rat was measured by means of the apparatus described by Buttle and his colleagues[10], and 0·1 ml. of a 3 per cent solution of formaldehyde was injected into the plantar aponeurosis of the foot. A second dose of griseofulvin was administered 3 hours after the formalin injection.

Control groups of rats were also injected with formalin solution and received oral doses of 5 per cent gum acacia alone. The changes in volume of the injected feet were noted in each treated and control rat at periods of 4½ and 6 hours after the formalin injection. For some experiments, the rats were adrenalectomised before being used in the tests.

The "cold stress" test. Normal and adrenalectomised mice were tested for protection against cold stress by a method previously described[11]. The animals were maintained on a pelleted diet and tap water.

Protection of histamine-sensitised mice. Parfentyev and Goodline[12] showed that mice were sensitised to the lethal effect of injected histamine by the intravenous injection of *H. pertussis* vaccine and that the sensitivity was reversed by the administration of cortisone. This method was used

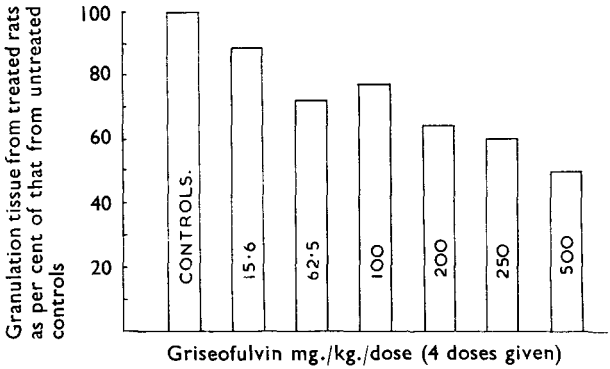

FIG. 1. The inhibitory effect of griseofulvin on the formation of granulation tissue around subcutaneously implanted cotton pellets. Griseofulvin administered orally daily for 4 consecutive days. Each column represents amount of granulation tissue, expressed as a percentage of that from untreated control animals. Groups of 5 or 10 rats used; four cotton pellets implanted in each rat.

to compare the effect of griseofulvin in oral doses up to 200 mg./kg., with that of cortisone by subcutaneous doses up to 100 mg./kg. Female albino mice of the A2G strain[13] (15–16 g.) were used and maintained on a pelleted diet and tap water.

The liver glycogen-deposition test. The actions of griseofulvin and cortisone on the deposition of glycogen in the livers of fasting adrenalectomised mice were compared by the method of Venning, Kazmin and Bell[14].

Effects on the adrenal cortex. The alterations in weight and histological appearance of the adrenal cortex after prolonged administration of griseofulvin and cortisone in rats were examined by a method previously described[15]. In similar experiments the effect of prolonged daily administration of griseofulvin was compared with that of Cortrophin ZN.

RESULTS

In the cotton pellet test on rats, griseofulvin inhibited the formation of granulation tissue around subcutaneously implanted cotton pellets. The combined results of several experiments are shown graphically in Figure 1,

from which it can be seen that griseofulvin produces a graded reduction in formation of granulation tissue over the oral dose range 15·6–500 mg./kg. daily for 4 consecutive days. Griseofulvin is less active than cortisone in the cotton pellet test. Figure 2 shows the results of three experiments in

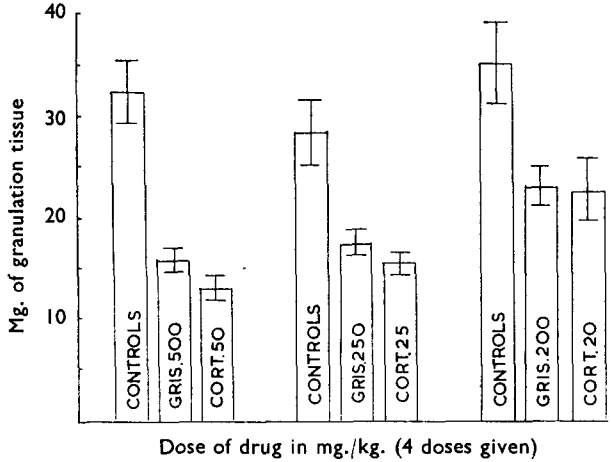

FIG. 2. Comparison between effects of griseofulvin (Gris.) and cortisone acetate (Cort.) on formation of granulation tissue around subcutaneously implanted cotton pellets. Both drugs orally administered daily for 4 consecutive days. Each column represents mean weight of granulation tissue in a group of five rats; four cotton pellets implanted in each rat; the vertical lines represent the standard errors.

TABLE I

THE EFFECT OF GRISEOFULVIN ON SKIN SENSITIVITY TO TUBERCULIN IN TUBERCULIN-POSITIVE GUINEA PIGS

Substance	Dose level (mg./kg./dose*)	Route of administration	No. of observations	Mean percentage reduction in tuberculin sensitivity and range
Griseofulvin	250	Oral	1	85
Griseofulvin	100	Oral	2	75 (62–87)
Griseofulvin	50	Oral	6	66 (58–82)
Griseofulvin	25	Oral	2	47 (36–57)
Griseofulvin	10	Oral	2	22 (20–23)
Controls—no treatment	0	—	8	0
Hydrocortisone acetate ..	25	Subcutaneous	5	68 (54–78)

* Three doses of griseofulvin were given orally, 18 and 2 hours before and 6 hours after intradermal injections of tuberculin.

which an attempt was made to compare directly the two compounds at equivalent response levels. The results indicate that griseofulvin has about one-tenth the activity of cortisone acetate in this test, since 200 mg./kg. griseofulvin produced a response similar to that of 20 mg./kg. of cortisone acetate; 250 mg./kg. griseofulvin was equivalent to 25 mg./kg. cortisone acetate and 500 mg./kg. griseofulvin was similar in its effect to 50 mg./kg. cortisone acetate.

In tuberculin-positive guinea pigs, the oral administration of griseofulvin caused a reduction in skin sensitivity to tuberculin, the percentage reduction being graded to the amount of griseofulvin (Table I). In this test

oral griseofulvin appears to have about one-third the activity of subcutaneous hydrocortisone, since a 25 mg./kg. dose of the latter caused 68 per cent reversal and 50–100 mg./kg. of griseofulvin gave the same effect. In other experiments, not recorded in detail here, it was found that

TABLE II

COMPARISON BETWEEN EFFECTS OF GRISEOFULVIN AND CORTISONE ACETATE ON INDICES OF ADRENOCORTICAL ACTIVITY IN THE RAT

Test	Griseofulvin		Cortisone acetate	
	Dose	Result	Dose	Result
(1) Protection of adrenalectomised mice against cold stress	5 g./kg. orally in 2 doses	No protection	62·5 mg./kg. orally in 2 doses	Protection
(2) Protection of mice sensitised to histamine (by *B. pertussis* vaccine) against intraperitoneal challenge with histamine	200 mg./kg. orally in 3 doses	No protection	100 mg./kg. subcutaneously in 2 doses	Protection
(3) Deposition of glycogen in livers of fasting adrenalectomised mice	2·5 g./mouse orally in divided doses	No glycogen deposition	5 mg./kg. orally	Deposition of glycogen
(4) Effect of prolonged administration on:				
(i) Weight of adrenal gland	2 g./kg. orally daily for 6 weeks	(i) No effect	200 mg./kg. orally daily for 6 weeks	(i) Decrease in adrenal weight
(ii) Adrenal tissue (histological examination)		(ii) No effect		(ii) Atrophy of zona fasciculata; depletion of sudanophilic lipoid

TABLE III

EXPERIMENTS TO DETERMINE WHETHER THE ANTI-INFLAMMATORY ACTIVITY OF GRISEOFULVIN ON THE RAT IS INDEPENDENT OF THE PITUITARY-ADRENAL AXIS

Test	Griseofulvin+	Cortisone acetate+	Cortrophin-ZN*
(1) Cotton pellet test in adrenalectomised rats Cotton pellet test in normal rats	Inhibition of formation of granulation tissue to an equal extent	Inhibition of formation of granulation tissue to an equal extent	—
(2) Formalin foot tests in adrenalectomised rats Formalin foot test in normal rats	Inhibition of formalin-induced swelling to an equal extent	Inhibition of formalin-induced swelling to an equal extent	—
(3) Protection of normal mice against cold stress	No protection	Protection	—
(4) Effect of prolonged administration on adrenal weight in normal rats	No effect	Adrenal atrophy	Adrenal hypertrophy

+ Dose levels as in Table II.

* Five I.U./rat subcutaneously daily for 6 weeks.

subcutaneous griseofulvin had a much smaller effect, presumably owing to the slow absorption of the insoluble antibiotic from the site of injection.

The results by the formalin foot technique were inconsistent. Griseofulvin given orally to rats at the high dose levels of 2 × 5,000 mg./kg. and 2 × 2,500 mg./kg. inhibited the formalin-induced swelling of the rats'

feet but at lower doses, down to 125 mg./kg., the results were variable. It appears that the formalin foot technique is not sufficiently sensitive to assist in evaluating this action of griseofulvin, and the results are not therefore reported here in detail.

The results of the "cold stress" tests in adrenalectomised mice, the histamine sensitisation tests in mice, the liver glycogen-deposition tests and the tests on the weight and histological appearance of the adrenal cortex are summarised in Table II. They show that, although cortisone acetate gives a positive result in all of these tests, griseofulvin at much higher dose levels has no activity.

Griseofulvin and cortisone acetate were further compared by cotton pellet tests and formalin foot tests in *adrenalectomised* and normal rats and in *normal* mice for protection against cold stress. Griseofulvin was also compared with Cortrophin ZN for any possible effect in producing hypertrophy of the adrenal cortex on prolonged administration to normal rats. The results are summarised in Table III.

Discussion

Griseofulvin was shown by the cotton pellet method in rats and the tuberculin skin sensitivity method in guinea pigs to have "anti-inflammatory" activity. This evidence was supported by some of the results of the formalin foot tests. The activity was about one-third to one-tenth that of cortisone acetate, depending on the method.

Our further experiments questioned whether the action of griseofulvin was due to a cortisone-like activity or to an entirely different mechanism. From the results of the cold-stress tests in adrenalectomised mice, the histamine sensitisation tests in mice and the liver glycogen-deposition tests in adrenalectomised mice and from the studies on the weight and morphology of the adrenal cortices of rats (Table II), it was evident that the anti-inflammatory effect of griseofulvin could not be due to any "cortisone-like" action.

Another possible explanation for the action of griseofulvin was that the substance might owe its activity in the anti-inflammatory tests to an activation of the pituitary-adrenal axis of the experimental animals. Tests by the cotton pellet and formalin foot methods in normal and adrenalectomised rats, by cold stress tests in normal mice and for a possible effect in producing hypertrophy of the adrenal cortex on prolonged administration in normal rats (Table III) showed that this could not be occurring. Whereas in the cotton pellet and formalin foot tests griseofulvin and cortisone acetate were active whether the animals were adrenalectomised or not, normal mice (with their adrenals intact) were not protected against cold stress by griseofulvin but were by cortisone. Moreover, in normal rats prolonged administration of cortisone produced atrophy of the adrenals and Cortrophin-ZN caused hypertrophy, but griseofulvin had no effect.

It is therefore clear that the anti-inflammatory action of griseofulvin does not depend on stimulation of the adrenal gland or on potentiation of its normal steroid secretion. In the cold stress test, if griseofulvin had

any action in increasing either output or utilisation of adrenocortical secretion, this would be reflected by protection of the animals. Moreover, since griseofulvin on prolonged administration to rats does not cause hypertrophy of the adrenals as judged by weight and tissue structure, it appears that it can neither cause an increased secretion of pituitary corticotrophin nor exert a "corticotrophin-like" action of its own.

It thus appears likely that griseofulvin owes its anti-inflammatory activity to some direct action of unknown nature at the site of inflammation.

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