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DEVELOPMENT OF A NEW TABLET FORMULATION OF THEOPHYLLINE : IN VITRO AND IN VIVO STUDIES

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ABSTRACT

The preparation of a new scored 250 mg theophylline tablet is described, for which effects of particle size of the active principle, aspects of granulation and changes in tabletting settings were investigated.

In vitro studies showed the dissolution rate from tablets prepared from theophylline of commercial quality (50 µm) or of selected particle size (30 µm) to be faster than that from tablets prepared from micronized theophylline (10 µm). In vivo studies in dog showed that only the tablet from theophylline of selected particle size has the same bioavailability as an aqueous solution.

The scale up study showed that the characteristics of the tablets, including dissolution rate, are independent of the formulation factors.

AIMS OF THE STUDY

This work was undertaken to prepare a new theophylline tablet having a rapid rate of dissolution and, if possible, the same bioavailability as an aqueous solution, or one at least equivalent to those of the more efficient formulations available within the EEC. The additional requirements for this formulation were that the tablets should be easy and reproducible to manufacture and that the new formulation should allow the dose of theophylline to be adjusted to the individual needs of the patients by means of scored tablets, possibly even by multiple scoring. The size of the tablet

0363-9045/83/0903-0399$3.50/0

was also taken into account to encourage patient compliance during treatment.

MATERIALS AND METHODS

Raw Materials and their Characterization

Anhydrous theophylline[1], which complies with the European Pharmacopoeia monograph; four samples having the following characteristics were investigated :

- a sample of commercial quality, mean particle size 50 µm (batches R966 and R1027),
- a sample having a carefully selected particle size (CSPS) with a mean particle size of 30 µm, obtained from commercial quality theophylline by a controlled milling process (batch R1028),
- a micronized sample, mean particle size 10 µm (batch 51647),
- a spray dried sample, mean particle size 40 µm (batch L8008).

All the other materials conformed to the USP XX and European Pharmacopoeia monographs.

Particle size determination was carried out using an Alpine[2] air jet sifter as described in the French norm AFNOR[3] NFX 11-640. Dissolution profiles for theophylline were measured in 750 ml of 0.1N HCl at 37 ± 0.5°C using the apparatus 3 described in the USP XX. It was not possible to use the same apparatus for both the raw materials and for the theophylline tablets (apparatus 2), because of "powder-caking". This was in spite of efforts to apply a method published recently[1].

X-Ray diffraction patterns from a powdered specimen under vacuum was obtained with a Guinier de Wolff camera using the Cu Kα radiation at 15.418 nm. The measurement of intensities on the films was by means of a microphotometer.

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[1]Finorga, F-38670, Chasse sur Rhône.

[2]Alpine, D-8900, Augsburg.

[3]AFNOR, F-92080, Paris La Défense.

Tablet Preparation

Mixing was by means of a Z arm type mixer[4] having a capacity of 1, 5 or 30 liters, or a 'hurling and whirling' type mixer[5], capacity 50 and 130 liters, drying by fluid bed dryers, first in an Aeromatic[6] ST2, then in an ST15 and granulation with an oscillating granulator (Erweka FGS)[7] fitted with a screen having a 1 mm mesh. Tabletting was carried out with a reciprocating single punch tabletting machine (Frogerais AM)[8] fitted with 11 mm diameter flat punches which were equipped with strain gauges on the upper and lower punches and a displacement transducer on the upper punch. Tablets were also prepared on a rotating 15 stations machine (Frogerais MR15)[8] with 11 mm diameter flat punches equipped with strain gauges on the compression roll.

Tablet Weight was measured for 50 tablets with an accuracy of ± 0.1 mg on an electronic weighing unit connected to a computer (Mettler HL32[9] + Hewlett Packard 97S[10]) which calculated the variation in weight.

Tablet Hardness was measured on Schleuniger[11] apparatus.

Tablet Friability was measured on 10 tablets by a Roche friabilator during a 15 min period at 30 r.p.m.

Tablet Disintegration was studied as described in the USP XX and the European Pharmacopoeia.

Dissolution Testing was carried out using the USP XX apparatus 2. The medium used was 500 ml of 0.1N HCl at 37 ± 0.5°C. Paddle

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[4]Guittard-Perkins, F-77500, Chelles.

[5]Gebrüder Lödige, D-4790, Paderborn.

[6]Aeromatic AG, CH-4132, Muttenz.

[7]Erweka, D-6056, Heusenstamm.

[8]Ets. Frogerais, F-94596, Rungis.

[9]Mettler AG, CH-8606, Greifensee, Zürich.

[10]Hewlett Packard, OR 97330, Corvallis, USA.

[11]Dr. D. Schleuniger, CH-8033, Zürich.

stirring speed was 56 r.p.m. A 3 ml sample was filtered through a 0.45 µm Millipore membrane filter [12] and diluted. The amount of drug in solution was estimated using an ultraviolet spectrophotometer at 276 nm. Samples withdrawn were not replaced by an equal volume of dissolution medium.

Bioavailability Studies

"In vivo" tests were carried out in 3 beagle dogs weighing 11.8-12.4 kg. Dogs were fasted (water ad libitum) for 12 hr before and 8 hr after drug intake. They received single 250 mg oral doses of theophylline : an aqueous solution (30 ml, batch 1), three tablet formulations prepared with the raw materials tested and two Theolair® tablets [13] (2 x 125 mg, batch 79G03). The administration was according to a cross over design. Tablets were given with 30 ml of water.

Venous blood (6 ml samples) was collected before each administration, then at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, 7, 8, 24, 28, 32 and 48 hr after. Blood was immediately centrifuged, plasma was separated and stored at -20°C until analyzed. A period of two weeks was allowed between each administration.

Analytical Method

Theophylline was measured in plasma by High Performance Liquid Chromatography (HPLC). 1 ml of plasma was spiked with 200 µl of an aqueous solution of the internal standard, diazepam (50 mg/l); 1 ml of 1N HCl was added and the solution was extracted with 10 ml of chloroform/isopropanol (95/5, v/v). Tubes were shaken for 30 min then centrifuged at 1000 g. The organic phase was filtered on hydrophobic paper Whatman 1PS [14], then evaporated to dryness at 30°C under vacuum.

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[12] Millipore, Mass. 01730 Bedford, USA.

[13] Theolair ®, Riker Laboratories, Brussels, Belgium.

[14] Whatman, F-45210, Ferrières.

The residue was taken up into 150 µl of isopropanol. A Varian Aerograph[15] 8500 HPLC equipped with a Varichrom UV detector set at 270 nm, was used. The stainless steel column (15 cm x 0.46 cm i.d.) was packed with Sil 60 D 5 CN[16]. The mobile phase used was hexane/isopropanol/methanol (90/10/0.5, v/v) at a flow rate of 1 ml/min at 20°C.

Under these conditions, the HPLC retention times were 7 and 8.5 min for diazepam and theophylline respectively.

Quantification of theophylline was obtained by the height ratio method (height theophylline/height diazepam) and the calibration curve gave a linear response for plasma concentrations of theophylline ranging from 0.2 to 40 mg/l. The recovery of theophylline from plasma was 85 $\pm$ 5 % and the lower limit of sensitivity was 0.1 mg/l for theophylline and the internal standard. The main theophylline metabolites (3-methylxanthine, 1-methyluric acid and 1,3-dimethyluric acid), caffeine and theobromine did not interfere with the assay.

Pharmacokinetic Analysis

Pharmacokinetic analysis of the plasma curves was carried out using the G-PHARM interactive program[2] according to a one compartment open model. The parameters were calculated as follows :

$t_{1/2}$ abs = half-life of the absorption phase (hr)

t_{max} = time of the peak plasma concentration (hr)

C_{max} = peak plasma concentration (mg/l)

$t_{1/2}$ β = half-life of the elimination phase (hr)

$AUC_\infty = AUC_{48} + \frac{C_{48}}{\beta}$ = area under the plasma concentration/time curve extrapolated to infinity, determined by the trapezoidal rule. AUC_{48} and C_{48} represent the area and the plasma concentration values at 48 hr.

[15] Varian, CA 94303, Palo Alto, USA.

[16] Chrompack, F-91440, Orsay les Ulis.

$$F = \frac{AUC_\infty \text{ tablet}}{AUC_\infty \text{ aqueous solution}} = \text{availability of the tablet relative to the aqueous solution.}$$

Statistical Analysis

A two-way analysis of variance (formulation, dog) was used to compare the values of $t_{1/2}abs$, t_{max}, C_{max} and AUC_∞ obtained after the 5 doses. Since the variance observed for the C_{max} values was not homogeneous (Bartlett's test : $p < 0.05$), this parameter was analyzed by a non parametric test described by Friedman[3]. The AUC_∞ values were compared using symmetrical confidence intervals as for bioequivalence trials[4].

RESULTS AND DISCUSSION

Raw Materials

X-Ray diffraction patterns showed that the crystalline characteristics of the commercially available theophylline products remained the same before and after milling or micronisation. The results of the diffraction study agreed with those reported in the ASTM records 27 - 1977[5].

The physical characteristics of the raw materials were investigated further. The four batches of theophylline were compared by electron microscopy (Fig. 1 a & b), by analysis of particle size and by dissolution kinetics (Fig. 2).

The mean particle size of commercially available theophylline (50 µm) was reduced to about 30 µm by a carefully controlled milling process, but the dissolution kinetics were similar for the two samples.

After micronisation, the mean particle size of theophylline was decreased to 10 µm, however, analysis by electron microscopy (Fig. 1 b) showed agglomerates of the particles (200-300 µm) which could explain the slower rate of dissolution of this theophylline product.

Despite the reduction in particle size obtained after spray drying (40 µm), the dissolution rate of theophylline was slower

than from the other three products considered. This was probably due to a poor wettability of this powder. For this reason and because of problems associated with the industrial preparation, this product was not further investigated.

Formulation

The preparation of 250 mg tablets was commenced by first using commercially available theophylline. After some compressibility tests, the preparation of tablets by a wet granulation process was preferred to that of direct tabletting. In order to improve the compressibility of theophylline and to prepare a tablet having an acceptable disintegration/dissolution performance, several diluents currently in use were tested with different binders at various concentrations : the best results were achieved with calcium phosphate dihydrate as diluent and water as wetting agent. The formulation study was then completed by selection of the disintegrant and lubricant, and the determination of their optimal concentrations. Granulation, drying, lubrication and tabletting were satisfactory as shown by the pharmaceutical characteristics (table 1) and dissolution kinetics (Fig. 3) of the tablet. With the same raw materials and under the same operating conditions, the variability of the results at this early stage of development was low either for the same batch (tests 1 & 2), or for a different batch (test 3) of theophylline.

Compression cycles showed a good compressibility and satisfactory lubrication of the tablet : an example of a typical record is given in figure 4. The shape of the tablets prepared was discoid with a diameter of 11 mm and a thickness of 3.2-3.5 mm. The tablet was scored on one face and can therefore be easily administered or divided into two parts [6, 7].

Under the experimental conditions described above, the dissolution kinetics of the tablet prepared were similar to those of two batches of Theolair® tablets (batch 79 G08 and batch 79 F26) (Fig. 3). A compression test has shown that it is possible to prepare a capsule shaped tablet (17 x 6.4 mm).

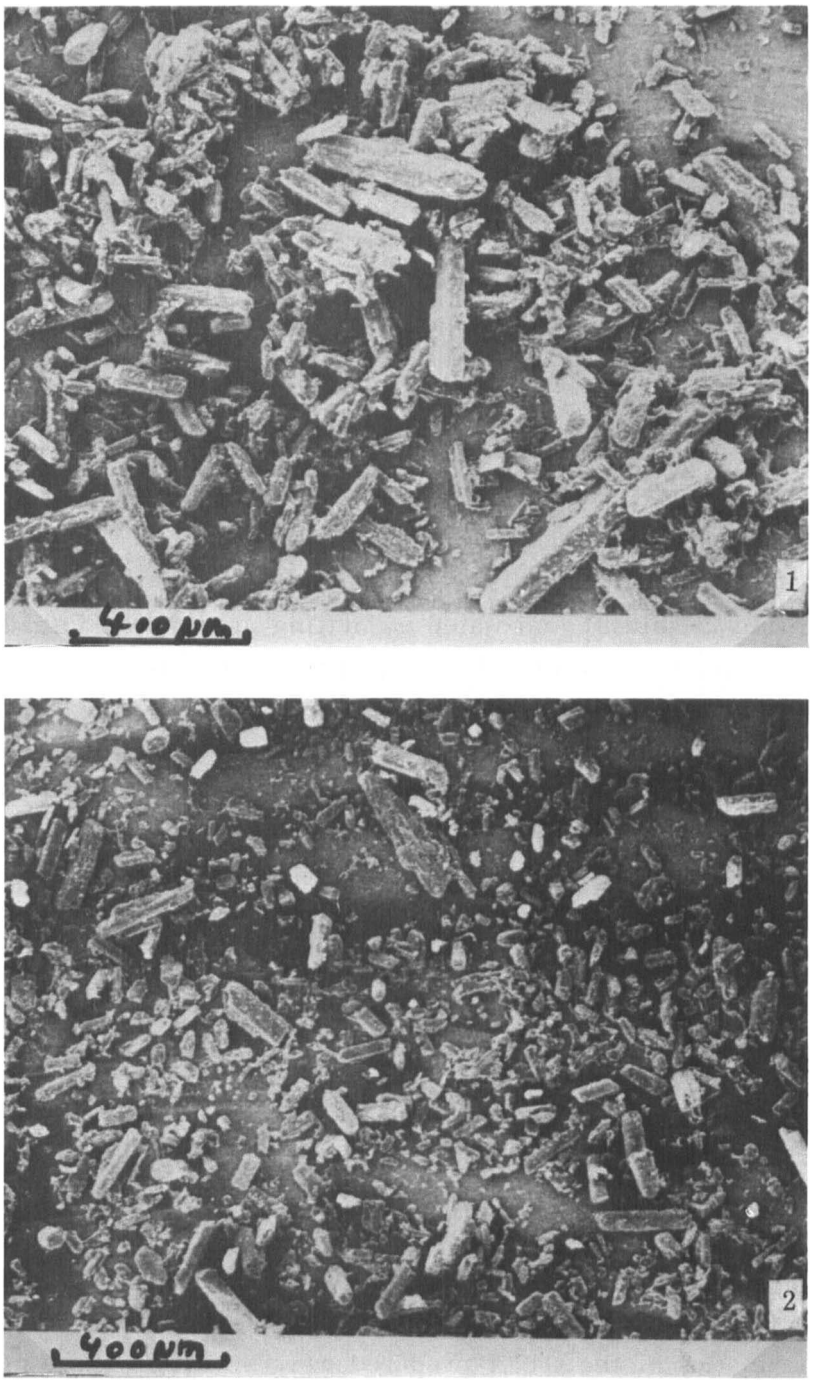

FIGURE 1. a.

Electron photomicrographs of theophylline.
1 : commercial quality - 2 : selected particle size

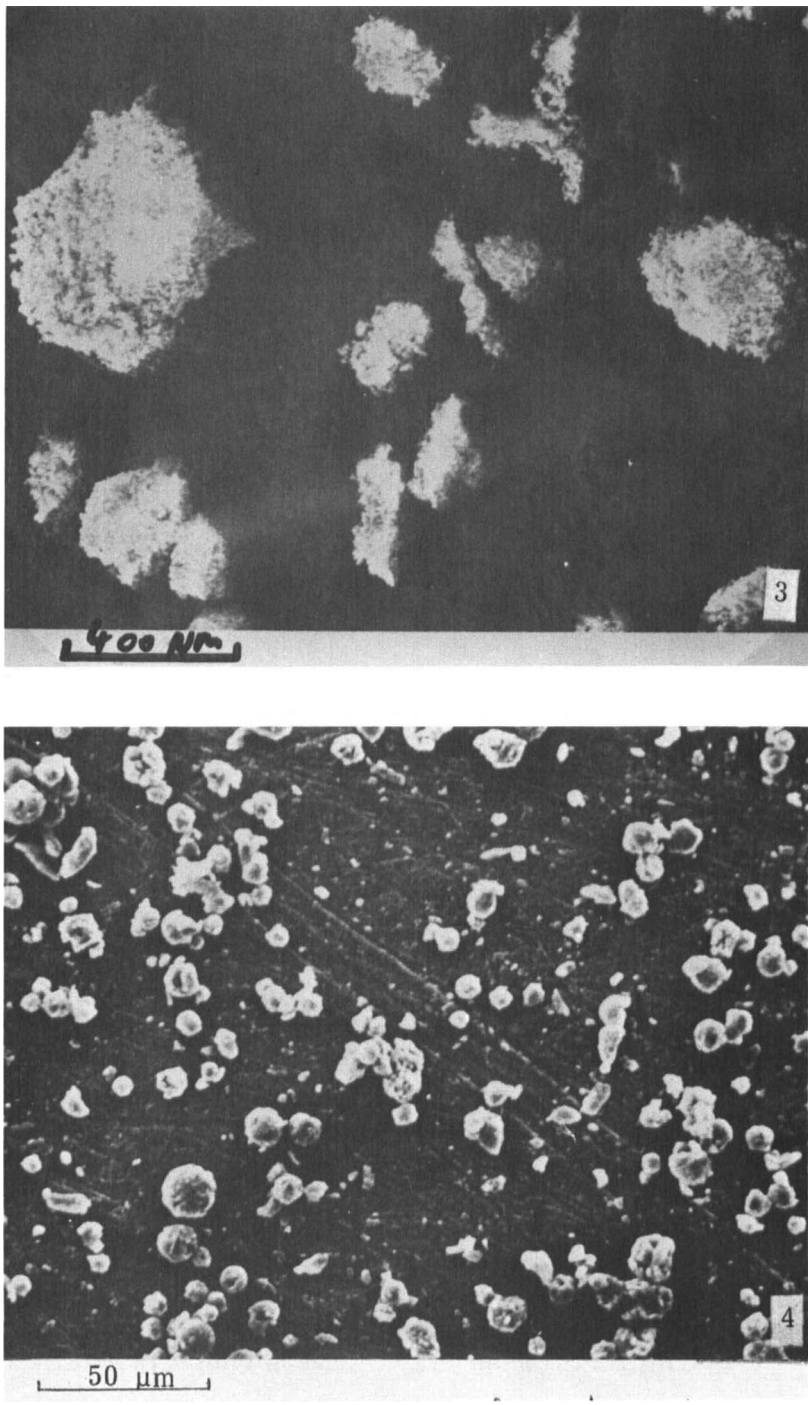

FIGURE 1.b.

Electron photomicrographs of theophylline
3 : micronized - 4 : spray-dried

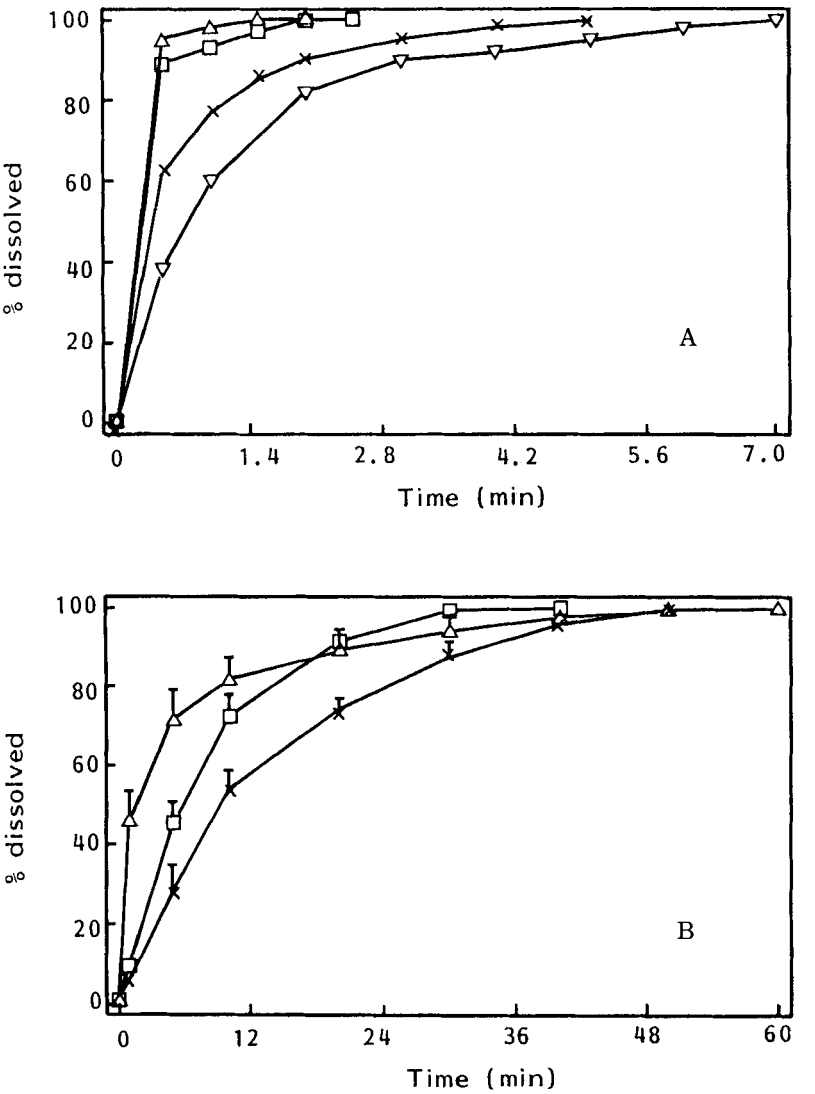

FIGURE 2

Dissolution profiles of theophylline. Commercial quality (△), selected particle size (□), micronized (x), spray-dried (▽). A : theophylline raw material; B = theophylline tablets.

TABLE 1

Characteristics of tablets A obtained from 2 batches of theophylline tests 1 & 2 relate to the batch R966 and test 3 to the batch R1027.

	test 1	test 2	test 3
Compression force kN	9.0	8.8	6.6
Hardness daN ± S.D.	14.4 ± 0.5	15.5 ± 0.8	12.4 ± 0.9
Friability %	0.6	0.4	0.5
Mean weight ± S.D.	400.9 ± 2.6	398.9 ± 3.6	405.2 ± 4.4
Weight CV %	0.7	0.8	1.1
Disintegration time (sec)	32	33	32

Influence of Theophylline Batch in the Formulation

At this point of the development, the tablets containing the milled (tablet B) and the micronized theophylline (tablet C) were formulated. The pharmaceutical properties of the two tablets were compared with those obtained with tablets prepared from commercial quality theophylline (tablet A) (table 2).

The results showed that the differences between the three formulations were negligible. As observed for the raw materials, the dissolution rate of tablets A and B was higher than that of tablet C (Fig. 2). On the basis of the dissolution kinetic experiments, the formulations A and B provided a faster release of theophylline.

Bioavailability Study

Before starting the study to scale up the formulation, the "in vivo" characteristics of the three different tablets were investigated. The bioavailability of tablets A, B and C relative to an aqueous solution (250 mg theophylline) and to Theolair® tablets (2 x 125 mg theophylline) was determined in three dogs.

Plasma concentrations of theophylline were best fitted to a one compartment open model. The pharmacokinetic parameters ob-

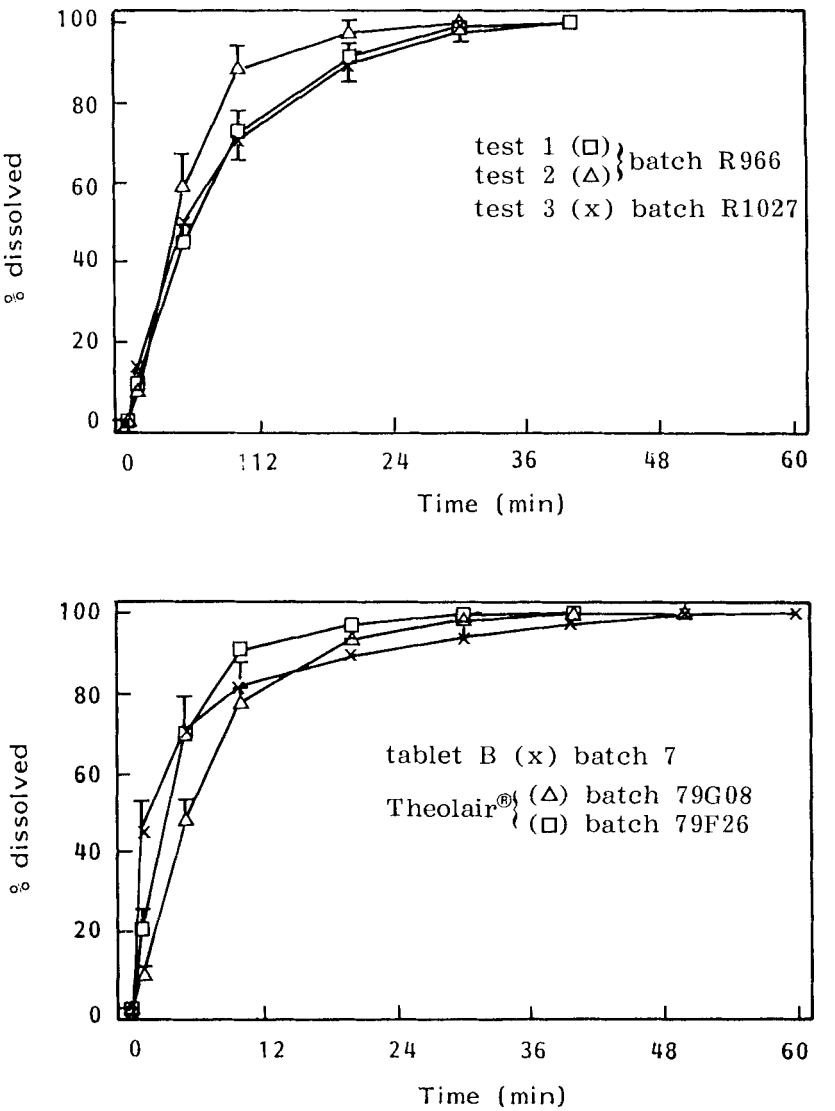

FIGURE 3

Dissolution profiles of theophylline tablets. Reproducibility of tablet A characteristics (top); comparison of Theolair® and tablet B (bottom).

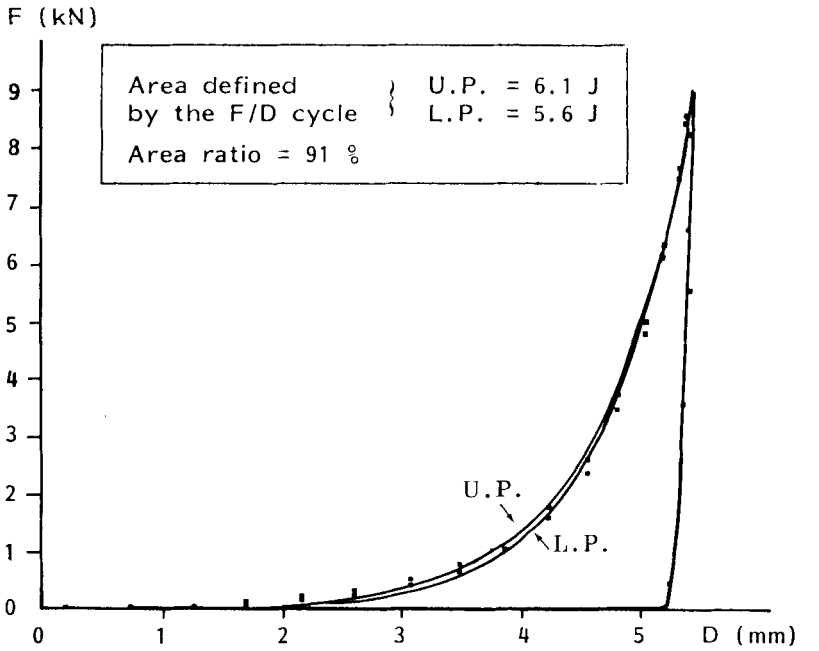

FIGURE 4

Force (F) applied on powder by the upper punch (U.P.) and stress transmitted through the powder to the lower punch (L.P.) as a function of the displacement of the upper punch (D).

TABLE 2

Effect of theophylline particle size on the properties of the tablets.

	Theophylline raw materials		
	commercial quality tablets A	selected quality tablets B	micronized quality tablets C
Compression force kN	8.8	6.8	6.7
Hardness daN ± S.D.	14.0 ± 0.6	11.9 ± 0.6	12.5 ± 0.7
Friability %	0.8	0.5	0.3
Mean weight ± S.D.	401.7 ± 3.4	403.3 ± 4.2	405.6 ± 3.1
Weight CV %	0.9	1.0	0.8
Disintegration time (sec)	30	28	40

TABLE 3

Pharmacokinetic parameters of theophylline from 3 dogs after single oral administration of an aqueous solution and 4 tablet formulations (250 mg theophylline) (mean ± S.D.)

Parameter	Aqueous solution	Tablet A (50 µm)	Tablet B (30 µm)	Tablet C (10 µm)	Theolair®
$t_{1/2}^{abs}$ (hr)	0.40 ± 0.47	0.73 ± 0.62	0.39 ± 0.25	0.52 ± 0.12	0.64 ± 0.30
t_{max} (hr)	1.7 ± 2.0	2.2 ± 1.6	1.8 ± 0.6	2.2 ± 0.7	2.8 ± 1.4
C_{max} (mg/l)	23.3 ± 9.6	18.4 ± 3.4	21.8 ± 1.2	16.6 ± 4.5	21.1 ± 0.7
$t_{1/2}$ β (hr)	6.8 ± 0.3	6.2 ± 2.2	6.2 ± 1.9	6.1 ± 1.4	5.7 ± 0.6
AUC_{∞} (mg.hr/l)	237 ± 75	163 ± 30	228 ± 66	181 ± 49	221 ± 67
F	--	0.71 ± 0.09	0.97 ± 0.16	0.77 ± 0.14	0.94 ± 0.19

tained are given in Table 3. The drug was rapidly absorbed after each administration, the half-life of absorption ($t_{1/2}$abs) was generally less than 1 hr and the time of the peak plasma concentration (t_{max}) occurred between 1 and 3 hr. Peak plasma concentrations ranged from 12.1 to 33.7 mg/l. The availability (F) of tablet B relative to the aqueous solution (0.97 $\pm$ 0.16) was superior to that of tablets A (0.71 $\pm$ 0.09) and C (0.77 $\pm$ 0.14) and was similar to that of Theolair® tablets (0.94 $\pm$ 0.19). The analysis of variance showed a significant difference between the AUC∞ values of the aqueous solution and tablet A ($p < 0.01$) or tablet C ($p < 0.05$). There was no significant difference between aqueous solution, tablet B and Theolair®.

No difference between formulations was found for the other pharmacokinetic parameters ($t_{1/2}$abs, t_{max}, C_{max}). Plasma elimination of theophylline showed half-lives ($t_{1/2}\beta$) of 6-7 hr for the five formulations tested. Westlake's test for the determination of the symmetrical confidence intervals (95% probability) where aqueous solution was chosen as the reference compound, showed a 21% confidence interval for tablet B, 23% for Theolair®, 47% and 39% for tablets A and C respectively. Confidence intervals near 20% or lower are generally accepted to establish the bioequivalence between two formulations. From these results it appears that aqueous solution, tablets B and Theolair® are bioequivalent, whilst the bioavailability of tablets A and C is lower.

Scale up

After completion of the "in vitro" and "in vivo" experiments, tablet B was chosen for the scale up study of the formulation. Firstly the influence of calcium phosphate, which is the major excipient (> 30%) of the formula was investigated. The characteristics of the formulation were not modified when four different batches of calcium phosphate were used as supplied from three different suppliers (table 4 and Fig. 5). The scale up study showed that for tablet B, granulation and compression characteristics were unaffected by the change in operating conditions during mixing which

TABLE 4

Effect of different batches of calcium phosphate (from different sources) on the properties of the tablets.

	Supplier A		Supplier B	Supplier C
	type A1	type A2		
Compression force kN	8.8	9.4	8.8	9.0
Hardness daN ± S.D.	15.5 ± 0.8	14.7 ± 0.6	15.8 ± 0.8	14.4 ± 0.5
Friability %	0.9	0.6	0.5	0.6
Mean weight ± S.D.	398.9 ± 3.6	402.0 ± 4.3	392.2 ± 4.6	400.9 ± 2.6
Weight CV %	0.9	1.1	1.2	0.7
Disintegration time (sec)	33	45	31	32

TABLE 5

Effect of the type and size of mixer on the properties of the tablets.

	Z arm mixer			Lödige mixer	
	1 l	5 l	30 l	50 l	130 l
Compression force kN	6.6	6.7	7.2	8.8	6.8
Hardness daN ± S.D.	12.4 ± 0.9	12.3 ± 0.7	13.2 ± 0.5	14.0 ± 0.6	13.7 ± 1.1
Friability %	0.5	0.5	0.5	0.8	0.9
Mean weight ± S.D.	405.2 ± 4.4	401.2 ± 2.2	402.0 ± 2.6	401.7 ± 3.4	404.5 ± 5.0
Weight CV %	1.1	0.5	0.6	0.9	1.2
Disintegration time (sec)	32	25	27	30	20

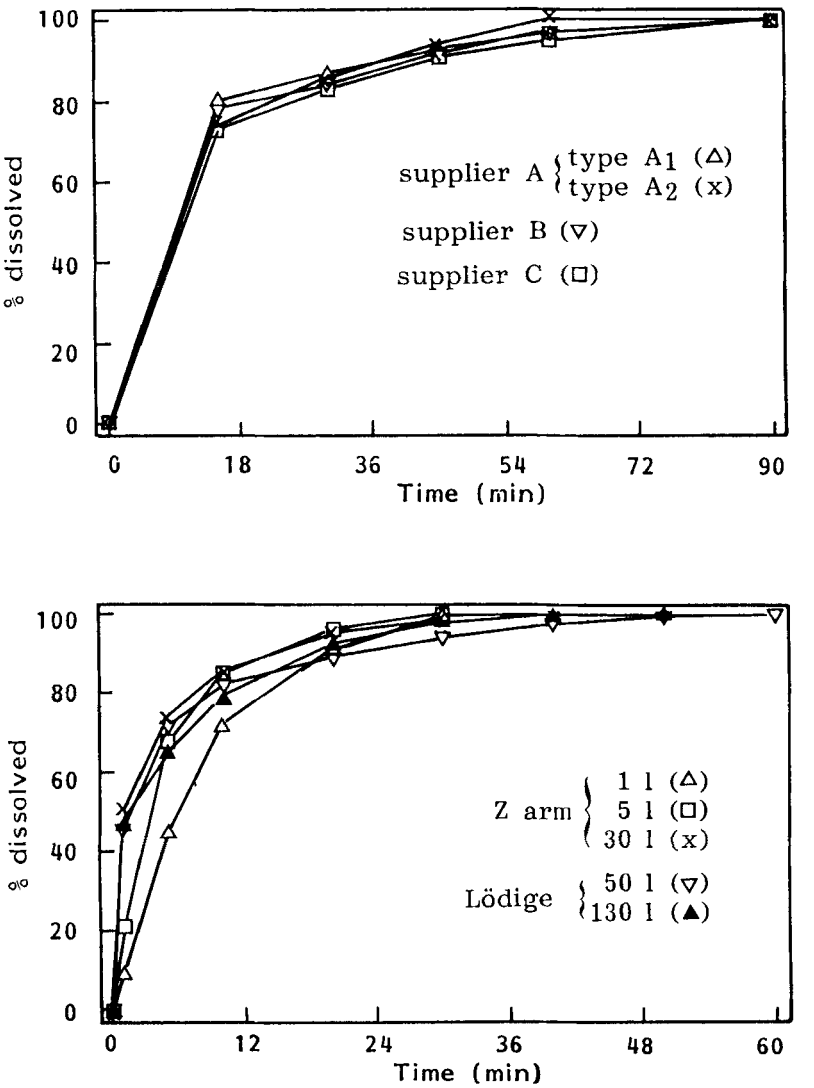

FIGURE 5

Effect of calcium phosphate (top) and mixer type (bottom) on dissolution profiles of tablets B.

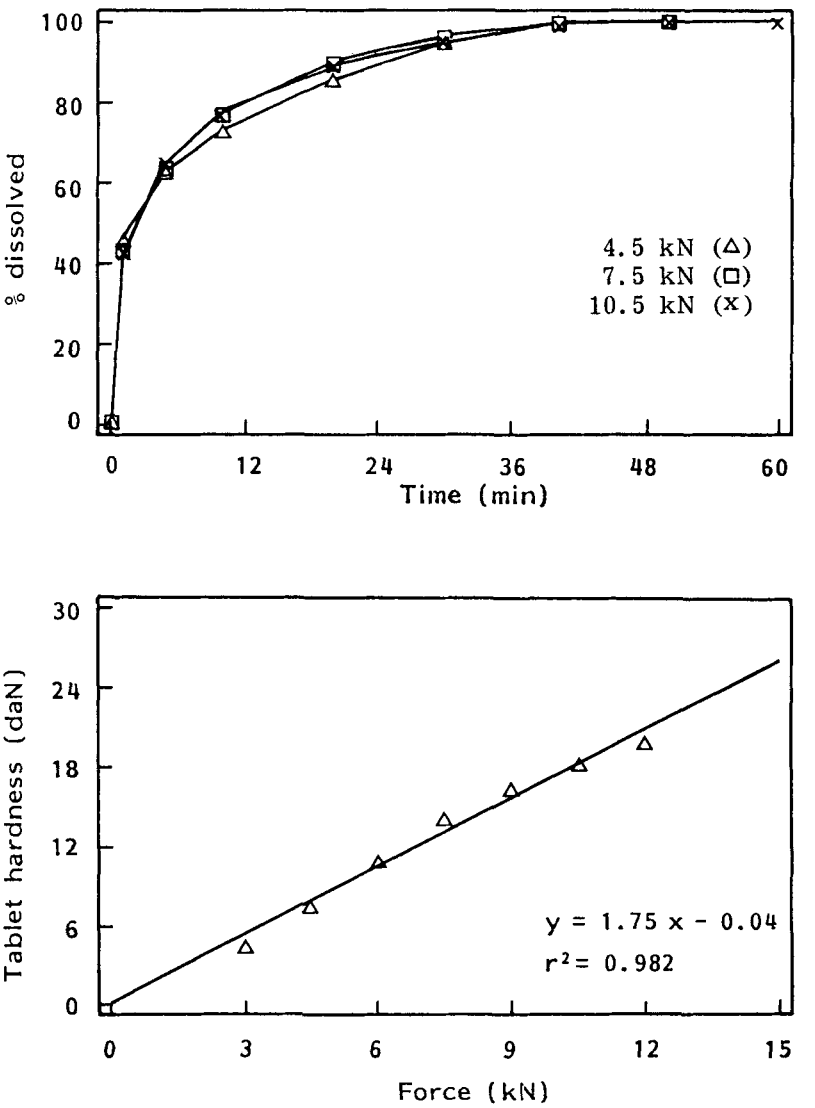

FIGURE 6

Effect of compression force on dissolution profiles (top) and tablet hardness (bottom).

were dictated by charges in size of the mixer used : Z arm mixers of 1, 5 and 30 liters, or Lödige mixers of 50 and 130 liters respectively. Table 5 and figure 5 show the results obtained for the characteristics of the granulated powders prepared, together with their dissolution kinetics. Powder tabletting was carried out under the same conditions using a rotating machine. Tablet hardness and dissolution kinetics were measured as a function of the compression force applied by the tabletting machine. The compression force was increased by steps of 1.5 kN, from 3 to 12 kN (a 7.5 kN value has previously been considered satisfactory for this formulation). In this range, tablet hardness increased linearly with the compression force, the relationship being expressed in the following equation obtained by linear regression :

$$y = 1.75\ x - 0.04 \quad (r^2 = 0.982,\ p < 0.01)$$

Dissolution kinetics remained unchanged for tablet compression force values between 4.5 and 10.5 kN (Fig. 6). It is important to underline that this formulation requires a relatively weak compression force for the preparation of the tablets (6.5 to 8.5 kN) which avoids a high consumption of energy when working under extreme conditions.

CONCLUSION

The first requirement for a new formulation is to make the drug completely available at the site of absorption. This will facilitate constant absorption of the drug and thereby provide a high systemic availability. When the absorption of the drug and not the release from the formulation represents the limiting step, the bioavailability of the formulation should be equivalent to that of an aqueous solution. In order to obtain a dissolution rate of theophylline higher than its rate of absorption, several parameters including particle size of raw materials, granulation, and tablet formula were investigated.

The studies on dissolution rate showed that the release of theophylline from tablet A (theophylline of commercial quality) and

tablet B (theophylline of selected particle size) was faster than from tablet C (micronized theophylline), whereas no difference was observed between tablets A and B. The "in vivo" study showed that only tablet B has the same bioavailability ($F = 0.97 \pm 0.16$) as an aqueous solution, whilst the bioavailability of tablet A ($F = 0.71 \pm 0.09$) and tablet C ($F = 0.77 \pm 0.14$) was lower than that of tablet B and the aqueous solution.

Therefore, "in vitro" experiments do not give an accurate prediction of the bioavailability of theophylline from a new formulation. Nevertheless, estimations from dissolution rate studies may enable the number of formulations tested to be reduced before proceeding to an "in vivo" evaluation.

The rapid and complete absorption of theophylline from tablet B and the bioequivalence with an aqueous solution have been confirmed in a study carried out in healthy adult subjects, after a single oral dose[8].

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