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In vivo study of sustained-release formulations containing amoxicillin and Gelucire 64/02

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Summary

Formulations prepared by fluid-bed coating of direct acting granules with Gelucire 64/02 have shown adequate sustained-release properties in vitro. The in vivo study described demonstrates that the amount of unaltered amoxicillin excreted via urine decreases progressively as the ability of the formulations to sustain release increases. No differences are observed in MRT or VRT. The results obtained show the presence of an absorption window for this aminopenicillin. An assessment is made of the topographic representations proposed by various authors to predict the in vivo behaviour of controlled-release formulations and also potential problems in the bioavailability.

Introduction

Fluid-bed coating is widely used for preparing sustained-release systems (Brossard and Lefort des Ylouses, 1984). Nevertheless, few studies have used lipidic excipients, particularly Gelucire, for coating (Terrier et al., 1975a; Terrier, 1976; Mehta, 1989). The bioavailability of the active principle from the formulations obtained was scarcely considered in these studies when, in fact, it is very relevant, since these sustained-release oral dosage forms can give variable or insufficient absorption. It is well known that with these dosage forms liberation, and hence, subsequent absorption of the active principle are slowed markedly; physiological factors may therefore influence and cause considerable variability.

Among these factors, one of the most important is the pH. Release independent of the pH of the medium is always the ideal situation, since it results in absorption that is more independent of the physiological factors (Skelly, 1986).

From this, it has been demonstrated (Skelly, 1986; Skelly et al., 1986a,b) that dissolution testing in various media of different pH is essential. Using a single medium of constant or varying pH can lead to errors concerning the possible bioe-

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quivalence of two formulations. Furthermore, with this sort of topographic representation, possible problems in the absorption of sustained- or delayed-release oral dosage forms may be predicted.

As well as the pH, two other fundamental factors are the gastric emptying and intestinal transit times. The time a dosage form is in the stomach depends, principally, on the presence of food and the characteristics of the dosage form, above all whether it is multiparticular or monolithic. Because of these variations in gastric emptying time, the absorption rate and the quantity absorbed of the active principle can vary, especially if the release characteristics of the active principle are pH dependent. The intestinal transit time is 2–4 h according to the latest studies, and not 8 h as previously thought. This reduction could be an important limitation in the development of sustained-release systems (Davis, 1986; Moës, 1989).

Finally, whether or not the bioavailability is adequate will depend in some way on the absorption characteristics of the active principle. The mechanism of absorption, the presence of an absorption window, etc., are important aspects to consider. It is believed that the development of many sustained-release systems is due to absorption of many active principles in the colon being greater than previously suspected (Taylor, 1986).

Materials and Methods

Formulations were prepared by coating direct-acting granules of amoxicillin with an organic solution of Gelucire 64/02. The preparation and in vitro study of the formulations are detailed elsewhere (Delgado Charro and Vila Jato, 1991). Formulations containing 18, 23 and 28% of Gelucire were used and the reference formulation was a capsule containing 500 mg of amoxicillin trihydrate. In 16 healthy volunteers of both sexes with no history of kidney disease, the bioequivalence was studied according to a 4×4 Latin square with four replicates. Subjects fasted for the administration which was accompanied by 200–250 ml of water, and continued fasting for 2 h. The wash through period was 1 week. Urine was collected at 1, 2, 3, 4, 5, 6, 8, 10 and 12 h and the quantity of amoxicillin excreted determined as per the method of Smith et al. (1967), of which the validity was confirmed in earlier studies (Vila Jato et al., 1980; Llabrés et al., 1982a; Vila Jato and Delgado Charro, 1990).

The excretion data were used to calculate the three statistical moments (Yamaoka et al., 1978):

E_{12} = total excreted in 12 h in mg

$$\mathrm{MRT} = \left[\int_0^{12} (\mathrm{d}E/\mathrm{d}t)t\,\mathrm{d}t\right]/E_{12}$$

$$\mathrm{VRT} = \left[\int_0^{12} (t - \mathrm{MRT})^2(\mathrm{d}E/\mathrm{d}t)\right]/E_{12}$$

where $\mathrm{d}E/\mathrm{d}t$ is the rate of excretion of amoxicillin in urine. The statistical testing of the parameters was by the corresponding MANOVA and Roy's test, in accordance with the report of Vila Jato et al. (1980).

Results

The release characteristics of the active principle are demonstrated by the topographic representations in Figs 1–3. In each case, the release is very fast at pH 1.2, slower at pH 7.5 and ex-

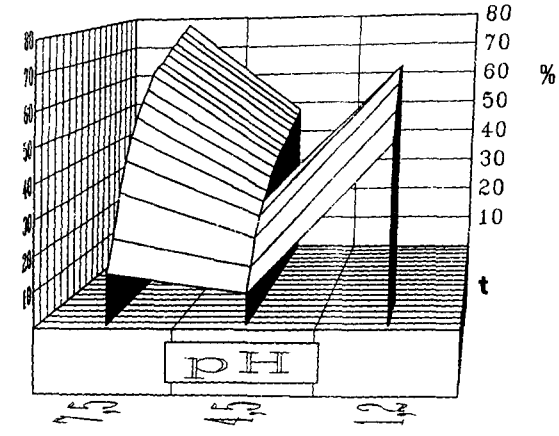

Fig. 1. Topographical dissolution characterization (as a function of time and pH) of the formulation containing 18% of Gelucire 64/02 and prepared by fluid-bed coating method.

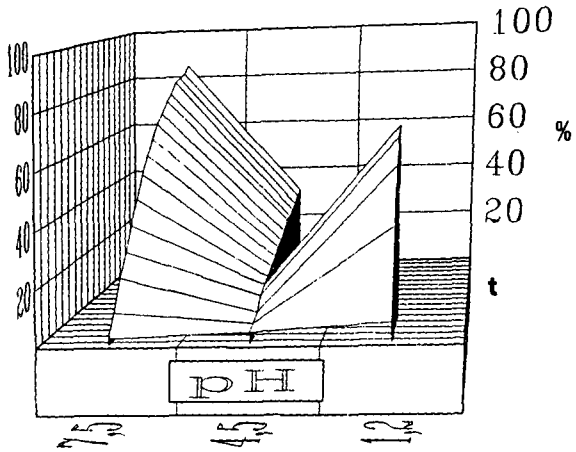

Fig. 2. Topographical dissolution characterization (as a function of time and pH) of the formulation containing 23% of 64/02 Gelucire and prepared by fluid-bed coating method.

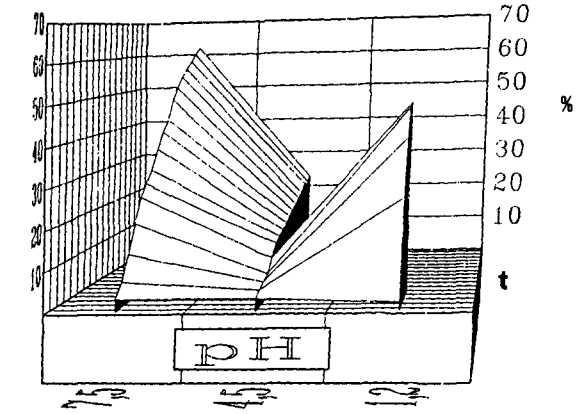

Fig. 3. Topographical dissolution characterization (as a function of time and pH) of the formulation containing 28% of Gelucire 64/02 and prepared by fluid bed coating method.

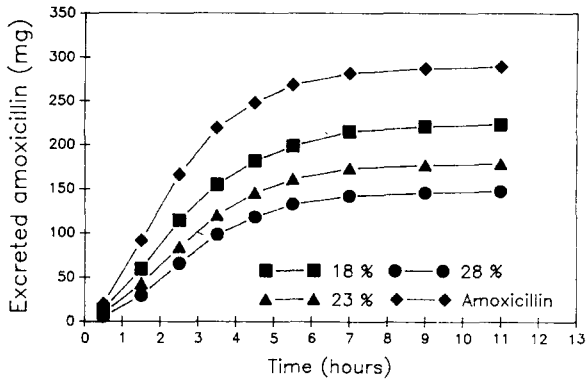

Fig. 4. Mean cumulative curves for the urinary excretion of unchanged amoxicillin.

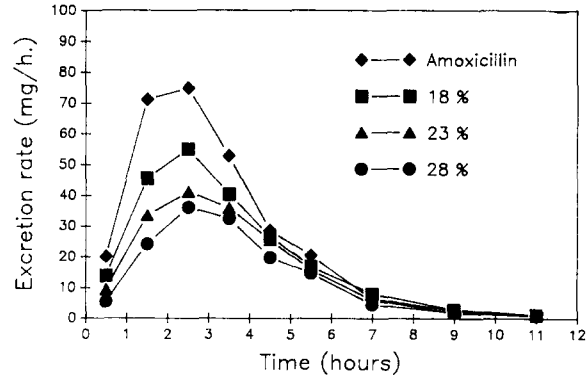

Fig. 5. Mean curves of amoxicillin urinary excretion rate.

tremely slow at pH 4.5. The in vivo results are reflected in the distributive and accumulated curves of amoxicillin excretion (Figs 4 and 5). The amoxicillin eliminated after 12 h was 58.6% for

TABLE 1

Amoxicillin mean excretion rate (mg / h)

Time (h)	Level of Gelucire 64/02 in formulation			
	28%	23%	18%	Ref.
0.5-	5.55 ± 2.31	9.44 ± 5.94	14.01 ± 5.76	20.21 ± 16.75
1.5	24.24 ± 10.01	33.56 ± 12.35	45.64 ± 13.47	71.28 ± 35.36
2.5	36.19 ± 17.21	41.26 ± 16.99	55.15 ± 20.88	75.04 ± 20.33
3.5	32.50 ± 14.12	35.91 ± 11.11	40.50 ± 11.31	52.93 ± 13.45
4.5	19.89 ± 9.96	25.52 ± 13.61	26.78 ± 12.70	28.74 ± 13.86
5.5	14.72 ± 11.05	15.82 ± 8.54	16.96 ± 8.79	20.59 ± 10.51
7.0	4.57 ± 2.43	6.06 ± 3.61	8.10 ± 6.58	6.58 ± 3.05
9.0	1.89 ± 0.90	1.81 ± 0.89	2.97 ± 3.07	2.64 ± 1.13
11.0	0.92 ± 0.76	0.86 ± 0.71	1.22 ± 1.44	1.13 ± 0.75

TABLE 2

Total recovered amoxicillin (mg) 12 h after administration

Volunteers	Gelucire 64/02 level			
	28%	23%	18%	Ref.
1	205.42	167.50	179.35	259.46
2	59.21	89.35	176.56	198.37
3	162.07	214.55	234.88	235.46
4	232.46	248.74	278.40	392.83
5	160.37	107.99	140.50	232.94
6	135.72	205.15	215.89	275.79
7	182.81	197.65	244.66	358.43
8	116.65	197.98	239.22	275.23
9	106.69	138.49	274.55	298.31
10	88.11	124.36	162.93	295.86
11	104.95	220.06	290.46	306.79
12	168.99	261.68	160.79	265.94
13	161.96	156.98	272.46	342.06
14	238.06	248.39	250.61	329.42
15	124.45	165.43	239.89	293.16
16	101.14	126.87	213.62	330.22
X	146.82	179.45	223.42	293.14
σ	51.40	52.96	46.99	50.29

TABLE 3

MRT values obtained

Volunteers	Gelucire 64/02 level			
	28%	23%	18%	Ref.
1	3.42	3.13	3.55	3.51
2	2.49	2.78	2.80	2.83
3	3.59	3.33	3.66	3.78
4	3.45	3.40	3.05	2.57
5	3.85	3.82	3.59	3.34
6	3.66	3.29	3.27	3.25
7	3.53	3.22	2.94	2.61
8	3.72	3.65	3.21	2.61
9	3.77	3.58	3.63	3.73
10	3.29	3.37	2.74	2.32
11	3.51	3.48	3.33	3.16
12	3.98	3.55	2.54	2.97
13	4.13	3.55	5.09	3.38
14	3.78	4.36	3.99	3.64
15	3.50	3.87	3.50	3.38
16	2.71	2.77	2.75	2.39
X	3.52	3.45	3.35	3.09
σ	0.42	0.39	0.64	0.48

the reference formulation and decreased with the proportion of Gelucire, reaching 29.3% for the formulation prepared with 28% Gelucire. There was no change in the profile of the distributive curve corresponding to this decrease. For the four formulations, t_{max} remained the same but none show a slower decrease of the excretion rate (Table 1).

MANOVA gave results in total accordance with these observations. The only effect of a greater percentage of Gelucire in the formulation is to decrease the amount of active principle absorbed without prolonging the action. No differences were found between the MRT and VRT of the four formulations (Tables 2–6).

These results led us to expect in vitro-in vivo correlations. The percentage dissolved in gastric juice at 30 min and the accumulated amount excreted at different sampling times do show correlation (Fig. 6 and Table 7).

The length of intestinal reserve is negative for the three formulations tested (Ho et al., 1983). This type of result is consistent with active princi-

TABLE 4

VRT values obtained

Volunteers	Gelucire 64/02 level			
	28%	23%	18%	Ref.
1	3.98	3.92	4.66	3.69
2	2.61	5.24	3.85	2.07
3	4.26	2.93	4.22	3.48
4	3.07	4.37	3.07	3.40
5	4.83	3.89	3.81	3.73
6	4.44	4.04	3.47	3.65
7	2.91	4.38	3.49	3.17
8	4.40	3.20	3.22	2.67
9	4.19	4.10	4.20	3.99
10	3.79	3.24	3.68	2.94
11	3.36	3.41	3.29	3.67
12	4.14	4.03	3.45	3.46
13	4.63	4.25	7.05	3.91
14	4.10	3.99	4.01	3.80
15	3.56	4.31	3.98	3.47
16	3.39	2.86	2.83	3.01
X	3.85	3.88	3.89	3.38
σ	0.64	0.62	0.96	0.51

TABLE 5

Results of MANOVA

Source	Matrix	Sums of squares and products		
		192475.67		
Treatments	***H***	−885.62	4.56	
		−636.51	2.55	−636.52
		89968.83		
Subjects	***I***	356.92	14.20	
		139.47	8.90	12.84
		62132.22		
Error	***E***	110.35	28.03	
		105.52	5.70	17.87
		344576.72		
Total	***T***	−418.35	46.80	
		−391.51	17.16	−391.51

The greatest eigenvalue of $\boldsymbol{HE}^{-1}$ is $C_s = 3.54$ and $C_s / C_s + 1 = 0.779$. The parameters for its distribution are $s = 3$, $m = -1/2$ and $n = 20.5$. Since the critical value of this statistic is 0.257 for $\alpha = 0.01$; null hypothesis is rejected at the $\alpha = 0.01$ level.

TABLE 6

Roy's test and minimum significant difference for the statistical moments ($\alpha = 0.01$)

	Minimum difference	Gelucire 64/02 level			
		Ref.	18%	23%	28%
E_{12}	61.07	293.14	223.42	179.45	146.82
MRT	1.29	3.09	3.35	3.45	3.52
VRT	1.03	3.38	3.89	3.88	3.85

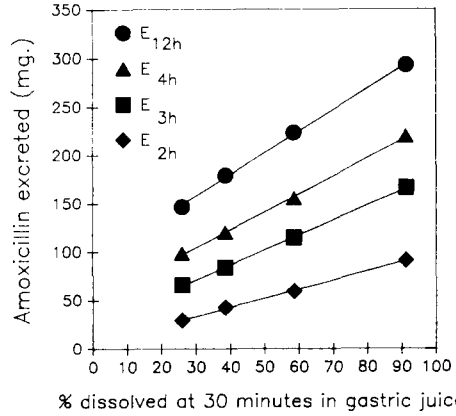

Fig. 6. In vitro-in vivo correlations. AUC_{12h}, AUC_{4h}, AUC_{3h} and AUC_{2h} correspond to the amoxicillin recovered at 12, 4, 3 and 2 h after administration. % Dis_{30m} denotes the percentage dissolved in gastric juice at 30 min.

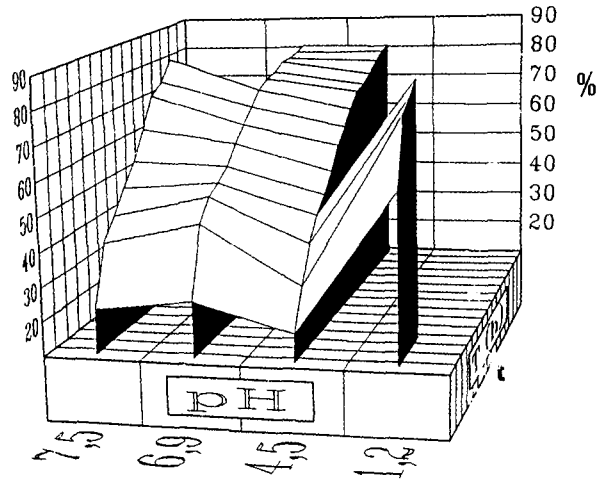

Fig. 7. Topographical dissolution characterization (as a function of time and pH) of the formulation containing 20% of Gelucire 64/02 and prepared by evaporation method.

TABLE 7

In vitro-in vivo correlations obtained

$AUC_{12h} = 2.2192\%Dis_{30m} + 91.8197$
$r = 0.9993$ $F_{(1,2)} = 1502.0$ $\alpha < 0.01$

$AUC_{4h} = 1.8542\%Dis_{30m} + 49.0174$
$r = 0.9995$ $F_{(1,2)} = 1967.7$ $\alpha < 0.001$

$AUC_{3h} = 1.5428\%Dis_{30m} + 25.2411$
$r = 0.9999$ $F_{(1,2)} = 8061.6$ $\alpha < 0.01$

$AUC_{2h} = 0.9337\%Dis_{30m} + 5.9544$
$r = 0.9994$ $F_{(1,2)} = 1748.1$ $\alpha < 0.01$

AUC_{12h}, AUC_{4h}, AUC_{3h} and AUC_{2h} correspond to the amoxicillin recovered at 12, 4, 3 and 2 h after administration. $\%Dis_{30m}$ represents the percentage dissolved in gastric juice at 30 min.

ples which have an absorption window. Only the fraction that dissolves before passing the zone where absorption is preferential is available for absorption. After this section of intestine no further absorption is possible.

Discussion

The absorption characteristics of amoxicillin (Tsuji et al., 1981; Sjövall et al., 1985a,b; Hespe et

al., 1987; Sugawara et al., 1990), those of the gastrointestinal transit of multiparticular dosage forms (Davis, 1986) and those of pH-dependent release (Vila-Jato and Delgado Charro, 1990; Delgado Charro and Vila-Jato, 1991) need to be considered.

In previous studies (Vila-Jato and Delgado Charro, 1990), amoxicillin granulates containing 20, 30 and 40% of Gelucire 64/02 were prepared by the evaporation method. No sustained effect was found in vitro due to the rapid drug release in gastric medium. Consequently, in this study another preparation method was examined.

The in vitro dissolution behaviour of fluid-bed coated granulates of amoxicillin containing 18, 23, 28 and 30% of Gelucire 64/02 has been previously reported (Delgado Charro and Vila Jato, 1991). Drug release from the formulation containing 30% of Gelucire was extremely slow and therefore was not included in this study.

Amoxicillin release was rapid at pH 1.2, moderately fast at pH 7.5 and slow at pH 4.5. Amoxicillin has a strongly pH-dependent solubility (Tsuji et al., 1978), being a minimum between pH 4 and 6 and increasing rapidly beyond this range. The dissolution rate is also pH dependent, increasing as the pH decreases for pH $\leqslant$ 3 and not varying above pH 3.

The work of Davis (1986) on the gastrointestinal transit of dosage forms indicates a mean time of 30 min for 1 mm pellets to pass to the intestine when taken during fasting. Therefore, according to the diffusion law of Higuchi (1963), amoxicillin release is fast in the stomach, but very slow in the first section of the small intestine. Moreover, in accordance with the same diffusion model, increasing the percentage of Gelucire slowed amoxicillin release.

A number of investigations on the absorption process of aminopenicillins (Tsuji et al., 1981; Sjövall et al., 1985a,b; Hespe et al., 1987; Sugawara et al., 1990) have shown that this occurs mainly in the first section of the small intestine, possibly by a saturable process mediated by transporters. In the case of the formulations tested, the only amoxicillin available for absorption is that previously dissolved in the stomach. This may explain the in vitro-in vivo correlations found between the amount dissolved in gastric juice after 30 min and that excreted at different times (2, 3, 4, and 12 h).

Similar results were obtained with tablets (Llabrés et al., 1982a–c) prepared with amoxicillin and Precirol (Gelucire 64/02). In other studies, the bioavailability of amoxicillin and ampicillin was demonstrated to be dependent on k, the initial rate constant, reflecting gastric emptying and intestinal passage of the microcapsules (Goto et al., 1984; Uchida et al., 1986a,b, 1989).

Topographical representations can assist in predicting the in vivo behaviour of the formulations. They all show a strong effect by pH and extremely slow release at pH 4.5 (Figs 1–3). V-shaped release profiles have indicated low bioavailability for other active principles with a limited absorption zone (Skelly, 1986; Skelly et al., 1986a, b). Fig. 7 shows the profile for a formulation prepared with 20% Gelucire 64/02 by the evaporation method, and giving adequate bioavailability. In comparison with the other formulations, release is more rapid, and although pH-dependent, is not extremely slow at any pH (Vila Jato and Delgado Charro, 1990). This and many other studies have demonstrated the necessity for having pH-independent release profiles in order to achieve adequate and reproducible bioavailability. In these circumstances, the effects of gastrointestinal transit on release, and hence, on the absorption of active principles from sustained-release systems are reduced and, according to Brockmeier (1986), it is also possible to obtain better in vitro-in vivo correlations.

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