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CONTROLLED RELEASE OF GRISEOFULVIN FROM COPRECIPITATES WITH PHOSPHOLIPIDS

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ABSTRACT

The release of griseofulvin from griseofulvin:phospholipid coprecipitates has been studied as a function of 1) the fatty ester chain length of phosphatidylcholine, 2) the choice of solvent in forming coprecipitates, and 3) the ratio of phospholipid:cholesterol in the lipid component. Only coprecipitates containing 95%w/w and 80%w/w griseofulvin were examined. The release kinetics and total release after 60 min correlates with the phase transition temperatures of the various phosphatidylcholines. The addition of cholesterol diminishes the initial release rates but increases the total amount released after 60 min when the phospholipid:cholesterol mole ratio is greater than unity. The role of chloroform as an enhancer of the release process and the phospholipid as a solubilizer of griseofulvin is demonstrated.

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INTRODUCTION

A formulation approach which has been advocated for some time that alters the dissolution characteristics of a drug and its bioavailability is the solid dispersion system. A marked enhancement of *in vitro* dissolution and oral absorption of griseofulvin in dogs and humans has been reported with solid dispersions with polyethylene glycol 6000[1]. In general, the main interest has been focused on attempts to increase the dissolution properties of a hydrophobic drug by combining it with a large proportion of an inert water-soluble agent either as a fused melt or as a coprecipitate from a suitable organic solvent.[2] However, it has also been shown that the same approach can be applied to delay dissolution, thereby yielding a slow or sustained release of drug[3] with a subsequent increase in its oral absorption efficiency.[4-6] Recent evidence[7] has indicated that pronounced increases in the dissolution rate of griseofulvin and substantially larger amounts of drug in solution are obtainable from coprecipitates of griseofulvin and L-α-dimyristoylphosphatidylcholine of compositions which have a high proportion of drug, e.g. 95%w/w.[7]

The choice of phospholipids as co-dispersing agents is based on their potential advantages related to their liposome-forming behaviour. It is well known that under appropriate conditions, phospholipids can spontaneously form myelinic or liposomal structures in aqueous media and, in so doing, entrap various

solutes in either the aqueous compartments or in the bilayers. Furthermore, liposomal drugs may be protected against deleterious factors associated with the environment and, as a matter of course, be assisted in their transport across biological membranes by virtue of the biomembrane-liposome interaction.[8] Thus, a drug:phospholipid solid dispersion formulation may possess many of the attributes of an ideal drug delivery system.

Further investigations have now yielded data which suggest that the selection of suitable phospholipid compositions offers considerable versatility for obtaining the desired release properties of a drug, in particular, griseofulvin, when the system is prepared as a coprecipitate at a relatively high weight ratio of drug.

MATERIALS

Griseofulvin was obtained in a micronized state.[9] Pure synthetic phospholipids with label claim of 98 percent purity included: L-α-dimyristoylphosphatidylcholine[10] (DMPC), L-α-dipalmitoylphosphatidylcholine[10] (DPPC), and L-α-distearoylphosphatidylcholine[10] (DSPC). Cholesterol[10] (CHOL), 99+% pure, polyoxyethylene 40 stearate[11] (POS), polyethylene glycol (PEG) 4000[12] were used as received. Chloroform and methylene chloride were reagent grade solvents. Ethanol was USP grade and demineralized, distilled water was used throughout.

METHODS

Coprecipitates were prepared employing the solvent method[2]. The required amounts of the drug and lipids were weighed, dissolved in the appropriate solvent, then the solvent was evaporated over a warm water-bath assisted by a gentle stream of nitrogen. Further drying was carried out under vacuum over anhydrous calcium sulfate. All samples were examined within 24 hours after preparation following sieving at 80/120 mesh for size uniformity. Griseofulvin was similarly treated.

The release of griseofulvin from its solid state to the aqueous medium was measured using the spin-filter dissolution test apparatus[13]. A powdered sample equivalent to 50 mg griseofulvin was sprinkled on the surface of the stirred (600 RPM) buffer (900ml of HCl-KCl buffer at pH2) at 37°C. The aqueous medium was continuously circulated through a flow cell in a spectrophotometer and absorbance values at 293nm were recorded graphically and by a printer. The presence of the co-dispersing substance did not interfere with the analysis of griseofulvin. The results are averages of duplicate experiments of which variation from the mean was normally 5 percent or less.

RESULTS

The dissolution of griseofulvin from a lipid-containing coprecipitate is analogous to release of dispersed drug from a bioerodable matrix polymer drug delivery system. In both cases,

the rate and extent of drug dissolving is influenced by the erosion kinetics of the matrix material in each unit.

Effect of Phospholipid Composition:

The release of griseofulvin from 95%w/w and 80%w/w coprecipitates with DMPC, DPPC, or DSPC is shown in Figure 1. The dissolution of pure griseofulvin is also shown for comparison. It is apparent that the release rate and the amount released after 60 min are greater for all coprecipitates than that obtained by the dissolution of griseofulvin. Furthermore, the release of drug decreases with an increase in the fatty ester chain length of the phospholipid. An increase in the content of the phospholipid in the coprecipitate leads to a greater release of griseofulvin with each phospholipid except DSPC. If it is assumed that DPPC and DSPC behave like DMPC in the formation of the crystalline lattice of the coprecipitate, the decreased release of griseofulvin when combined with a longer chain agent is likely determined by the relative ease of dispersion of the phospholipid upon contact with the aqueous phase, which, in turn, is related to its phase transition temperature (Tc) and its corresponding physical state at 37°C. The Tc of DMPC, DPPC, and DSPC is 23°, 41°, and 58°C[14], respectively. Thus, DSPC would not spontaneously disperse because its Tc is too far above 37°C. Therefore, increasing the amount of DSPC in the coprecipitate makes little difference in the release of griseofulvin.

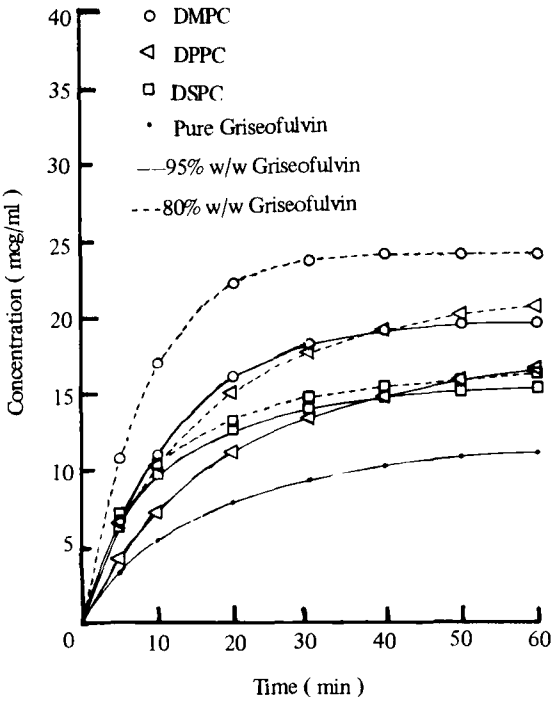

FIGURE 1

Release of Griseofulvin from Griseofulvin:Phospholipid Coprecipitates.

Influence of the Coprecipitating solvent:

It is known that griseofulvin forms a chloroform solvate[15] and when combined with DMPC in a coprecipitate chloroform augments the release of griseofulvin upon contact with aqueous solution[7]. However, the dissolution of solvated griseofulvin alone is essentially the same as micronized griseofulvin. The results of further studies to establish the role played by the coprecipitate-forming solvent on release of griseofulvin are given in Table 1.

TABLE 1

Effect of the Coprecipitate-Forming Solvent on the Release of Griseofulvin from 80 Per Cent Griseofulvin:DMPC Coprecipitates

Coprecipitate-Forming Solvent	Initial Release Rate[a] (mcg/ml/min)	Amount Released after 60 min(mg)
Chloroform	2.18 (0.68)	21.8 (10.2)
Methylene chloride	0.89 (0.63)	10.5 (10.3)
Ethanol	0.32 (0.21)	9.1 (8.1)

[a]Average release rate during the first 5 min.
Numbers in brackets correspond to solvent-treated griseofulvin shown for comparison.

Thus, it is found that the use of methylene chloride as a solvent has little effect on altering the release of griseofulvin from coprecipitates of 80%w/w griseofulvin:DMPC whereas the use of chloroform produces a very significant effect. Using ethanol as a solvent in forming the coprecipitate or in treating griseofulvin appears to cause a slight reduction in the release or dissolution of griseofulvin. Differential thermal analysis of coprecipitates yielded a solvent endothermic peak from all chloroform-treated samples and not from other solvent-treated samples[7]. These results suggest that chloroform-solvated griseofulvin combines with phospholipid in a manner that provides an appropriate orientation of the phospholipid molecules to enable rapid dispersion into bilayers when contact is made with

water. The formation of myelinic structures and liposomes within 2 min of making contact with water has been observed previously.[7] Ethanol or methylene chloride do not yield coprecipitates in which the phospholipid is readily available for dispersion in water, consequently, release of griseofulvin is poor from these samples. Thus, residual chloroform in the coprecipitate may be considered an enhancer of the release of griseofulvin and phospholipid in aqueous media.

Comparison of the Effect of Various Agents:

The release of griseofulvin from 95%w/w coprecipitates prepared from chloroform with agents other than phospholipid is described in Table 2.

The addition of PEG 4000 or CHOL produces little improvement in release over the dissolution of griseofulvin because neither of these agents is able to reduce surface tension or solubilize drug. Rapid dissolution of griseofulvin:PEG 4000 solid dispersions has only been shown at much higher concentrations of carrier.[16]

Griseofulvin:POS coprecipitates released approximately twice the amount of griseofulvin after 60 min compared with the other agents (except DMPC) as well as a significantly higher initial release rate. POS possesses pronounced surface activity[17] and, therefore, increases the effective surface area of griseofulvin for dissolution. The critical micelle concentration of POS is 0.014%w/v at 37°C[17], thus, solubilization does not play a role in

TABLE 2

Comparison of Various Agents Coprecipitated with Griseofulvin on Release of Griseofulvin[a]

Agent	Initial Release Rate[b] (mcg/ml/min)	Amount Released after 60 min(mg)
POS	2.72	22.3
DMPC	1.26	17.7
PEG 4000	0.77	11.1
CHOL	0.76	9.5
No agent	0.68	10.2

[a]95%w/w griseofulvin coprecipitates.
[b]Average release rate during the first 5 min.

dissolution of griseofulvin (maximum obtainable concentration in the dissolution fluid is 2.8×10^{-4}%w/v).

Cholesterol is a lipid which retards dissolution of some drugs[3] and does not significantly alter the release of griseofulvin (Table 2). On the other hand, a phospholipid such as DMPC increases the release of griseofulvin without itself possessing appreciable surface activity, but it does form liposomes into which griseofulvin may partition. Thus, the mechanism of release may be described as involving two stages: 1. spontaneous dispersion of phospholipid and 2. partitioning of griseofulvin into rapidly-formed phospholipid bilayer structures.

Controlled Release from Griseofulvin: Lipid Coprecipitates:

The release of griseofulvin from 95%w/w and 80%w/w coprecipitates as a function of the ratio of DMPC:CHOL is graphically illustrated in Figure 2 and Figure 3, respectively. The addition of CHOL has a variable effect on the release of griseofulvin depending on the ratio of DMPC:CHOL in the coprecipitate. With 80%w/w coprecipitates having a high mole ratio of DMPC:CHOL (1:0.33), the release rate is similar to that obtained when DMPC is the only lipid present (Figure 3). However, with 95%w/w coprecipitates the introduction of CHOL decreases the release rate of griseofulvin. Furthermore, after approximately 20 min the release curve corresponding to the griseofulvin:DMPC coprecipitate begins to plateau, whereas the curve corresponding to a coprecipitate containing a DMPC:CHOL ratio of 1:0.33 continues to increase and the curve does not plateau even at 60 min. At a mole ratio of DMPC:CHOL, 1:1, the release rate at early times is further reduced but again the concentration of griseofulvin in solution continues to rise with time. The decrease of the release rate from coprecipitates becomes more pronounced after the addition of CHOL when there is a smaller amount of total lipid material present. In a similar fashion, coprecipitates containing a preponderance of CHOL in the lipid component, e.g. DMPC:CHOL, 1:3, release griseofulvin at even slower rates but continue to release the drug after 60 min. In summary, the effect of adding CHOL to the lipid component of

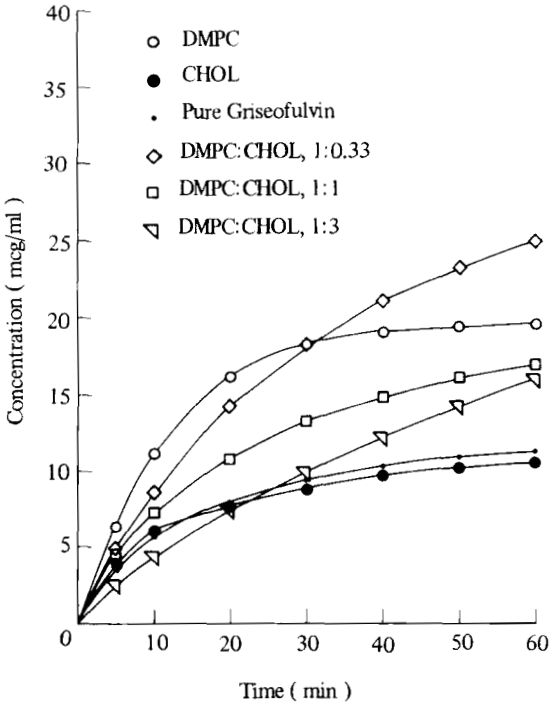

FIGURE 2

Release of Griseofulvin from 95%w/w Griseofulvin:Lipid Coprecipitates.

the coprecipitates is to decrease the release rate at the early times but to increase the amount of drug released after 60 min. The magnitude of these effects appears to be related to the total amount of lipid in the coprecipitate.

A quantitative comparison of these and additional data are shown in Table 3.

It can be seen that the inclusion of a smaller amount of CHOL (DMPC:CHOL, 1:0.2), or a larger amount of CHOL (DMPC:CHOL, 1:5), does not drastically alter the rate of release or the

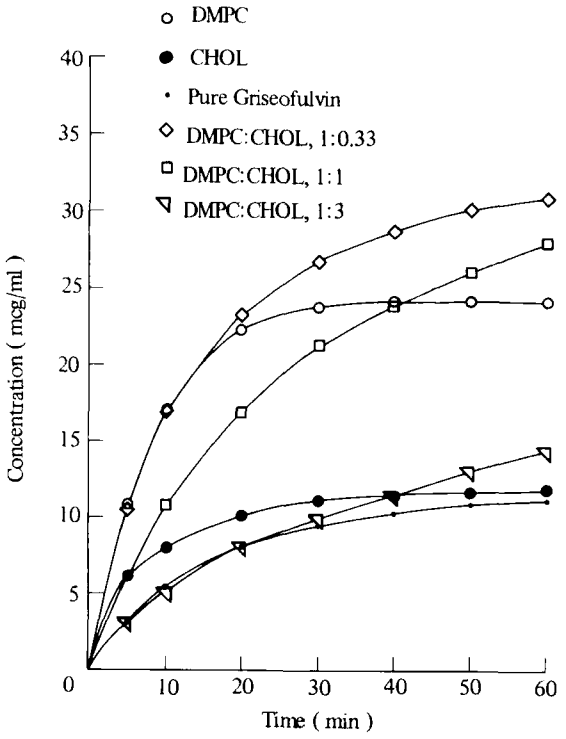

FIGURE 3

Release of Griseofulvin from 80%w/w Griseofulvin:Lipid Coprecipitates.

amounts of griseofulvin released, respectively. However, it should be noted that the fraction of griseofulvin released after 60 min from coprecipitates containing a phospholipid is considerably greater than the fraction of pure griseofulvin dissolved. Furthermore, these levels are increased by including CHOL in the coprecipitate.

DISCUSSION

The formulation of drugs, particularly those having low water solubility, in solid dispersion systems to provide a fast release

TABLE 3

Release of Griseofulvin from Griseofulvin:Lipid Coprecipitates

Gris Composition	DMPC:CHOL (mole ratio)	Initial Release Rate[a] (mcg/ml/min)	Fraction Released after 60 min (%)
100%	-	0.68	20.4
95%w/w	1:0	1.26	35.5
	1:0.2	0.76	46.3
	1:0.33	0.98	45.0
	1:1	0.90	30.6
	1:3	0.50	28.8
	1:5	0.44	29.3
	0:1	0.76	19.1
80%w/w	1:0	2.18	43.6
	1:0.2	1.84	52.7
	1:0.33	2.10	55.6
	1:1	1.28	50.4
	1:3	0.62	25.9
	1:5	0.60	27.0
	0:1	1.20	21.8

[a]Average release rate during the first 5 min.

of a drug is a common practice. In order to achieve this, a large proportion of a water-soluble carrier such as polyethylene glycol or polyvinylpyrrolidone is often used in the dosage form.[16] In contrast, a sustained release of drug from lipid-containing solid dispersions is possible, although this is also from formulations possessing high lipid:drug ratios.[3] The results obtained using griseofulvin:phospholipid coprecipitates indicate that substantially higher release rates of drug can be

achieved with as little as 5%w/w phospholipid and much higher drug concentrations in the aqueous medium can be obtained within one hour compared with that obtained by the dissolution of plain griseofulvin.

The importance of the role of chloroform in preparing the coprecipitates and the inclusion of a phospholipid is underlined by the results of several studies. Firstly, it was demonstrated that physical mixtures of griseofulvin and phospholipid do not improve the dissolution characteristics of griseofulvin[7] and a simple monotectic phase diagram is obtained indicative of the absence of any solid solution or eutectic formation. Furthermore, powder X-ray diffraction measurements on coprecipitates always revealed the existence of griseofulvin crystallites. A correlation was found between the heats of fusion of coprecipitates of varying composition, but not of physical mixtures, and release of drug. Secondly, when chloroform is replaced by methylene chloride or ethanol, coprecipitates offer no advantage over physical mixtures or plain drug. When phospholipid is replaced by PEG 4000 or CHOL, again little advantage is obtained from using coprecipitates of high drug content. Thirdly, it was observed under a microscope that a crystal of coprecipitate placed in water spontaneously dispersed, such that over a period of about two minutes a large number of elongated, myelinic structures had formed and protruded out from the crystal surface.[7] This behavior was not observed with

physical mixtures or with coprecipitates prepared using a solvent other than chloroform.

Combining CHOL with DMPC alters the behavior of the phospholipid in releasing griseofulvin in such a way to provide varying release patterns depending on the ratio of DMPC:CHOL. This offers the possibility of preparing a controlled release formulation of griseofulvin to meet a particular need.

CONCLUSIONS

Coprecipitates of griseofulvin:phospholipid of high drug content can be prepared which provide rapid release and extended release of drug in aqueous media at 37°C. Further control over the release kinetics can be achieved by incorporating the appropriate amount of CHOL. The mechanisms involved are related to a complex arrangement of griseofulvin, phospholipid, and chloroform in the coprecipitate crystal which permits the phospholipid to spontaneously disperse upon contact with aqueous solution. This enhancing effect of chloroform could possibly be achieved with some other agent.

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