RESEARCH PAPERS

SOME FACTORS INFLUENCING THE ABSORPTION OF GRISEOFULVIN FROM THE GASTROINTESTINAL TRACT

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Some surface-active agents enhance the absorption of griseofulvin from suspensions administered to rats and man. The method of incorporating the agent into the suspension is important. No similar effect is observed with tablets of griseofulvin in man. The effect of particle size on the absorption of griseofulvin when administered as a suspension and as tablets has been studied in a statistically designed experiment in man. The results show that doses of 0·5 g. griseofulvin of surface area 0·35 m.²/g. or 0·25 g. of material with a surface area of 1·5 m.²/g. give similar concentrations of griseofulvin in the blood.

MANY papers have been published on the systemic use of griseofulvin for fungal infections without any conclusion being drawn as to the optimum dosage régime. Atkinson, Bedford, Child and Tomich (1962) in a study of the concentrations of griseofulvin in the blood of man after different dosing schedules showed that a given amount of griseofulvin administered in divided doses would be expected to be more effective than a large single dose. They also observed a variation between patients in the concentrations of griseofulvin in the blood.

As a further study of the absorption of griseofulvin, experiments have been conducted in animals and man to examine the effect of surface-active agents incorporated either in suspensions or in tablets of the antibiotic. We understand that the studies of Atkinson, Bedford, Child and Tomich with preparations of different particle sizes have been extended to include griseofulvin with a surface area of 5·0 m.²/g.

EXPERIMENTAL

Estimation of Griseofulvin

The concentration of griseofulvin in blood was determined by a modification of the spectrophotofluorometric method described by Bedford, Child and Tomich (1959). The oxalated blood sample (1 ml.) was extracted with ether (10 ml.) by shaking for 1 min. after which an aliquot (9 ml.) of the ether extract was evaporated to dryness and the residue dissolved in ethanol (3 ml.). The fluorescence of the alcoholic solution was measured using a Locarte Photofluorometer with an ultra-violet selecting filter on the primary side and a combination of two filters transmitting light between 420 mμ and 470 mμ on the secondary side.

Measurement of Surface Area

Surface area measurements were made using a modification of the air permeability method described by Rigden (1943).

Preparation of Griseofulvin Suspensions

Two formulation techniques were used to prepare the suspensions of griseofulvin. *Technique A.* The griseofulvin and the appropriate surface-active agent were mixed together before the addition of water. *Technique B.* The surface-active agent was dissolved in water to give the desired final concentration and the griseofulvin then added.

Preparation of Tablets

In the experiments to determine the effect of the surface-active agent Perminal BXN on the absorption of griseofulvin, tablets of griseofulvin were prepared in which (*a*) starch was the only excipient and (*b*) the griseofulvin was mixed with Perminal BXN before the addition of the starch paste.

Effect of Particle Size on the Absorption of Griseofulvin

Suspensions of griseofulvin of three surface areas were prepared by different milling techniques. The surface area of the griseofulvin in the finest suspension was believed, from study of photomicrographs, to be greater than 5·0 $m.^2/g.$ though no direct measurement was used. This suspension, after dilution, was freeze dried to give a powder of 3·0 $m.^2/g.$ from which tablets were prepared.

The suspension of 1·5 $m.^2/g.$ griseofulvin was freeze dried to give material with the same surface area from which tablets were prepared.

The coarse suspension of griseofulvin with a surface area of 0·35 $m.^2/g.$ was oven-dried to give a preparation with the same surface area.

The tablets prepared from each of the three preparations of griseofulvin contained the same excipients and had a Monsanto hardness of 3–4.

Animals

Male, specific rat-pathogen free, albino rats of the Alderley Park, I.C.I. Ltd. strain (120–150 g.) were used. Animals in groups of 40 were each given oral doses of griseofulvin (50 mg./kg.) presented as a 1 per cent suspension containing 0·5 per cent of a surface-active agent. Eight rats from a group were killed at each selected time interval and their blood separately analysed for griseofulvin.

Experiments in Man

Healthy, adult, male volunteers were used. Suspensions containing 2 per cent griseofulvin and 0·04 per cent of surface-active agent were prepared by formulation technique A; each man received the equivalent of 0·5 g. griseofulvin on each occasion. Blood samples were taken by venepuncture.

To determine whether Perminal BXN incorporated in tablets influenced the absorption of griseofulvin, 1 g. griseofulvin was administered to each man in a cross-over trial once with and once without Perminal BXN.

The effect of particle size on absorption was examined in 30 male volunteers who were each given griseofulvin on three occasions at weekly intervals; the concentration of griseofulvin in their blood was measured

4, 8, 25 and 49 hr. after each administration. Five dose : particle size combinations were investigated as tablets and also as suspensions so that on each of the three occasions only three volunteers received the same dose of a particular surface area either as tablets or as a suspension.

Surface-active Agents

Myrj 52, Arlacel 20, Tween 20, Tween 65 and Tween 80 are produced by the Atlas Powder Company, Wilmington, Delaware, U.S.A. Perminal BXN, Agral 2, Dispersol LN, Lissapol NX and Lubrol W were obtained from Imperial Chemical Industries Limited, Dyestuffs Division, Manchester, England. Lecithin RG was manufactured by The Glidden Company, 1825 N. Laramie Avenue, Chicago, Illinois, U.S.A., Goulac by Production Chemicals (Rochdale) Limited, Manchester, England, Pluronic F68 by Wyandotte Chemicals Corporation, Wyandotte, Michigan, U.S.A., and Aerosol OT by American Cyanamid, Pearl River, New York, U.S.A. Nekal was obtained from Ayerst Laboratories Incorporated, New York, U.S.A.

Results and Discussion

Many observations have been made on the effect of adjuvants on the absorption of drugs from the gastrointestinal tract. Kozlik and Mosinger (1957) showed that the surface-active agent, sodium lauryl sulphate, increased the rate of absorption of glucose from the gastrointestinal tract

TABLE I

THE ARITHMETIC MEAN CONCENTRATIONS OF GRISEOFULVIN (μG./ML.) AND THE STANDARD ERROR, IN THE BLOOD OF RATS AFTER ORAL ADMINISTRATION OF 50 MG. GRISEOFULVIN/KG.; THE DRUG WAS ADMINISTERED AS A SUSPENSION PREPARED BY FORMULATION TECHNIQUE B*

Surface-active agent	Chemical constituent	Hr. after administration				
		2	4	6	7·5	24
Perminal BXN	Butylated sodium naphthalene sulphonate	1·9 ± 0·8	2·5 ± 1·0	2·0 ± 0·2	0·8 ± 0·1	0
Lecithin (Glidden R.G.)	Phosphatidylcholine	1·6 ± 0·8	1·6 ± 1·0	1·3 ± 0·4	1·1 ± 0·3	0·3 ± 0·3
Sod. Lauryl sulphate B.P.	—	0·8 ± 0·5	1·5 ± 0·4	0·7 ± 0·3	0·4 ± 0·2	0·2 ± 0·2
Aerosol O.T.	Dioctyl sodium sulphosuccinate	0·9 ± 0·2	0·8 ± 0·5	0·4 ± 0·3	0·1 ± 0·2	—
Pluronic F68	Polyoxyethylene stearate	1·5 ± 0·6	1·5 ± 0·3	0·8 ± 0·4	0·4 ± 0·2	—
Myrj 52	Polyoxyethylene stearate	0·9 ± 0·1	1·0 ± 0·4	0·6 ± 0·3	0·6 ± 0·5	0·2 ± 0·1
Arlacel 20	Sorbitan monolaurate	0·6 ± 0·3	1·0 ± 0·6	0·8 ± 0·6	0·4 ± 0·4	0·2 ± 0·1
Tween 20	Polyoxyethylene sorbitan monolaurate	1·4 ± 0·6	1·8 ± 0·6	1·6 ± 0·6	0·3 ± 0·3	0
Tween 65	Polyoxyethylene sorbitan tristearate	1·1 ± 0·2	1·1 ± 0·4	1·0 ± 0·6	1·0 ± 0·4	0·1 ± 0·1
Tween 80	Polyoxyethylene sorbitan mono-oleate	1·3 ± 0·4	1·5 ± 0·4	2·3 ± 0·8	1·8 ± 0·7	—
Goulac	Calcium lignosulphonate	1·7 ± 0·5	1·5 ± 0·4	1·4 ± 0·3	2·4 ± 1·0	—
Lubrol W	Cetyl alcohol/ethylene oxide condensate	1·2 ± 0·3	1·0 ± 0·2	2·0 ± 0·9	1·0 ± 0·2	—
Nekal	Propylated sodium naphthalene sulphonate	0·7 ± 0·1	1·2 ± 0·4	1·2 ± 0·5	0·7 ± 0·2	0·2 ± 0·1
Agral 2	Propylated sodium naphthalene sulphonate	0·2 ± 0·3	0·6 ± 0·4	1·1 ± 0·7	0·8 ± 0·5	0
Dispersol LN	Methylene dinaphthalene sodium sulphonate	0·2 ± 0·2	0·8 ± 0·3	1·4 ± 0·3	1·5 ± 0·4	0
Lissapol NX	Polyethylene oxide condensate of nonyl phenol	2·4 ± 1·0	2·6 ± 0·8	2·4 ± 0·3	2·0 ± 0·5	—

* See experimental section.

whereas Nissim (1960) reported that the cation trimethylhexadecylammonium inhibited the absorption of glucose but did not inhibit the absorption of methionine or sodium butyrate in the rabbit. The results of experiments in rats on the influence of surface-active agents on the absorption of griseofulvin from suspensions are shown in Table I. Some surface-active agents affected the concentrations of griseofulvin found in the blood and therefore apparently the rate and extent of absorption of the drug. Perminal BXN and Lissapol NXA, both containing an aromatic nucleus, consistently gave rise to higher concentrations of griseofulvin in the blood than did the other agents examined. As far as could be determined by appropriate control experiments the substance extracted from the blood of these animals for measurement was griseofulvin uncontaminated by the surface-active agent used. It was further observed that the surface-active agent could affect the pattern of absorption as may be seen by comparing the results obtained with Dispersol LN and Aerosol OT. The method of incorporation of the surface-active agent into the suspension of griseofulvin also influenced the apparent absorption of the drug (Table II). In this experiment the standard error of the observed concentrations was found to increase with the mean level of the results and it was appropriate therefore to use the geometric mean rather than the arithmetic mean and to consider the standard error as a percentage rather than in μg./ml. as previously. The standard error of a single observed concentration of griseofulvin was ± 60 per cent and the standard error of the means (Table II), which are based on the results

TABLE II

THE GEOMETRIC MEAN CONCENTRATIONS OF GRISEOFULVIN (μG./ML.) IN THE BLOOD OF RATS AFTER ORAL ADMINISTRATION OF 50 MG. GRISEOFULVIN/KG.

Surface-active agent	Formulation technique*	Hr. after dosing				
		2	4	6	7·5	24
Lecithin Glidden R.G. ..	A	1·49	1·39	1·20	1·00	0·23
Perminal BXN..	A	1·79	1·91	1·84	0·74	—
Lecithin Glidden R.G. ..	B	1·06	1·44	0·90	0·53	—
Perminal BXN..	B	0·72	1·31	1·63	1·24	—

* See experimental section.

from 8 animals, was therefore ± 18 per cent. The concentration of griseofulvin in the blood was maximal between 2 and 6 hr. after dosing, after which it decreased and had virtually reached zero by 24 hr. As there was no significant difference in the mean concentrations at 2, 4 and 6 hr. for any particular agent and method of incorporation, the average concentration over this period was used to assess the statistical significance of the observed differences between the agents and the formulation technique.

Technique A gave rise to higher concentrations of griseofulvin in the blood than technique B for both lecithin and Perminal; the mean difference in concentration, for both substances, was 40 per cent which is significant at the 95 per cent level. Using the formulation technique A, in which the surface-active agent and the griseofulvin were mixed together

before addition of water, the mean concentration with Perminal was 36 per cent higher than with lecithin; this difference was also statistically significant (95 per cent confidence limits 4 per cent and 79 per cent). To establish whether or not this effect in rats would be observed in man an experiment was conducted using suspensions of the same preparation of griseofulvin containing either Lecithin Glidden R.G. or Perminal BXN formulated according to technique A (Table III). Over the period 2 to

TABLE III

THE CONCENTRATIONS OF GRISEOFULVIN (μG./ML.) IN THE BLOOD OF MAN AFTER ORAL ADMINISTRATION OF 0·5 G. THE GRISEOFULVIN WAS FORMULATED BY TECHNIQUE A* AS SUSPENSIONS INCORPORATING EITHER PERMINAL BXN OR LECITHIN GLIDDEN R.G.

Volunteer code	Surface-active agent	Hr. after dosing			
		2	4	7	24
A	Perminal BXN	0·9	1·1	0·8	0·1
B	,,	1·0	1·2	0·7	0·0
C	,,	1·4	2·1	1·4	0·5
D	,,	1·4	1·7	1·2	0·0
E	,,	0·7	1·2	1·2	0·6
F	,,	0·9	1·2	0·7	—
A	Lecithin Glidden R.G.	0·7	1·0	0·9	0·0
B	,,	1·0	0·9	0·6	0·0
C	,,	0·6	0·4	0·2	0·0
D	,,	1·0	1·3	1·2	0·0
E	,,	0·7	0·5	0·8	0·0
F	,,	1·0	1·3	0·9	0·0

* See experimental section.

7 hr. after dosing the mean concentration when using Perminal was 50 per cent higher than with lecithin. This was comparable to the increase shown in rats, though it was not itself quite significant at the 95 per cent level. Three subjects had detectable amounts of griseofulvin in their blood 24 hr. after receiving the Perminal preparation but not with the lecithin preparation.

TABLE IV

THE CONCENTRATIONS OF GRISEOFULVIN IN THE BLOOD (μG./ML.) OF MALE VOLUNTEERS AT INTERVALS AFTER RECEIVING 1 G. GRISEOFULVIN AS TABLETS FORMULATED EITHER WITH OR WITHOUT PERMINAL BXN

Volunteer code	Occasion One					Occasion Two				
	Formulation	Hr. after dosing				Formulation	Hr. after dosing			
		2	4	7	24		2	4	7	24
G	Perminal BXN	0·4	1·1	1·5	0·3	No agent	0·1	0·6	0·9	0·3
H	,,	1·4	1·2	1·2	0·1	,,	0·5	0·7	0·9	0·4
I	,,	1·7	2·2	2·0	0·3	,,	0·9	1·5	1·7	0·3
J	,,	0·7	0·9	0·6	0·1	,,	0·1	0·9	1·2	0·4
K	,,	0·8	0·7	0·9	0·2	,,	0·7	0·4	0·8	0·5
L	,,	1·2	1·5	1·3	0·2	,,	1·0	0·8	0·9	0·7
M	No agent	0·6	0·6	1·3	0·4	Perminal BXN	0·4	0·6	1·3	0·4
N	,,	0·3	0·2	0·1	0·0	,,	0·7	0·4	1·0	0·4
O	,,	0·1	1·4	1·7	0·5	,,	0·0	1·1	0·8	0·2
P	,,	0·6	0·5	0·5	0·2	,,	0·5	0·7	1·6	0·5
Q	,,	0·9	1·5	1·2	0·3	,,	0·8	1·3	1·5	0·6
R	,,	1·2	0·8	0·6	0·0	,,	0·8	0·7	1·0	0·5

These experiments indicate that Perminal BXN and Lecithin (Glidden R.G.) when incorporated into suspensions of griseofulvin most probably

exert the same influence on the intestinal absorption of the antibiotic in both man and rats. How these agents promote the absorption of griseofulvin is unknown.

The effect of incorporating Perminal BXN into tablets of griseofulvin was determined in a cross-over trial with 12 adult male volunteers. The results (Table IV) were subjected to an analysis of variance using "available griseofulvin" values which were calculated from the area under the blood concentration: time curve over the period 0–24 hr. No statistically significant difference was observed between the tablets containing Perminal BXN and those without a surface-active agent.

Although the tablets of griseofulvin contained the same relative proportion of Perminal BXN as the suspensions we were unable to demonstrate any enhancement of absorption compared with tablets without a surface-active agent. No explanation is advanced for the difference in results between suspensions and tablets.

TABLE V

THE MEAN "AVAILABLE GRISEOFULVIN" VALUES (μG. GRISEOFULVIN/ML. BLOOD HR.) CALCULATED OVER THE PERIOD 0–25 HR. FROM THE RESULTS FOR 30 MALE VOLUNTEERS AFTER RECEIVING DIFFERENT FORMULATIONS OF GRISEOFULVIN. THE STANDARD ERROR OF THESE VALUES WAS 1·01

Formulation	Dose	Surface-area ($m.^2/g.$)			
		0·35	1·5	3·0	5·0
Suspension	0·5 g.	12·2	17·4	—	20·5
Tablets	0·5 g.	15·6	21·0	15·0	—
Suspension	0·25 g.	—	11·4	—	14·4
Tablets	0·25 g.	—	13·4	10·2	—

In the experiments on the absorption of griseofulvin by man the volunteers apparently differed in the extent to which they either absorbed or excreted griseofulvin: in the experiments with rats (Tables I and II) the concentrations of griseofulvin found in the blood varied with the occasion. To study the effect of particle size on the absorption of griseofulvin by man it was therefore necessary to design an experiment in which allowance could be made in the statistical analysis for any possible effect caused by different volunteers having different absorption patterns, and for any bias in the results obtained on the different occasions. As a precaution the volunteers were randomly assigned a code number so that any unexpected effect attributed to age or other variable would affect all treatments equally. From the results the "available griseofulvin" values (that is, the area under the blood concentration: time curve) for the periods 0–25 hr. (Table V) and 0–49 hr. (Table VI) were calculated. These sets of results were each analysed statistically as it was considered possible that the effect of the 5·0 or the 3·0 $m.^2/g.$ griseofulvin might be to maintain a higher concentration of griseofulvin in the blood for a longer time. However, both sets of calculations presented the same result so that the following comments apply to both.

The analysis confirmed apparent differences in the results obtained on the separate occasions and also revealed a difference in the mean "available griseofulvin" values from one volunteer to another. Within the scope of this experiment it is difficult to assess the true magnitude of this volunteer effect but the statistical approach used in the analysis of this experiment indicated that the highest "available griseofulvin" value obtained, on a standard formulation, was about three times greater than the lowest. A difference of this kind between patients is obviously of clinical importance and emphasises the necessity of ensuring that each patient receives an adequate dosage of the antibiotic to allow for his particular absorption characteristics.

TABLE VI

THE MEAN "AVAILABLE GRISEOFULVIN" VALUES (μG. GRISEOFULVIN/ML. BLOOD HR.) CALCULATED OVER THE PERIOD 0–49 HR. FROM THE RESULTS FOR 30 MALE VOLUNTEERS AFTER RECEIVING DIFFERENT FORMULATIONS OF GRISEOFULVIN. THE STANDARD ERROR OF THESE VALUES WAS 1·46

Formulation	Dose	Surface-area (m.²/g.)			
		0·35	1·5	3·0	5·0
Suspension	0·5 g.	17·0	22·4	—	27·8
Tablets	0·5 g.	20·8	28·2	20·4	—
Suspension	0·25 g.	—	13·4	—	17·8
Tablets	0·25 g.	—	17·2	14·2	—

When the griseofulvin preparations of different particle size were administered as suspensions a linear relationship was observed between the logarithm of the surface area and the mean "available griseofulvin" value. This observation does not disagree with that reported to us by Atkinson, Bedford, Child and Tomich although they reported a slightly greater effect. This linear relationship, however, was not observed with tablets as the tablets of griseofulvin with a surface area of 3·0 m.²/g. gave unexpectedly low values. Photomicrographs of the disintegrated tablets did not indicate any extensive aggregation of particles, so this result would appear to be due to some unknown factor in the preparation of the dried powder from the suspension. However, the results obtained with griseofulvin of surface area 1·5 and 0·35 m.²/g. bear the same relationship to each other whether administered as tablets or as suspensions. Although the ratio of the "available griseofulvin" values obtained with 1·5 m.²/g. material to those obtained with 0·35 m.²/g. griseofulvin was less than two, 0·25 g. of the finer material gave almost the same "available griseofulvin" value, a reflection of similar blood levels, as 0·5 g. of the coarser material. No information is available on the concentrations of griseofulvin in the blood which are required for a therapeutic effect nor is it known whether peak or protracted concentrations are important. However, this experiment shows that a similar clinical response should be obtained with 1·5 m.²/g. griseofulvin, administered orally, at half the dosage required for griseofulvin with a surface area of 0·35 m.²/g.

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