J. Pharm. Pharmacol. 1990, 42: 729–731
Communicated April 20, 1990

Distribution of griseofulvin in the rat: comparison of the oral and topical route of administration

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Abstract—Effective penetration of griseofulvin across the dermal barrier has been achieved using an anhydrous solvent system of benzyl alcohol (10%), acetone (40%), and isopropanol (50%). There were quantitative differences in the relative accumulation of griseofulvin in skin compared with internal organs, when the topical and oral routes of administration were compared. The topical route enhanced localized concentrations of griseofulvin at the site of application, and these persisted for several days. After daily topical application a steady state was reached at day 3, when the diffusion across the skin barrier and epidermal loss seemed to equal the total amount applied to the skin surface. The application of griseofulvin topically, required a much smaller amount of drug to achieve similar integumentary levels compared with the amount required orally.

Even though the published literature on topical griseofulvin almost conclusively supports the view that it is as effective as oral griseofulvin, oral therapy remains the one currently available (Wozniak et al 1970; Knight 1974; Epstein et al 1975; Zarowny et al 1975; Abdel-Aal et al 1977; Cartwright 1978; Post & Saunders 1979). This could reside in the difficulties in preparing a stable solution of griseofulvin which is sparingly soluble in ethanol, chloroform, DMSO, dimethylformamide and acetone and, because of its strong hydrophobic character, its insolubility in polar solvents. Certain organic acids such as succinic acid enhance its solubility but most organic solvents are irritating to tissues and therefore are not biocompatible. More recently, ointments containing griseofulvin have been prepared using an aprotic solvent, glycerinformal to which monoglycerides of medium chain length fatty acids 1:1, were added to enhance the permeability of the solute (Franz et al 1981).

The potential of using topical griseofulvin as an adjuvant to orally administered drug has also been explored. Patients with microsporosis of the scalp were treated concurrently with oral griseofulvin and a topical 5% solution in DMSO. This therapy shortened the period of treatment and enabled the dose to be reduced (Medvedeva & Timofeeva 1980).

In the present studies we have used mixtures of solvents in proportions that allow for rapid evaporation of the more volatile components and transfer of the solute to the less volatile component, which will carry the active drug across the dermal barrier.

Materials and methods

Most of the metabolic studies were performed on 200–300 g Fischer rats. In a later study hairless Sprague-Dawley rats were compared with hooded Long-Evans rats, in the same weight range. For most topical studies two rats were used for each time interval, with four sites of application in each animal. Rats were housed in stainless steel metabolic cages and faeces and urine collected separately. Radioactive griseofulvin (spec. act. 41·3 mCi mg^{-1} radiochemical purity 99%) was obtained from Amersham International, UK.

For topical application, griseofulvin was dissolved in an anhydrous carrier solvent system containing benzyl alcohol (10%) acetone and isopropanol (Nimni 1989). The benzyl alcohol, because of its high boiling point, is considered as the stable solvent carrying the griseofulvin through the epidermis to the basal layer and across it, and the acetone and isopropanol are present as fugitive solvents to allow initial spreading of the preparation. For oral administration the fugitive solvents were replaced by olive oil such that 0·4 mL could be fed via gastric intubation.

Before topical application, the dorsal area of the rats was shaved and divided into quadrants by marking with indelible ink. The topical dose applied to each site was 25 μL, a total of 100 μL per rat. As there were four sites the 100 μL covered an area approximately 6 cm^2. A slightly larger area of skin was excised at the time of death to measure residual radioactivity. The average total dose per rat administered orally and the combined topical dose per rat varied in each experiment between 3 and 10 μCi and are specified in the text. Rats were killed each day to determine rates of skin penetration and residual radioactivity, and at the end of the metabolic study.

The amount of radioactivity present in skin and internal organs was determined following extraction with an organic solvent. For this purpose the tissue (250 mg) was homogenized in 1 mL of water using a Polytron homogenizer. This suspension was vortexed for 1 min at full speed after the addition of 5 mL of benzene. The emulsion was centrifuged for 15 min at 4000 rev min^{-1} and sample counted using a Beckman liquid scintillation counter.

Results and discussion

Urinary excretion of [^{3}H]griseofulvin. A comparison of the urinary excretion profiles following topical or oral administration of griseofulvin is shown in Fig. 1. The average amount of radioactivity applied topically to the young rats was 9·2 μCi divided among four sites in the dorsal area. A similar dose was given orally. In this study approximately 17·6% of the administered radioactivity was recovered in the urine for each group, 80% of which was recovered during the first 48 h, whereas

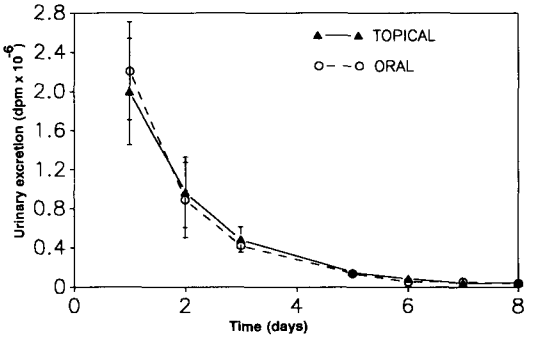

FIG. 1. Urinary excretion of ^{3}H following either topical (▲) or oral (○) administration of equivalent amounts of [^{3}H] griseofulvin (9·2 μCi).

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Table 1. Concentrations of ^3H (d min^{-1}) present at the site of application (dorsal skin) and internal organs as a function of time following topical administration of [^3H]griseofulvin (Total dose = $6{\cdot}72 \times 10^6$ d min^{-1}).

	Number of days after application			
Organ	1	2	4	8
Dorsal skin	3 041 600 ± 591 500	1 401 100 ± 469 000	657 950 ± 480 200	20 100 ± 12 700
Liver	17 300 ± 1200	4200 ± 300	2000 ± 600	ND
Kidney	2900 ± 40	2500 ± 80	4000 ± 140	1400 ± 90
Sem. ves.	2000 ± 100	2100 ± 50	1700 ± 20	1300 ± 130
Brain	ND	ND	ND	ND
Ventral skin	7900 ± 450	16 900 ± 600	4300 ± 2300	1200 ± 60

ND = Non detectable.

following oral administration, a total of 18·2% was recovered, 82% of which was during the first 48 h. Therefore the patterns of urinary excretion in Fischer rats following the two routes were essentially similar.

Concentration of [^3H]griseofulvin in skin and internal organs following topical application. The total amounts of griseofulvin present at the site of topical application (dorsal dermis) and organs of the rats at various intervals following application are shown in Table 1. The amount of [^3H]griseofulvin applied to each rat was equivalent to $7{\cdot}72 \times 10^6$ d min^{-1} divided among four sites. After 1 day, this was reduced to $3{\cdot}04 \times 10^6$ or approximately half of the applied dose. Even after 4 days almost 10% of the dose applied was still present in the skin, whereas in liver, kidney and seminal vesicles, there were negligible amounts of the dose, and in the brain, none. The ventral skin concentration was also low, but this site tended to become contaminated with urine as the rats moved about the cages. In general there was a several hundred fold accumulation of drug in the area of application compared with any of the organs tested. By the eighth day, as the drug disappeared from the skin (0·3% of dose) and residue tended to become evenly distributed throughout the organism, there was still a 13 to 16 fold difference in drug at the site of application compared with any of the organs tested.

Concentration of [^3H]griseofulvin in skin and internal organs following oral administration. A similar study following the oral administration of [^3H]griseofulvin showed contrasting results (Table 2). The drug reached close to maximum values in skin 1 day after administration, and seemed to be in equilibrium with the various organs tested. In no instance did the concentration in skin approach that following topical application (0·07% compared with 45% after 24 h). From these studies it may be concluded that to achieve skin concentrations of griseofulvin similar to those from topical administration, it would require oral doses orders of magnitude larger. Also when applied topically the accumulation at the site of application relative to its

Table 2. Concentrations of ^3H (d min^{-1}) present in skin and internal organs after oral administration of [^3H]griseofulvin (initial dose = $6{\cdot}12 \times 10^6$ d min^{-1}).

	Days after administration		
Organ	1	2	3
Dorsal skin	4500 ± 300	100 ± 140	5290 ± 600
Liver	11 100 ± 900	4200 ± 160	4000 ± 50
Kidney	4400 ± 140	4700 ± 640	4640 ± 220
Brain	4500 ± 120	4500 ± 140	5900 ± 560
Ventral skin	4460 ± 140	3940 ± 200	7840 ± 3200

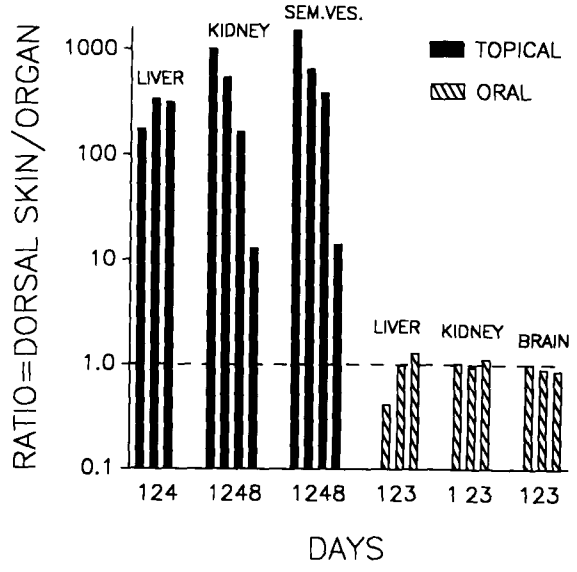

FIG. 2. The relative amounts of radioactivity present in skin and internal organs are compared. The skin/organ ratio reflects the relative concentration of drug in skin at different intervals following topical (black bars) and oral (dashed bars) administration.

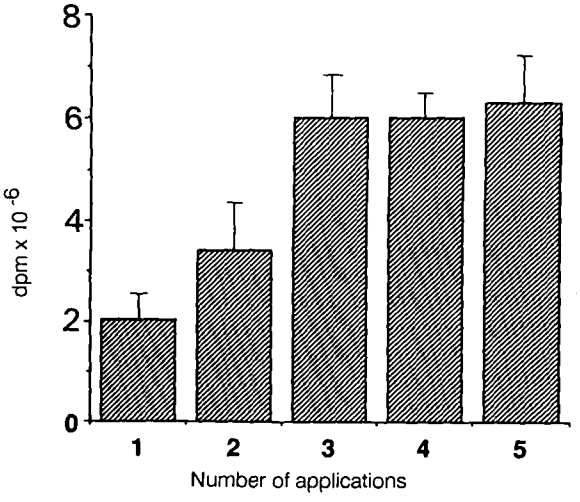

FIG. 3. Cumulative dose following daily successive applications at a single site in the dorsal aspect of young rats. Animals received consecutive applications of 25 μL containing $4{\cdot}44 \times 10^6$ d min^{-1} of [^3H]griseofulvin and were killed 24 h after the last dose. Vertical bars represent the standard deviation of 4 determinations.

Table 3. Comparison of percutaneous absorption of [^{3}H]griseofulvin by hairless (Sprague-Dawley) and normal (Long-Evans) rats, evaluated by the degree of urinary excretion of the drug. Each rat received a topical application of 5 μCi of [^{3}H]griseofulvin in a standard carrier vehicle containing 10% benzyl alcohol. (3 animals per group). Determinations were in duplicate.

	Urinary excretion (d $min^{-1} \times 10^{-5}$)	
Days	Hairless rats	Normal rats
1	9·12 ± 2·44	22·40 ± 5·58
2	6·10 ± 0·32	3·60 ± 0·84
3	2·82 ± 0·60	3·10 ± 0·58

concentration in internal organs is maintained over at least 8 days. In contrast, oral administration never resulted in a concentration higher in the skin compared with kidney, liver and brain (Fig. 2). Thus, when the infection is localized to a discrete and accessible area of the body, the topical route would have a clear advantage.

Concentration of [^{3}H]griseofulvin in skin following repeated topical application. Fig. 3. summarizes a study in which young Fischer rats received daily applications of topical griseofulvin at a single dorsal site on 1, 2, 3, 4, or 5 consecutive days. The animals were killed 24 h after the last dose and the residual radioactivity determined. At day 3 of continued application, a steady state of radioactivity was reached, so the concentration of griseofulvin in the skin reached equilibrium with the dose applied showing that steady levels of the drug can be maintained at the affected site by regular application.

Urinary excretion of [^{3}H]griseofulvin by normal rats compared with a hairless variety. As the rates of penetration of griseofulvin across the skin barrier in the rat could depend on the number of hair follicles, the rate of penetration across the skin of a standard rat (Long Evans) was compared with that for hairless rats of similar age (Sprague-Dawley). The results suggest that the normal rat absorbed the drug faster and to a greater extent than the hairless animal, approximately 20% of the applied dose being excreted in 24 h compared with only 8% in the hairless rat (Table 3). The initial rapid rate of penetration in normal rats, is probably aided by the presence of hair follicles. This suggests that persistence of griseofulvin in human skin after topical application may be higher than otherwise expected from the rat studies. As suggested by Rougier et al (1987) the hairless rat may be a better predictor of the behaviour of topically applied substances used in the treatment of human conditions, than the standard laboratory rat.

From these results it can be concluded that griseofulvin administered topically in a suitable solvent mixture could be expected to reach effective concentrations in integumental tissues at much lower doses than currently used orally.

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