ELSEVIER

International Journal of Pharmaceutics 110 (1994) 65–73

international journal of pharmaceutics

Absorption characteristics of novel 8-MOP semi-solid-lipid-matrix formulations: In vitro-in vivo correlation

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Received 10 January 1994; accepted 28 March 1994

Abstract

The results of an in vivo study of the absorption of 8-methoxypsoralen (8-MOP) in dogs from semi-solid lipid matrices are presented. The pharmacokinetic parameters obtained with different types of formulations were compared using ANOVA for pairwise comparison. The effect of the emulsifying agent added to the semi-solid matrix was investigated. The in vivo bioavailability data are in agreement with the release rate constants determined previously with a biphasic dissolution model.

Key words: Keywords: 8-Methoxypsoralen; Bioavaibility; Semi-solid lipid matrices; In vitro – in vivo correlation

1. Introduction

Recently, a technique has been described (Kinget and De Greef, 1994) for the assessment of the in vitro release of drugs from semi-solid lipid matrices (SSLM). Interest in this type of excipient has continued to increase, since it has clearly been demonstrated by several authors that, in the presence of lipid material, compounds with a very low water solubility, such as 8-methoxypsoralen (8-MOP), are more effectively absorbed. In this article, the results of an in vivo study of the absorption of 8-MOP in dogs from SSLM are presented. The main objective of these studies was to determine whether a correlation exists between the previously reported in vitro results and the in vivo situation. Indeed, in almost every instance, the in vivo release of active substances from pharmaceutical formulations determines the time course of the therapeutic effect. This is the underlying reason for the growing interest in the in vitro determination of dissolution rates, both as a tool in the development of new formulations and in the control of the quality of manufactured batches.

However, the in vitro dissolution study of a dosage form is of less value if the results do not reveal information about the corresponding dissolution in vivo. Exact replication of the in vivo situation is therefore not necessary, the important issue being whether changes in dissolution in vitro or in vivo are reflected by corresponding changes in the counterpart.

It has been suggested (Huttenrauch and Speiser, 1985) that intra- and inter-subject varia-

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SSDI 0378-5173(94)00101-A

tions are too large to allow systematic in vivo predictions based on in vitro results. This view has never been generally accepted, nevertheless, in spite of the long search for in vitro-in vivo correlations, the discussion about methods and goals has not led to a full consensus. The present work may contribute to the discussion by revealing possibilities when etablishing this correlation.

2. Materials and methods

2.1. Dosage forms

The ingredients, composition and preparation of the SSLM studied have been described previously (Kinget and De Greef, 1994). For the sake of better understanding, the compositions (Table 1) are repeated in this article. The SSLM formulations were prepared 1 week before administration. The relative bioavaibility was assessed vs a solution with the following composition (Stolk, 1982): 8-MOP, 100 mg; propylene glycol, 25 ml; ethanol, 5 ml; syrup, 50 ml; water, q.s. to 100 ml. This solution is supersaturated and should be used within 3 h after preparation. Meladinine and Oxsoralen are commercial brands of Basotherm (Germany) and Gerot Pharmazeutica (Austria), respectively. Meladinine is presented as tablets while Oxsoralen are liquid-filled soft gelatin capsules, both containing 10 mg 8-MOP per unit.

Table 1
Composition of semi-solid liquid matrices

Type of SSLM	Composition
Lipo	white beeswax, corn oil 1:9
Lipo-Cremo	cocoa butter, Cremophor RH 60, corn oil 18:27:55 (Cremophor RH 60 = PEG-60 hydrogenated castor oil)
Lipo-Leci	white beeswax, corn oil, Epikuron 145V 14.5:83:2.5 (Epikuron 145V = concentrated soya phospholipids)
Meta P+F	Metarin P, Metarin F 1:1 (Metarin P = oily concentrate of soya lecithin; Metarin F = natural lecithin fraction)
Cremo RH 60	Cremophor RH 60
Gelu 44/14	Gelucire 44/14 (Gelucire = saturated polyglycolysed glycerides)

2.2. In vivo study

Because of the size of the dose, which must be administered to rabbits in order to obtain measurable plasma levels (Kinget and Van Roelen, 1994), dogs were preferred for this in vivo study.

Six healthy beagle dogs with a mean weight of 17 kg were used. Each dog received each formulation three times. The interval between two administrations varied from 8 to 10 days. 20 h before administration of the dosage form, no food was given but free access to water was allowed before and during the experiment. Blood samples were taken after 20, 40, 60, 90, 120, 180, 240 and 360 min. The plasma samples were centrifuged and stored at −20°C until analysis. It has been shown that, under these conditions, 8-MOP remains unchanged in plasma (Stolk, 1982). In order to avoid the possibility that no plasma level could be detected in the case of low bioavailability, a dose of 4 mg/kg (Van Boven et al., 1985) body weight was given.

2.3. Drug analysis

The different plasma samples of the dogs were analysed according to the HPLC method described by Van Boven et al. (1985), but using 5-MOP as internal standard. For a concentration of 200 ng/ml, the recovery of 8-MOP equalled 98% and the relative standard deviation 5%. For a concentration of 1000 ng/ml, the values were 99 and 5%, respectively. In the case of 5-MOP, the corresponding values were 101 and 4% for a concentration of 400 ng/ml.

2.4. Data analysis

The bioavailability of the formulations is described in terms of the rate and extent of drug absorption using three characteristic pharmacokinetic values: C_{max}, T_{max} and the area under the plasma concentration-time curve (AUC) up to 3

and 6 h; 3 h was selected because in PUVA therapy this period is decisive. The AUC values were calculated on the basis of the trapezoidal rule. In order to evaluate the bioequivalence, an analysis of variance (ANOVA) for pairwise comparison (SAS general linear model procedure) was conducted, with as independent variables the dogs and the dosage forms and as dependent variables the pharmacokinetic parameters.

The standard deviation of the mean (SE) given in the graphs was calculated using the formula: $SE = SD/\sqrt{n}$, where SD is the standard deviation and n the number of experiments.

3. Results and discussion

3.1. In vivo

The plasma concentration-time curves after oral administration of two SSLM formulations, Lipo and Gelu 44/14, to dogs are shown as examples in Fig. 1 and 2.

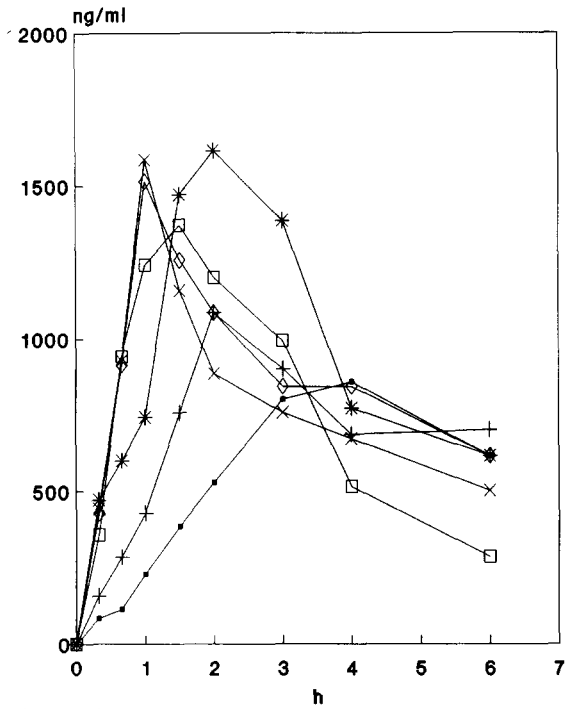

Fig. 1. Individual plasma concentration-time profiles of 8-MOP in six dogs after oral administration of Lipo capsules.

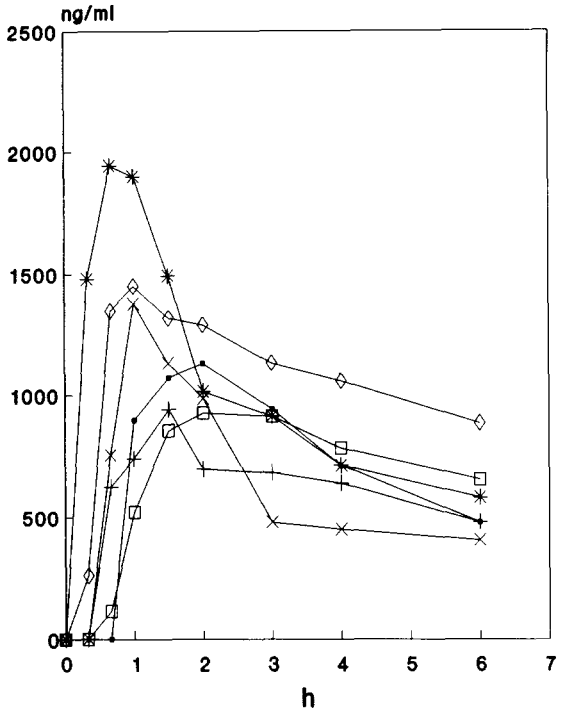

Fig. 2. Individual plasma concentration-time profiles of 8-MOP in six dogs after oral administration of Gelu 44/14 capsules.

Table 2 lists the mean values of the pharmacokinetic data.

Within a first series of five formulations, statistical analysis by pairwise comparison revealed significant differences (Tables 3 and 4) in T_{max} and C_{max} ($p < 0.005$) and AUC_{0-3} and AUC_{0-6} ($p < 0.05$) (Fig. 3 and 4).

The time to peak was significantly shorter for Lipo-Cremo and longer for Lipo. A low T_{max} is desirable in PUVA therapy as is a high but sharp peak value. C_{max} and AUC_{0-3} of Lipo-Cremo and Oxsoralen were significantly greater than for Lipo-Leci and Meladinine; the difference with Lipo was less pronounced.

Within the group of SSLM, the C_{max} values were in the following rank order: Lipo-Cremo $\gg$ Lipo > Lipo-Leci. Different factors are probably the reason for the superiority of Lipo-Cremo. It was shown microscopically (Kinget and De Greef,

Table 2
Pharmacokinetic parameters obtained for various 8-MOP formulations (mean ± SE)

Type of formulation	Symbol	T_{max} (min)	C_{max} (ng ml^{-1})	AUC_{0-3} (ng h ml^{-1})
Series I				
Lipo	A	195 (134)	1456 (564)	3089 (1330)
Lipo-Leci	B	118 (80)	1850 (385)	2404 (852)
Lipo-Cremo	C	42 (29)	2235 (751)	4392 (1620)
Meladinine	D	138 (63)	1210 (265)	2500 (845)
Oxsoralen	E	75 (56)	2150 (860)	4100 (1930)
Series II				
Solution	F	42 (7)	3550 (410)	7510 (1094)
Met P + F	G	205 (42)	1650 (170)	1510 (1100)
Cremo RH 60	H	48 (18)	2032 (510)	4410 (1100)
Gelu 44/14	I	60 (24)	2304 (225)	4330 (370)

1994) that large crystals of 8-MOP were still present in Lipo and Lipo-Leci. Furthermore, in studies with isolated gut preparations, a number of authors (Franz and Vanderscher, 1981; Loyd et al., 1981) observed increased absorption from micellar solutions, as can be formed by Cremophor RH 60 present in Lipo-Cremo. The same effect was not found with Lipo-Leci containing Epikuron 145V. This might be due to the typical characteristics of phospholipids, which on mixing with fatty substances give rise to w/o emulsions, when in contact with water. This emulsion type is known to cause delayed drug release.

A high value for T_{max}, as observed with Lipo, was also reported (Francois et al., 1982) for fat-containing capsules. The value of C_{max} found for Lipo lies between those of Lipo-Cremo and Lipo-Leci. Due to the absence of an emulsifier in this formulation, in situ emulsification is not favoured, which leads to a delay in T_{max} but without a pronounced influence on the extent of absorption as for Lipo-Leci.

The influence of the emulsifying agent was studied by means of the second series of SSLM in comparison to a solution of 8-MOP and Oxsoralen. The total variance between the results obtained must again be attributed to the subjects and dosage forms. The pairwise comparison (Tables 5 and 6) demonstrated the solution to have superior bioavailability compared with all other formulations.

C_{max} and T_{max} were significantly smaller and greater, respectively, for Met P + F (Fig. 3) than for the other dosage forms. The AUC_{0-3} of RH 60 and Gelu 44/15 was significantly larger than for Met P + F (Fig. 4).

Table 3
Results of pairwise comparison of T_{max} and C_{max} for formulations of series I

	T_{max}					
	Formulation	A	B	C	D	E
Lipo	A	x	–	T	–	T
Lipo-Leci	B	–	x	S	–	–
Lipo-Cremo	C	S	–	x	T	–
Melanidine	D	–	–	S	x	–
Oxsoralen	E	T	S	–	T	x
	C_{max}					

S = $p < 0.05$, T = trend, – = no significant difference.

Table 4
Results of pairwise comparison of AUC_{0-3} and AUC_{0-6} for formulations of series I

	AUC_{0-3}					
	Formulation	A	B	C	D	E
Lipo	A	x	–	–	–	–
Lipo-Leci	B	–	x	–	–	T
Lipo-Cremo	C	–	T	x	T	–
Melanidine	D	–	–	–	x	T
Oxsoralen	E	–	–	–	–	x
	AUC_{0-6}					

S = $p < 0.05$, T = trend, – = no significant difference.

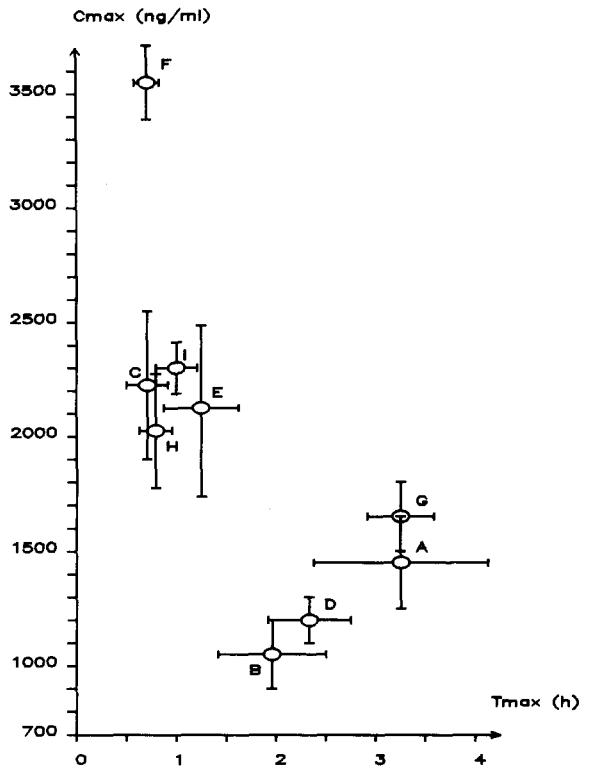

Fig. 3. Mean C_{max} and T_{max} ($\pm$SE) for the different formulations; for notation, see Table 2.

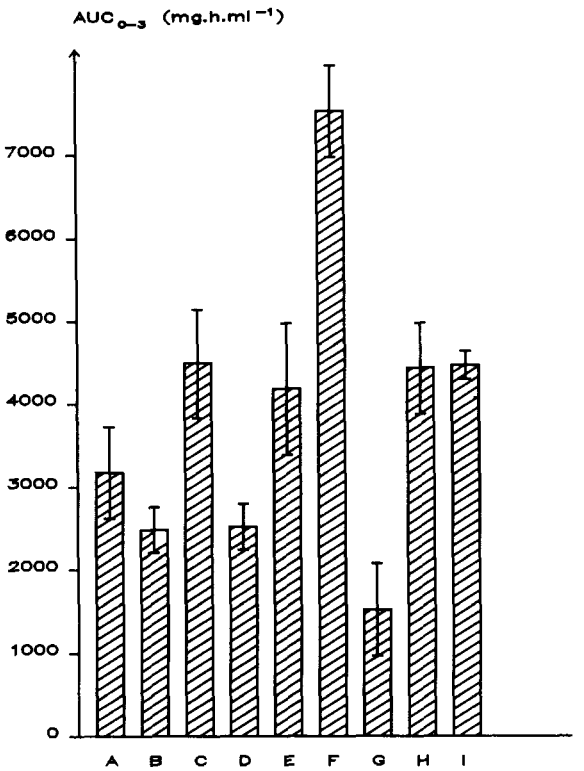

Fig. 4. Mean AUC_{0-3} ($\pm$SE) for the different formulations; for notation, see Table 2.

The much greater plasma concentrations determined with the solution are consistent with results reported elsewhere (Stolk, 1982). However, compared to the solution the C_{max} of Oxsoralen was significantly lower whereas the active ingredient was still dissolved in this product. However, due to the much smaller volume of solvent present in the capsules, the dissolved material probably precipitated in situ rapidly. The observation of high plasma concentrations remains an indication of the microcrystallinity of the precipitate.

The delay in absorption for Met P + F remains remarkable. The plasma concentrations were comparable with those of Oxsoralen but the T_{max} was much higher. Heun (1986) reported that such a plasma profile is due to a delay in gastric emptying. It is unknown to us whether phospholipids cause such an effect. However, in vitro, a highly viscous mass forms when in contact with water. Delayed gastric emptying has been reported (Djimbo, 1985) for high molecular weight polyethylene glycol masses which are also not readily dispersible in water. This delayed gastric emptying can increase dissolution time and thereby explain the high T_{max}. Moreover, lecithin

Table 5
Results of pairwise comparison of T_{max} and C_{max} for formulations of series II

	T_{max}					
	Formulation	E	F	G	H	I
Oxsoralen	E	x	S	–	–	–
Solution	F	–	x	S	–	–
Met P+F	G	S	S	x	S	S
RH 60	H	–	S	–	x	–
Gelu 44/14	I	–	S	–	–	x
	C_{max}					

S = $p < 0.05$, T = trend, – = no significant difference.

is known to increase the solubilising capacity of bile salts by the formation of mixed micelles with lecithin (Rosoff and Serajuddin, 1980; Imai et al., 1983).

The bioavailability of Cremo RH 60 and Gelu 44/14 was somewhat lower than for the solution but significantly different from the other formulations. Microscopic examination did not reveal solid paricles in these matrices. The formation of solid solutions with surfactants has already been described (Attwood and Florence, 1983). To our knowledge, this is not known to occur for Gelucires and needs further investigation. Besides the positive effect on dissolution resulting from the formation of solid solutions, the influence of the surface-active agent cannot be ignored.

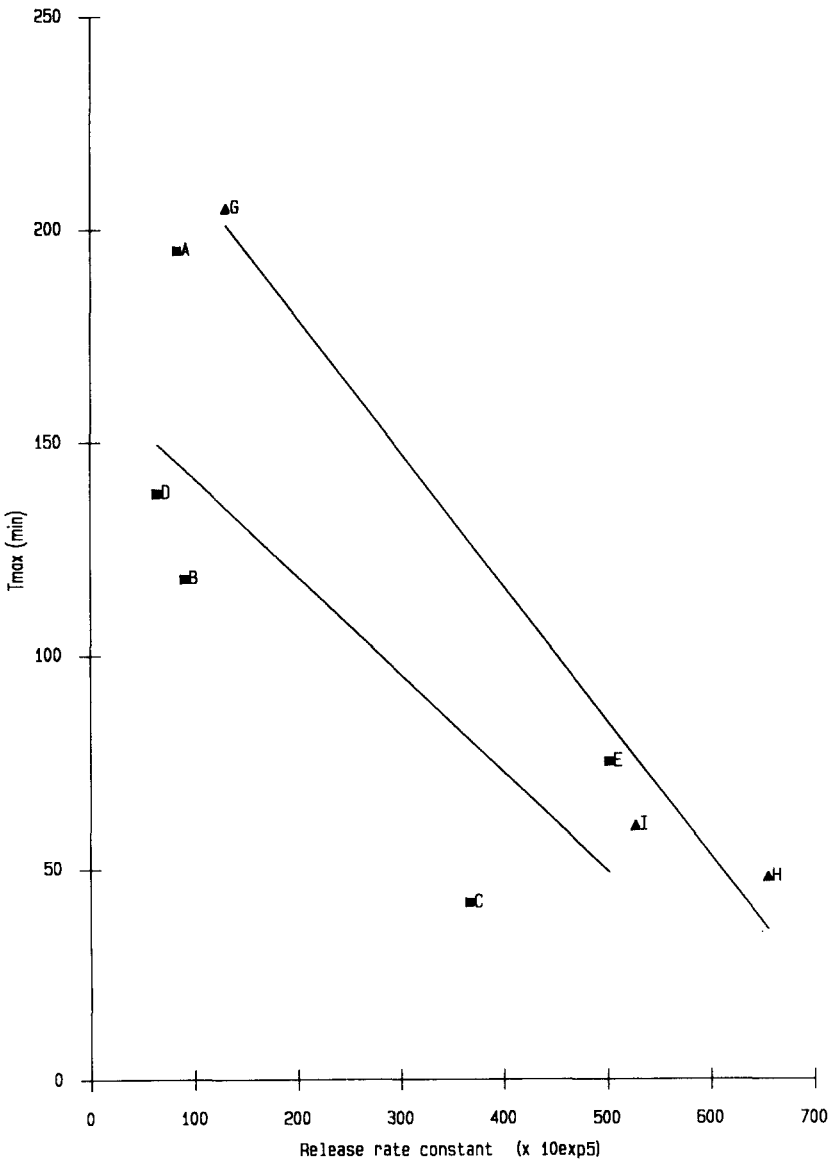

Fig. 5. Relationship between T_{max} and in vitro release constants for the various 8-MOP formulations.

3.2. In vitro-in vivo correlation

In our previous paper (Kinget and De Greef, 1994), a difference in release profile between four SSLM was established using a biphasic dissolution model. Since considerable effort is currently being directed at establishing in vitro-in vivo correlations, the relationship between data on in vitro dissolution and in vivo bioavailability is attracting a great deal of attention. Therefore, it was considered necessary to investigate the in vitro-in vivo correlation and the effects of the release rate in relation to 8-MOP absorption in man.

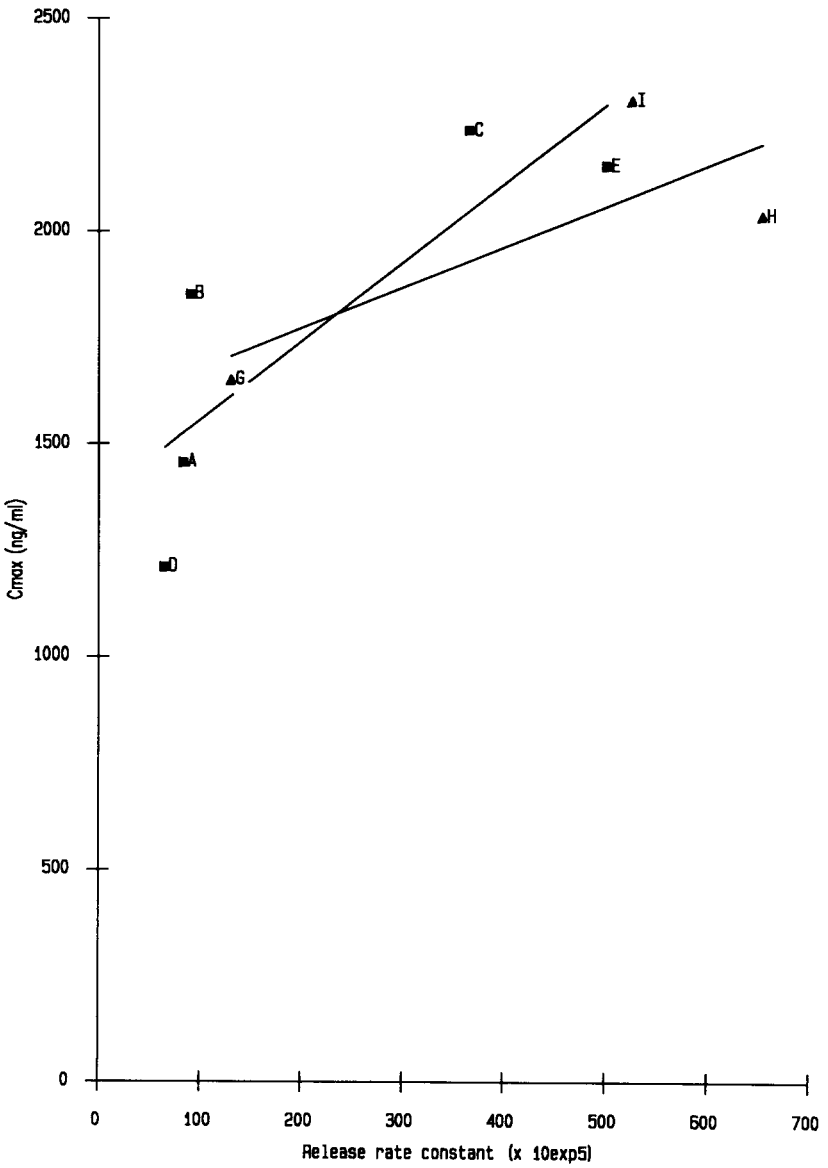

Fig. 6. Relationship between C_{max} and in vitro release constants for the various 8-MOP formulations.

Table 6
Results of pairwise comparison of $AUC_{0\to3}$ and $AUC_{0\to6}$ for formulations of series II

	$AUC_{0\to3}$					
	Formulation	E	F	G	H	I
Oxsoralen	E	x	S	–	T	T
Solution	F	S	x	S	S	S
Met P + F	G	–	S	x	S	S
RH 60	H	T	S	–	x	–
Gelu 44/14	I	–	S	–	–	x
	$AUC_{0\to9}$					

S = $p < 0.05$, T = trend, – = no significant difference.

In Fig. 5–7 the relationships between T_{max}, C_{max} and AUC and the in vitro release constants are depicted. The in vitro dissolution and in vivo bioavailability data obtained with the various SSLM preparations generally agree. From Fig. 5–7, it is clearly evident that the linear correlation with T_{max} is rather poor while for AUC a closer relationship is obtained.

For the formulations of series I the data undoubtedly lend support to a trend in the relationship between the pharmacokinetic parameters and the in vitro release rate constants. Considering the variability of gastric emptying and the diversity of physiological conditions with an influence on the absorption of lipophilic products, it is understandable that no linear correlation but, rather, a trend has been found. With respect to the data resulting from formulations of series II, the correlation between in vivo and in vitro methods is of a higher degree. Both reference prod-

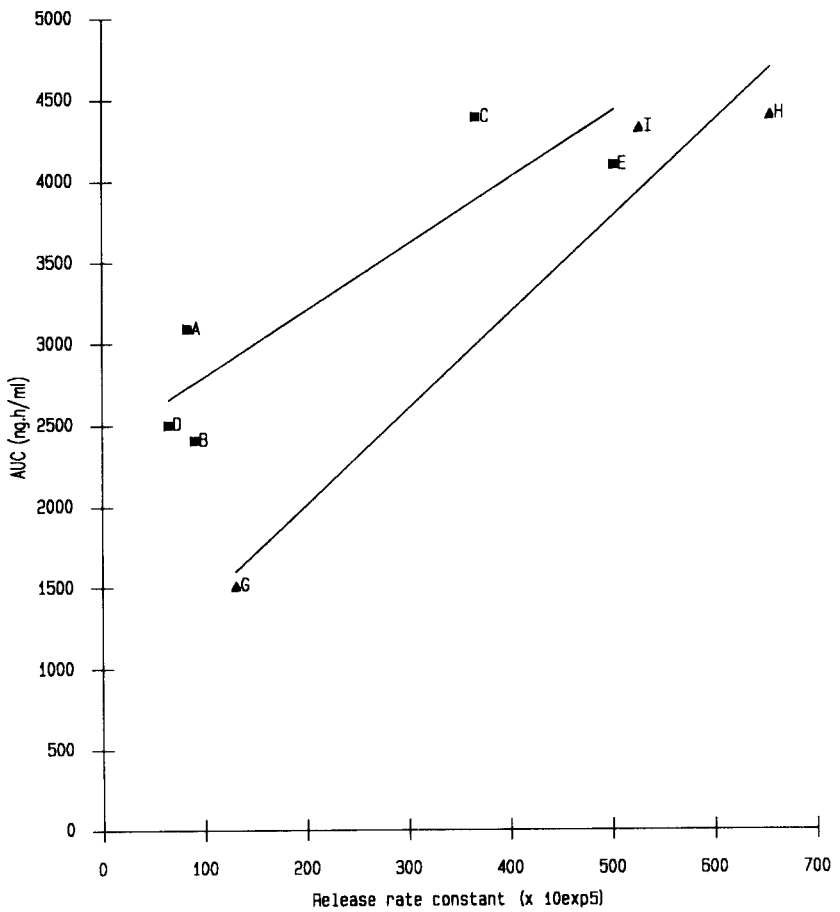

Fig. 7. Relationship between AUC and in vitro release constants for the various 8-MOP formulations.

ucts, Meladinin and Oxsoralen, well known for their low and high bioavailability, respectively, fit in the total picture.

4. Conclusions

The dissolution system, which we previously described, provides a suitable method for discrimination between variations in the formulation of SSLM. Although this in vitro system is not able to mimic very closely all physiological processes participating in the absorption of lipophilic compounds, a reasonably good correlation is obtained between the release rate constants and the pharmacokinetic parameters.

The absorption of 8-MOP in the GI tract is a complex process consisting of more than simple diffusive mechanisms. However, the trend in the correlation between in vitro and in vivo data supports the important role of dissolution in the total absorption process of lipophilic compounds. Future experiments with SSLM containing other drug molecules can improve the in vitro methodology and the in vitro-in vivo correlation.

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