THE EFFECTS OF GRISEOFULVIN ON EPITHELIAL CELLS IN TISSUE CULTURE*

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Griseofulvin, the newly available systemic antibiotic, shows clinical promise in the chemotherapy of superficial fungus infections (1). Oral, rather than topical, administration of this drug provides fungistatic levels effective clinically against the known species of dermatophytes. Some reports of toxicity have indicated that large doses of griseofulvin result in an arrest of mitosis in areas of high mitotic activity: e.g., bone marrow and intestine (2). There exists at present very little information concerning the effects of this agent on epithelial cells. One relatively simple yet controlled approach which may provide some information is an investigation of drug effects on cells in tissue culture. Accordingly, the short-term effects of griseofulvin on several epithelial-like cell lines maintained in culture were studied and contrasted with the effects of the antibiotic nystatin.

METHOD

The cell lines employed in this study included: HeLa, human carcinoma of cervix (Gey, 3); KB, human carcinoma of pharynx (Eagle, 4); Conjunctiva, adult human (Chang, 5); Human Skin (Perry, 6); Intestine, human embryonic (Henle, 7); Liver, adult human (Chang, 5); Bone Marrow, H-946, a human epithelial-like cell (Bozeman, 8). All of the cell lines have been maintained in the laboratory growing attached to glass. They were removed from the glass, either mechanically or with Versene, suspended in nutrient medium, and in one ml aliquots were pipetted as uniform cell suspensions into sterile, rubber-stoppered tubes. The tubes were incubated at 37 C. in an almost horizontal stationary position. The basic nutrient was Medium 199 (Microbiological Associates, Inc., Bethesda, Md.) to which were added horse serum (10%), penicillin (100 units/ml), streptomycin (20 units/ml) and nystatin (50 units/ml). Addition of these antibiotics did not influence the effects of griseofulvin or nystatin and consequently they were added routinely to minimize contamination with bacterial and mycotic species. One to five days after subculturing the cells when a continuous sheet of growth was present, the nutrient was replaced with fresh medium containing appropriate concentrations of the antimycotic drugs. Griseofulvin (Grifulvin Lot BS-8804),* was added in concentrations ranging from 1 to 5000 micrograms/ml and nystatin (Mycostatin, Upjohn) in concentrations as high as 2000 micrograms/ml. The latter drug contained 2500 units/mg. Both drugs were added as the sterile powder suspended in the nutrient medium and, due to limited solubility, were added as suspensions in the higher concentrations. All experiments were conducted at least in quadruplicate. Daily observations were made on the unfixed living cultures with the optical microscope.

RESULTS

Griseofulvin in relatively small concentrations caused cellular injury and inhibited growth of all the epithelial-like cell lines tested in this short-term study. Larger concentrations led to complete destruction of the cells. The concentrations of griseofulvin required to produce these effects are shown in Table I. Total destruction refers to the minimal concentration which destroyed essentially all of the cells in the roller tube; inhibition of growth refers to the minimal concentration which prevented cell growth and is roughly analogous to the "minimal inhibition dose" (MID) (9); detectable injury refers to the minimal concentration which produced any effect observable with this technic, and is analogous to the "least injurious dose" (LID) (9).

Results comparable to those shown in Table I were obtained in the other epithelial-like cells studied, viz; intestine, bone marrow and liver. It is of interest to note that liver cells incorporated suspended particles of griseofulvin more quickly and to a greater extent than did any of the other cells. Nystatin (results shown in parentheses in Table I) was included for comparison of both the morphologic changes and the

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TABLE I

Toxicity of griseofulvin and nystatin on epithelial cells in culture

Cell Line	Total Destruction	Inhibition of Growth	Detectable Injury	Complete Recovery
HeLa	1000	100–150	40–60	60
KB	800	100	40	40–60
	(400)	(200)	(100)	(100–200)
Conjunctiva	1000	150	40	40
	(400)	(200–250)	(100–150)	(150)
Skin	800	100	20–30	35

All concentrations are in micrograms per ml. Numbers in parentheses refer to nystatin concentrations in micrograms per ml required to produce comparable effects. Nystatin activity was 2500 units per mg. Effects were recorded after 96 hours of exposure to the drug. Complete recovery refers to the greatest concentration which produced effects reversible within three weeks.

time required for development of these alterations. It is of interest that those concentrations of griseofulvin or nystatin which produced total destruction of cells did so rapidly, in approximately 24 to 48 hours. Smaller concentrations caused cessation of cellular growth which could be determined with this experimental protocol in 48 to 72 hours. The least injurious dose was routinely determined at four days.

The cells were observed for recovery from drug effects after exposure to either antibiotic for four days, with subsequent replacement of the nutrient medium every two to three days. Comparable rates of growth and absence of detectable morphological differences between experimental and control tubes were the principal criteria employed. Complete recovery (Table I) refers to the greatest concentration of antibiotic to which the cells were exposed and from which they subsequently recovered within three weeks.

DISCUSSION

The concentration of drug and the time required to produce its effect on the cells was similar for both griseofulvin and nystatin. Concentrations of nystatin producing injury to epithelial cells cultured in stationary tubes were smaller than have been previously reported as injurious to explants of human skin growing in rotating tubes (10). Differences in technic may account in part for this result; however, the growing sheet of epithelial cells resulting from an inoculum of cells in suspension is probably more sensitive to drugs than are the epithelial cells radiating out from an explant.

Griseofulvin exerts a fungistatic effect *in vitro* on the dermatophytes in concentrations as low as 0.14 to 0.46 micrograms/ml (1). The minimum concentration required to produce a detectable effect on epithelial cells growing in culture, when exposed to drug on a short-term basis, was approximately 20 micrograms/ml. Thus the ratio between the smallest concentration injurious to epithelial cells (20 micrograms/ml) and fungistatic concentration (0.14 to 0.46 micrograms/ml) is between 40:1 and 140:1. This large ratio corroborates the rapidly accumulating clinical observations that griseofulvin does not have a marked deleterious effect on epithelial cells.

The fact that griseofulvin is fungistatic to a number of saprophytic fungi and yeasts (11) suggests that this compound may be a desirable addition to tissue culture media. Epithelial cells, exposed to 5 micrograms/ml of griseofulvin, have been maintained in the laboratory for several months with no cellular abnormalities detectable to date. Griseofulvin may thus prove to be of importance in tissue culture studies as an antibiotic which can be routinely added for the prevention of mold contamination.

SUMMARY

The short-term effects of griseofulvin on epithelial cells in tissue culture have been studied and contrasted with nystatin. Like nystatin, griseofulvin can be classified as only moderately toxic to epithelial cells. The concentrations required to produce toxic effects on epithelial cells are very large when contrasted with fungistatic concentrations and consequently clinical evidence of deleterious effects on epithelial cells is not to be expected. The potential usefulness of griseofulvin as a routine addition to tissue culture media is discussed.

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