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IMPROVED DELIVERY OF 5-METHOXYPSORALEN

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ABSTRACT

The air suspension technique was adopted to deposit 5-methoxypsoralen (5-MOP) with different water-soluble polymers and/or surfactants on lactose. Formulations showing acceptable dissolution behaviour were evaluated for their absorption rates by demonstrating their toxicities compared to pure drug by the LD_{50} method. Plots of 5-MOP dissolved at different time intervals up to 120 minutes against the LD_{50} values showed rank-order correlation. Formulation contains 5-MOP, PEG 6000, dioctyl sodium sulfosuccinate and lactose, in 1:2:0.1:6.9 ratio, would be a formulation of choice.

INTRODUCTION

Photochemotherapy which is based on the combined use of orally administered 8-methoxypsoralen (8-MOP, Xanthotoxin) and subsequent longwave ultraviolet irradiation[1] has been shown to be a highly efficient treatment for severe psoriasis[2,3], a chronic, intractable skin disease common in 1-3% of the world's population[4].

A naturally occurring water-insoluble psoralen analogue, 5-methoxypsoralen (5-MOP, Bergapten) has been, recently, found to be a potential photosensitizing drug in oral photochemotherapy of psoriasis[5,6]. Honigsmann et al.[6] reported

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5-MOP, 0.6 mg/kg, to be as effective as 8-MOP in clearing psoriatic lesions. A higher dose of 5-MOP, 1.2 mg/kg, was even more effective. Therapeutic doses of 5-MOP did not lead to erythema, the acute side-effect of 8-MOP photochemotherapy. No nausea was experienced even after administration of the high doses of 5-MOP. 5-MOP photochemotherapy thus represents a real alternative to 8-MOP photochemotherapy, its advantages over 8-MOP being greater safety and patient acceptance.

Stolk et al.[7] determined the concentrations of 5-MOP in serum and urine after oral administration of micronized 5-MOP in gelatin capsules to ten psoriatic patients. They found very much individual variation in the time required for reaching peak serum levels. Similar individual variation was found by Steiner et al.[8] in their study with nine patients after administration of commercial 8-MOP powder capsules. These variations suggest great differences in the absorption rate, probably largely caused by poor-solubility[4,7].

Kreuter and Higuchi[4] reported that, for the therapeutic use of 8-MOP, bioavailability is of limited relevance because it is used as a "hit-and-run" drug. The desired photoreaction of the drug occurs only if the patient is exposed to ultraviolet irradiation. Therefore, the effectiveness of the drug is dependent on the drug concentration in the epidermis. For this reason, blood levels shortly before or at the time of irradiation (normally two hours after drug administration) are of main interest. They reported also that, to avoid a prolonged photosensitivity of the patient[9], prolonged absorption is not desirable.

Several investigators reported that drug absorption may be assessed indirectly by determining quantitatively the oral toxicity of the drug[10-12]. Sheth et al.[13] used LD_{50} values as an absorption index to evaluate various drug delivery systems of poorly-soluble drugs. They reported that the LD_{50} studies can be a practical rapid method of achieving comparative rating of drug formulations.

In the present study, air suspension technique was adopted to deposit 5-MOP with different water-soluble polymers and/or surfactants on lactose with the aim of improving

the dissolution characteristics of 5-MOP. Those formulations showing acceptable dissolution behaviour were then evaluated for their absorption rates by demonstrating their toxicites compared to pure drug by the LD_{50} method.

EXPERIMENTAL

Materials

The following materials were used: 5-Methoxypsoralen "5-MOP, Bergapten" (Memphis Chemical Co., Egypt), PVP 40000, PEG 4000, PEG 6000, lactose and polysorbate 80 (B.D.H., England), PEG 20000 (Hoechst, W. Germany), dioctyl sodium sulfosuccinate (E. Merck, W. Germany), and ethanol (Prolabo, France).

Preparation of 5-MOP Granules

A specified amount of lactose was introduced into the vertical container of the fluidized-bed apparatus (Uni-Glatt "Wurster" system, CH-4133, Binzen-Haltingen, W. Germany), fluidized from below by a stream of air. The exhaust filter was shaken from time to time to keep all the lactose inside the container. The temperature of the bed was maintained at 70°. The atomized compressed air was adjusted at 29 psi and the discharge volume of the spray liquid pump was adjusted at 25 rpm to give a suitable droplet size of the spraying solution. The fluidized lactose was then sprayed with a hot ethanolic solution of 5-MOP, alone, or with a water-soluble polymer and/or surfactant in different ratios to make 100 g of the finished granules (Table 1). The spraying was stopped at occasional intervals, and the product was fluidized to dry. When the granules have been formed and the granulating fluid was finished, the product was left to fluidize inside the apparatus for about 15 minutes for complete drying at the same elevated temperature.

Determination of Drug Content in the Prepared Granules

Exactly 100 mg of the prepared granules was dissolved in 1000 ml of distilled water. The amount of 5-MOP was spectrophotometrically determined[14] at 311 nm using Varian Series 634-Spectrophotometer. The employed water-soluble polymers, surfactants and lactose in the concentration present in the assay sample were found not to interfere with the spectrophotometric determination of 5-MOP at 311 nm.

Dissolution Studies

Erweka-Dissolution Tester (Type DT 6 RE, Erweka Apparatebau GMBH, W. Germany) was used to investigate the dissolution rate of 5-MOP and its granules in distilled water. The apparatus is essentially of USP XX Method 2 specifications. To maintain sink conditions, 10 mg of 5-MOP (particle size of about 10 μm as measured by microscopic method) or a quantity of the tested granules (30/40-mesh screen, BP) equivalent to 10 mg of 5-MOP was dissoluted in 900 ml of deaerated distilled water equilibrated at 37±0.5°. The formulation samples were gently spread over the surface of the dissolution medium and no aggregation of particles was observed. The paddle was immersed such that a distance of 2.5±0.2 cm was allowed between the blade and the inside bottom of the vessel and rotated at 100 rpm. At each time interval, an aliquot was withdrawn by a pipet through a 0.45 μm millipore filter and spectrophotometrically assayed for 5-MOP content at 311 nm. The volume taken was immediately replaced by an equal amount of deaerated distilled water preheated at 37°. Each test was run at least in duplicate.

LD_{50} Studies

On the basis of the dissolution data (Table 1) five granule formulations were selected along with pure drug to assess the relative rate of drug absorption. The investigated formulations were suspended in gum acacia solution as a 5% concentration of 5-MOP. Mice, 18-22 g, were used. Preliminary experiments were carried out to discover approximately that dose which killed all mice (LD_{100}), that which killed nothing (LD_0) and 4 doses in between. These experiments revealed that the LD_{100} was 6250 mg/kg, while the LD_0 was 625 mg/kg. Thus, the four doses chosen were 1250, 2500, 3750 and 5000 mg/kg. The four doses of each formulation were administered orally by intubation to 10 mice per dose level. Dosed mice were left 24 hours, after which, mortality was reported. The LD_{50} values were calculated adopting the method of Litchfield and Wilcoxon[15].

Oral Toxicity of Excipients

The excipients added to the formulations were evaluated in the same manner as the drug (Table 3).

RESULTS AND DISCUSSION

Dissolution Studies

The dissolution data (Table 1) revealed that granulation of 5-MOP with lactose, as water-soluble carrier, by the air suspension technique slightly increased the dissolution rate of 5-MOP. The deposition of 5-MOP and PVP 40000 or polyethylene glycols 4000, 6000 and 20000, in equal weight ratio, on lactose resulted in more increase of the dissolution rate. Doubling the weight ratios of the investigated polyethylene glycol polymers evidently increased the dissolution rate of 5-MOP. This increase in the dissolution rate may be explained on one hand by the increase in the surface area of 5-MOP as a result of its deposition on lactose by the air suspension technique and on the other hand, by the fact that polyethylene glycols being water-soluble polymers will dissolve readily in the dissolution medium, hence, encircles the crystallites of the medicament causing its wettability and, thus, enhances its dissolution[17].

Addition of polysorbate 80 or dioctyl sodium sulfosuccinate in equal weight ratio as the drug decreased the dissolution rate of 5-MOP. On the other hand, the addition of the investigated surfactants in lower ratios (Formulations 11 and 13) increased the dissolution rate of the drug. This increase in the dissolution rate may be attributed to the increased wettability of 5-MOP by the surfactants[17,18] in addition to the increased surface area of the drug due to its deposition on lactose by the air suspension technique. The presence of the surfactant during the deposition process might also cause a defect in the drug crystal structure and the crystal would become thermodynamically unstable and, hence, dissolve faster[18]. The possibility of the formation of a solid solution of the water-soluble surfactant in the drug crystal might also enhance the dissolution[17,18].

Deposition of 5-MOP, PEG 6000 and polysorbate 80 or dioctyl sodium sulfosuccinate on lactose, in 1:2:0.1:6.9 ratio, resulted in obvious increase in the dissolution rate of 5-MOP. Formulation 15 gave the highest progressive pattern of *in vitro* drug release (about fourfold that of pure drug).

TABLE 1
Dissolution Rate of 5-MOP Granules in Distilled Water at 37°C.

No.	Formulation*	Weight Ratio	Percent Dissolved (Min.)									
			5	10	15	30	45	60	75	90	105	120
1	I		0.9	1.7	2.4	4.5	6.1	7.2	8.9	10.1	11.1	12.5
2	I-II	1:9	1.0	1.8	2.5	5.1	6.7	8.5	9.5	10.8	12.3	13.6
3	I-II-III	1:8:1	1.1	1.9	2.9	5.6	7.3	9.2	10.1	11.3	13.0	14.5
4	I-II-IV	1:8:1	1.2	2.3	3.2	5.9	8.8	10.8	12.1	14.1	15.6	16.8
5	I-II-IV	1:7:2	1.9	3.7	5.4	10.3	14.1	17.5	21.1	24.8	27.9	30.5
6	I-II-V	1:8:1	1.2	2.3	3.4	6.2	8.9	10.9	12.4	14.6	16.4	17.6
7	I-II-V	1:7:2	2.3	4.4	6.2	11.6	15.9	19.6	23.2	27.3	29.9	33.8
8	I-II-VI	1:8:1	1.2	2.1	3.0	5.7	7.8	9.6	11.5	12.8	14.5	15.9
9	I-II-VI	1:7:2	2.0	3.9	5.7	10.8	14.4	18.7	22.2	26.2	28.6	31.6
10	I-II-VII	1:8:1	0.8	1.4	2.1	3.9	5.6	6.7	8.0	9.2	10.8	11.4
11	I-II-VII	1:8.9:0.1	1.6	3.0	4.5	8.1	10.1	12.1	14.3	16.7	19.3	21.6
12	I-II-VIII	1:8:1	1.0	1.6	2.1	4.2	5.9	7.1	8.6	9.9	11.8	12.3
13	I-II-VIII	1:8.9:0.1	1.8	3.4	4.9	9.1	12.3	14.6	17.3	20.3	23.4	25.8
14	I-II-V-VII	1:6.9:2:0.1	2.7	5.2	7.2	14.0	19.9	25.5	30.0	35.2	39.3	43.4
15	I-II-V-VIII	1:6.9:2:0.1	2.9	5.7	8.2	15.8	22.7	28.6	34.2	39.6	44.7	49.0

* 5-MOP (I), lactose (II), PVP 40000 (III), PEG 4000 (IV), PEG 6000 (V), PEG 20000 (VI), polysorbate 80 (VII), and dioctyl sodium sulfosuccinate (VIII).

TABLE 2

Acute Toxicity of 5-MOP in Mice

No.	Formulation*	Dose mg/kg	Number Dead/ Number Dosed	LD_{50} (mg/kg) mean (95% con. lim.)
1	I	5000	8/10	2700 (1731-4212)
		3750	6/10	
		2500	4/10	
		1250	2/10	
5	I-II-IV	5000	9/10	2500 (1667-3750)
		3750	6/10	
		2500	5/10	
		1250	2/10	
7	I-II-V	5000	9/10	2200 (1486-3256)
		3750	7/10	
		2500	5/10	
		1250	3/10	
9	I-II-VI	5000	9/10	2400 (1714-3360)
		3750	7/10	
		2500	5/10	
		1250	2/10	
14	I-II-V-VII	5000	9/10	2000 (1307-3060)
		3750	7/10	
		2500	6/10	
		1250	3/10	
15	I-II-V-VIII	5000	9/10	1750 (1094-2800)
		3750	8/10	
		2500	7/10	
		1250	3/10	

* Symbols and the weight ratio of excipients to 5-MOP are the same as in Table 1.

TABLE 3

Acute Toxicity of Excipients Used in 5-MOP Formulations

No.	Formulation Excipients*	Dose mg/kg		Number Dead/Number Dosed	
		5-MOP	Excipients**	Excipients & 5-MOP	Excipients Alone
5	II+IV	5000	35000 +10000	9/10	0/10
7	II+V	5000	35000 +10000	9/10	0/10
9	II+VI	5000	35000 +10000	9/10	0/10
14	II+V+VII	5000	34500 +10000 + 500	9/10	0/10
15	II+V+VIII	5000	34500 +10000 + 500	9/10	0/10

* Symbols and the ratio of excipients to 5-MOP are the same as in Table 1.

** Quantity of excipients contained in each formulation when 5000 mg/kg of 5-MOP was administered.

LD_{50} Studies

The LD_{50} method is based on the assumption that the toxicity of an orally administered drug is directly proportional to the amount of the drug in the body or its blood level, which, in turn, is directly proportional to its absorption rate from the GI tract[16]. Where this is true, the LD_{50} values may be used to assess relative absorbability.

Table 2 shows the resulting LD_{50} values of five selected formulations compared to pure drug for an evaluation of their absorption rate. Formulations containing 5-MOP with lactose and PEG 4000, PEG 6000 or 20000 showed a decrease in the LD_{50} with an assumed increase in the *in vivo* absorption rate[13]. Formulations containing 5-MOP

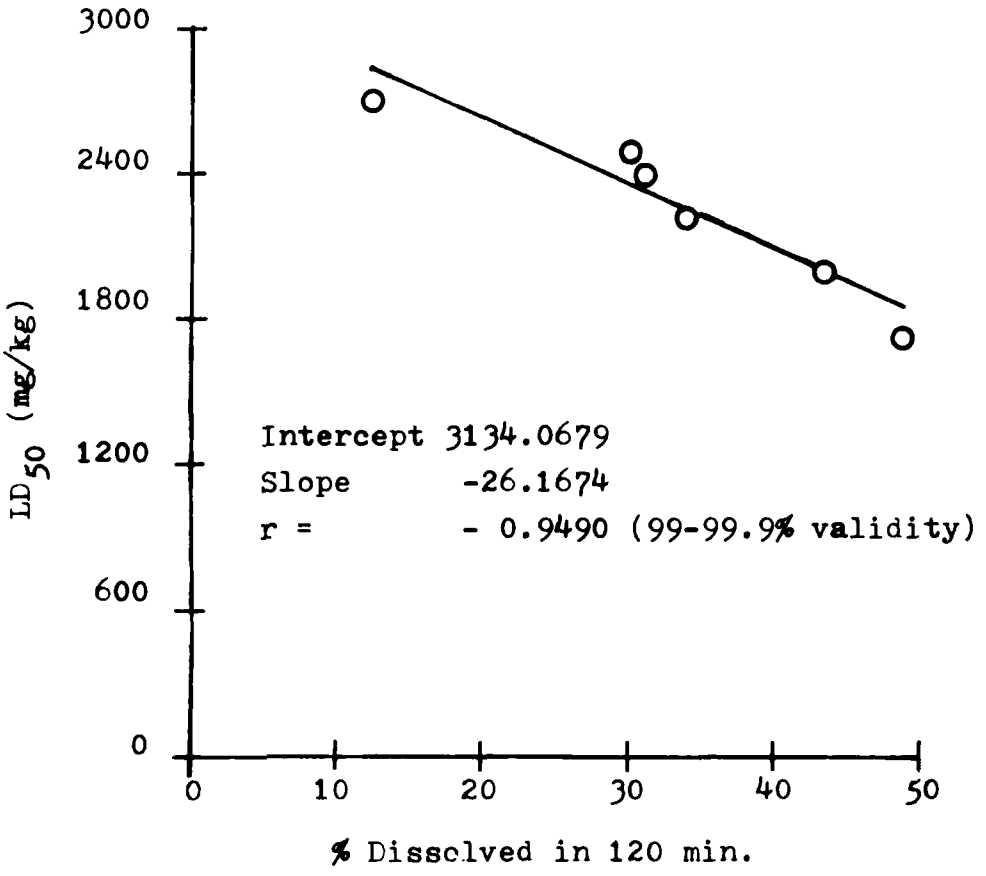

FIGURE 1

Correlation of dissolution rate with LD_{50} values.

The plot is expressed as regression line of the form y= mx + b; with y= LD50 value, x is the percent 5-MOP dissolved in 120 min., b is the y-intercept, m is the slope of the line; r is the correlation coefficient of the line.

with lactose, PEG 6000 and dioctyl sodium sulfosuccinate or polysorbate 80 showed a considerable decrease in the LD_{50} with an increase in toxicity. This increase in the oral toxicity may be attributed to the increased drug dissolution (Table 1).

As Sheth et al[13] reported, the value of the LD_{50} procedure can be appreciated readily if one considers a hydrophobic drug, like 5-MOP, where passive absorption was limited by the rate at which solution was effected in

the GI fluids. In this type of absorption pattern, any change in the rate of solution of drug in the GI fluids produces a corresponding change in its absorption rate.

The excipients used in the five selected formulations had no effect on the LD_{50} values in the maximum amount used in the formulation and, therefore, did not contribute to the toxicity (Table 3).

In vivo/In vitro Correlation

Figure 1 shows a good correlation (99-99.9% validity) between the percent of 5-MOP dissolved in 120 minutes and the LD_{50} values. Plots of 5-MOP dissolved in 5, 10, 15, 30, 45, 60, 75, 90 and 105 minutes against the LD_{50} values also showed rank-order correlation.

CONCLUSIONS

The results of this study suggest that the 5-MOP formulation prepared by air suspension technique with lactose as a water-soluble carrier, PEG 6000 as water-soluble polymer, and dioctyl sodium sulfosuccinate as surfactant would be a formulation of choice for further development work.

CORRESPONDENCE

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