ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 49 (2000) 35–39

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

Dissolution rate limited bioavailability of flutamide, and in vitro – in vivo correlation

Juhani Posti*, Kirsi Katila, Timo Kostiainen

Leiras Oy, Pharmaceutical R and D, Turku, Finland

Received 20 March 1999; accepted 21 August 1999

Abstract

Flutamide is, due to its properties as a practically water-insoluble, high-dose drug substance a typical representative of a drug with particle size limited dissolution rate and bioavailability. An in vitro dissolution test method comprising of standard paddle stirrer, round-bottomed vessel of 4 l capacity, and 0.1 N hydrochloric acid with 0.5% of sodium lauryl sulphate as dissolution medium, was developed. The dissolution method was shown discriminatory and predictive regarding bioavailability of conventional 250 mg flutamide tablets with different in vitro dissolution rates. Relative bioavailability of flutamide from the tablets, determined as AUC of the active metabolite 2-hydroxyflutamide in healthy male subjects, could be correlated with drug amount dissolved during 45 min in the in vitro test. Also bioavailability data from four different biostudies could be compared utilising the calculated ratios R_{AUC} and $R_{Diss.}$ for the test and reference formulations in the individual studies. It is suggested that the concept of R_{AUC} and $R_{Diss.}$ can also be applied to other orally administered, poorly soluble, high-dose drug substances with dissolution rate limited bioavailability.

Keywords: Flutamide; Dissolution rate; bioavailability; In vitro – in vivo correlation

1. Introduction

Flutamide (2-methyl-*N*-[4–nitro–3–(trifluoromethyl)phenyl]propanamide) is a non-steroidal compound with anti-androgenic properties. It is used in the palliative treatment of prostatic carcinoma. The usual oral dose is 250 mg three times daily. Following oral administration, flutamide is reported to be rapidly and completely absorbed with peak plasma concentrations occurring 1 h after a dose. It is rapidly and extensively metabolised; the major metabolite (2-hydroxyflutamide also called '*D*-flutamide' in literature) possesses anti-androgenic properties. The half-life of the metabolite is about 6 h [1].

Flutamide occurs as a pale yellow, crystalline powder of acicular particle shape. It is practically insoluble in water in the pH range of 1–6 but is freely soluble in methanol and ethanol [2]. Due to the poor solubility in water and the fairly high oral dosage, flutamide is a potential candidate for a drug substance with particle size limited dissolution rate and bioavailability. According to literature, drugs having limited solubility (below 1%) in the G-I fluids often exhibit poor or erratic absorption unless dosage forms are specifically tailored for the drug [3].

According to current EC guidelines, solid dosage forms should be tested for dissolution performance using one of the dissolution apparatuses described in the European Pharmacopoeia (EP). Justification for the use of a method other than EP must be put forward [4]. The United States Pharmacopeia (USP) suggests greater dissolution medium volumes up to 2000 ml to be allowed for drugs having limited solubility. The quantity of medium used should be not less than 3 times that required to form a saturated solution of the drug substance. Addition of surfactants to aid solubilisation of the drug must be balanced against giving excessive assistance at the cost of loss of the discriminatory power of the test; the use of hydroalcoholic media is generally not favoured [5]. According to the regulatory guideline effective in Germany, the dissolution medium must be free from surface active agents and organic solvents [6]. Rothe and Schellhorn have proposed the use of a standardised dissolution vessel allowing the use of medium volumes up to 4 l [7].

Based on determined solubility of flutamide in various aqueous and hydroalcoholic solvent systems, 0.1 N hydrochloric acid with 0.5% of sodium lauryl sulphate (SLS) was considered best suitable as the dissolution medium. Solubi-

* Corresponding author. Leiras Oy, P.O. Box 415, FIN-20101 Turku, Finland. Tel.: +358-2333-2607; fax: +358-2333-2570.

E-mail address: juhani.posti@leiras.fi (J. Posti)

0939-6411/00/$ - see front matter
PII: S0939-6411(99)00061-2

lity of flutamide in the chosen medium is about 1 g in 5.2 l, or 250 mg (corresponding to one tablet) in about 1.3 l (solubility at 25°C). Appropriate sink conditions during dissolution are thus maintained in the volume of 4 l, and a dissolution vessel of 4 l as proposed and described in [7] was chosen. Hydroalcoholic solutions were ruled out because the good solubility of flutamide in ethanol would very probably mask the effect of drug particle size on dissolution rate. Aqueous media with SLS have also been successfully used to study dissolution profiles of oral solid forms containing other sparingly water-soluble drug substances such as danazol, megestrol acetate, and prazosin HCl [8].

The aim of the present study was to find out whether drug particle size is one significant determinant in the dissolution rate of flutamide and to show, using the in vitro method developed and utilised at Leiras, that a quantitative correlation can be established between the dissolution rate in vitro, and bioavailability in vivo, of conventional flutamide tablets.

2. Materials and methods

2.1. Experimental flutamide tablet formulations

Flutamide synthesised at the Fine Chemical Plant of Leiras in Turku, Finland, was used in the test formulations prepared. Drug substance was used unmilled (particle size around 100–200 μm by microscopic examination) or milled to defined particle size (below 40 μm determined by laser diffraction method). Flutamide was formulated into conventional tablets, intended for immediate drug release and absorption, using conventional EP quality tablet excipients such as maize starch, microcrystalline cellulose, colloidal anhydrous silica, and magnesium stearate. All batches reported here were of the same qualitative and quantitative formulation (250 mg of flutamide and excipients up to a total tablet weight of about 780 mg). One laboratory batch was prepared using unmilled flutamide whereas milled drug substance was used for all other batches.

Disintegration times of the test formulations were typically about 5–10 min, independently of scale of preparation (Table 1).

2.2. Commercial reference tablets

Commercial lots of flutamide 250 mg tablets of the drug innovator (Schering-Plough, USA, marketed under trade names such as Drogenil®, Euflex®, Eulexin®, Flucinome® and Fugerel®), sampled from various markets, were used as reference in the comparative studies. For obtaining a reliable picture about the in vitro dissolution and bioavailability properties of the original product, several batches were collected from some markets. Technical determinations showed that tablets of the drug originator had same outer appearance and other general pharmaceutical properties irrespective of marketplace (Table 1). From the test results it is reasonable to conclude that original product of same or virtually same qualitative and quantitative composition is manufactured and distributed to the various markets.

2.3. In vitro dissolution test method

Dissolution medium: 4000 ml of 0.1 N hydrochloric acid with 0.5% sodium lauryl sulphate at 37 ± 0.5°C (Sodium lauryl sulfate NF; Purified water EP).

Apparatus: a cylindrical, round-bottomed glass vessel of 15.0 cm inner diameter immersed in a water bath at 37 ± 0.5°C. The dissolution medium was stirred with a paddle stirrer as described in EP third ed., 2.9.3. The paddle was positioned centrally, 2.5 cm from the bottom of the vessel.

Rotation speed: 50 rev./min.

General procedure: 10 ml samples of the dissolution medium were withdrawn at fixed time intervals, filtrated, and a 2.0 ml aliquot was diluted with pure dissolution medium and assayed by UV absorption at 295 nm for flutamide. The analytical method inclusive of stability of flutamide in test samples was validated. From the assay data, the cumulative amounts of flutamide dissolved were calculated. The results are presented as the average and range from three to six individual tablets, in percents from the declared flutamide content of the tablets.

2.4. Bioequivalence studies

Four separate bioequivalence studies were conducted. All studies were conducted at the Clinical Department of Leiras, in compliance with the principles laid down in the Nordic GCTP guidelines and the declaration of Helsinki, in 1964,

Table 1
General pharmaceutical characteristics of Flutamide 250 mg test and reference tablets

Tablet characteristics	Appearance	Average mass (mg)	Crushing strength N)[a]	Disintegration time[b]
Test tablets (Leiras)	Circular, yellowish tablets; diameter 12 mm; height ca 6 mm	ca. 780	ca 70–90	Ranging from ca. 4 to 13 min
Reference tablets (drug innovator)	Circular, pale yellow tablets with breakline; diameter ca 12.8 mm; height ca 5.5 - 5.7 mm	ca. 750	ca 90–110	ca. 10 min

[a] Determined as described in Ph. Eur. 3rd Ed. 2.9.8.
[b] Determined as described in Ph. Eur. 3rd Ed. 2.9.1 (in water).

as revised by the 29th World Medical Assembly, Tokyo, in 1975, and subsequent World Medical Assemblies. The studies were conducted on healthy male subjects. All studies were single-dose, cross-over studies with a nominal dose of 500 mg of flutamide (two tablets as a single dose), with a wash-out period of not less than 6 days between drug administrations. Blood samples were collected after 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 10, 12, 24 and 48 h following drug administration. The plasma was separated by centrifugation and stored frozen until analysis. Because absorbed flutamide is rapidly and extensively metabolised, only plasma levels of the pharmacologically active major metabolite 2-hydroxyflutamide (OH-flutamide) and of the other major metabolite 4-nitro-3-(trifluoromethyl)aniline (also called 'F-flutamine' in literature) were determined. However, only data for OH-flutamide were considered relevant and are reported in the present paper. For analysis, OH-flutamide was isolated from blood plasma by solvent extraction and quantitated by a specific HPLC method with UV detection using diazepam as internal standard. The bioanalytical method was developed and the analyses were conducted according to GLP requirements in the Bioanalytical Laboratory of Leiras. The analytical method was validated and showed the following performance characteristics: quantitation limit of OH-flutamide in plasma: 60 ng/ml; linear concentration range of determination: 60–3000 ng/ml; reproducibility of method determined as RSDs for peak height ratios of OH-flutamide and the internal standard: RSD = 4.5% at the concentration level 150 ng/ml and 0.8% at the level 1500 ng/ml. Calculated average peak concentrations of OH-flutamide in the biostudies ranged from about 1000 ng/ml to about 1400 ng/ml whereas individual peak concentration as high as about 3300 ng/ml (one observation only; all other peak concentrations were below 2200 ng/ml) or as low as about 300 ng/ml were observed. The validated range of determination of the analytical method may hence be considered appropriate for the studies.

The pharmacokinetic parameters $AUC_{0-24\ h}$ (in some cases AUC_{0-48h} = until last quantifiable level) by the trapezoidal method on untransformed data and C_{max} and t_{max} were calculated. The pharmacokinetic and statistical calculations were conducted by the Clinical Department of Leiras. For establishing an in vitro – in vivo correlation, only the mean AUC data for OH-flutamide were considered relevant and are reported here. The bioequivalence studies forming the basis of this report are summarised in Table 2.

2.5. Construction of an in vitro – in vivo correlation

The EC guideline for prolonged release oral preparations mentions three types of in vitro – in vivo correlations. Listed in order of decreasing predictive power these are: (a) comparison of the in vitro dissolution curve of the product with the in vivo dissolution curves generated by deconvolution of plasma level data; (b) comparison of the mean in vitro dissolution time of the product to either the mean in vivo residence time or the mean in vivo dissolution time derived by using the principles of statistical moment analysis; (c) comparison of the mean in vitro dissolution time to one mean pharmacokinetic parameter, e.g. t_{max} [9]. Similar correlation levels are also stated in the USP [5]. However, the flutamide tablets compared were formulated as 'immediate release' tablets and because the pharmacokinetic reference parameter were plasma levels of the OH-metabolite of the extensively metabolised parent drug substance (both first-pass and systemic metabolic processes are involved), none of the correlation methods proposed for oral prolonged release preparations appeared appropriate. Hence, mean AUCs for the OH-flutamide metabolite characterising the over-all relative bioavailability of the formulations were correlated with drug amounts dissolved (in percents from declared drug content) during 45 min in the in vitro test. The selected in vitro dissolution parameter is based on results of in vitro tests conducted during pharmaceutical development of the tablet formulations. It was also found that the dissolution profiles of all tablet formulations studied, inclusive of reference tablet batches, were smooth and continuous resembling that of a 1st-order release. Thus, the one-point results can be considered characteristic for the entire in vitro dissolution profile of the tablets concerned. Furthermore,

Table 2
Summary of bioequivalence studies[a]

Study Report no.	Tablet batch	AUC [SD] (μg/ml·h)	R_{AUC}	% dissolved at 45 min	R_{Diss}
1181 = Study I (n = 6)	T1 = 300189A, R1 = 8XCPA 10 (CA)	10.12 [3.2], 9.86 [2.8]	1.03	90, 86	1.05
1185 = Study II (n = 16)	T2 = 90198, R2 = 87J20 09 (DK)	12.63 [3.1], 8.77 [3.0]	1.44	79, 54	1.46
90533 = Study III (n = 24)	T3 = 890180, R3 = OXCPA 12 (CA)	11.12 [5.0], 7.96 [3.1]	1.40	73, 47	1.55
531–93525 = Study IV (n = 24)	T4 = 2C971/II, R4 = 93I10 19 (DE)	8.91 [4.0], 7.45 [3.2]	1.20	75, 70	1.07

[a] The mean AUCs refer to the active metabolite OH-flutamide following administration of single doses of 500 mg flutamide (=2 tablets with 250 mg flutamide). n, number of subjects included to the biostudy; T1 to T4, test batches manufactured by Leiras; R1 to R4, commercial reference batches collected from several markets: CA, Canada, DK, Denmark, DE, Germany; R_{AUC}, Ratio of mean AUC values Test preparation/Reference (T/R); R_{Diss}, Ratio of in vitro dissolution values T/R.

also the regulatory in vitro dissolution requirement for Flutamide 250 mg tablets of Leiras is based on drug amount dissolved during 45 min in the dissolution test.

Because different subject groups were used in the bioequivalence studies and because the studies were conducted and analysed at different time points, the resulting AUC values may show certain from study-to-study variability even for tablet batches with same dissolution characteristics and basically same relative bioavailability. To compensate for this variation, ratios of average AUC values 'test formulation vs. reference tablets' were calculated separately for each study. Similarly, the ratios of percentages dissolved for the same batches in the dissolution test were also calculated (Table 2).

3. Results and discussion

3.1. Effect of drug particle size on dissolution rate

Laboratory trial batches 300189A and 300189B had the same composition but were prepared using unmilled, coarse flutamide (batch 300189B showing ca 45% dissolution at 45 min) or finely milled drug substance (batch 300189A with ca. 90% dissolution during the same time). The latter batch was also included in a bioequivalence study (Table 2). It is concluded that particle size of this poorly soluble drug substance is important for its in vitro dissolution rate and, consequently, all further test tablet batches were prepared using milled flutamide only.

3.2. Characterisation of flutamide tablets studied

The formulations studied were all found to have very similar general pharmaceutical-technical properties, as reported in Table 1. Also the dissolution rates of the production batches of Leiras were consistently about 73% to 79% in 45 min (Table 2). Only the laboratory batch 300189A showed somewhat faster dissolution. It may hence be further concluded that the production batches showed consistent, reproducible dissolution rate but somewhat slower than the corresponding laboratory scale batch; such an observation is not uncommon in pharmaceutical industry.

Dissolution data for a number of flutamide 250 mg tablet batches of the drug innovator are presented in Table 3. The dissolution results are presented in a chronological order according to year of manufacture of the batches. The batches studied cover the time span from 1987 to 1993 (or 1994), as may be concluded from the batch numbers '87…' to '93…' (and '21' and '28').

In contrast to the generic test tablet batches quoted above, the reference tablets collected from various markets showed marked variability in their dissolution rates. This variability suggests that drug particle size or some other significant formulation variables were not adequately controlled at the manufacturer. The results also suggest that the variability is not distributed according to marketplaces, because batches showing good but also poor dissolution performance could be found from the same marketplace. The results suggest rather that there is a dependence between dissolution performance and time: batches manufactured during the same time period tended to have similar dissolution rates irrespective of marketplace.

Table 3
Drug released from drug innovator's Flutamide 250 mg tablets sampled from various markets[a]

Product/country	Batch no.	% Released in 45 min. (range)
Eulexin/Denmark	87G08 04	65 (62–70)
Eulexin/Denmark	87J20 09	54 (52–57)
Euflex/Canada	8XCPA 10	86 (85–88)
Eulexin/Denmark	88J28 14	77 (72–80)
Flucinome/Switzerland	88D27 05	94 (93–95)
Fugerel/Germany	89K09 10	51 (48–52)
Euflex/Canada	0XCPA 11	47 (44–51)
Euflex/Canada	0XCPA 12	47 (44–50)
Euflex/Canada	1XCPA 16	65 (62–72)
Flucinome/Switzerland	91A08 02	68 (63–73)
Drogenil/United Kingdom	92K04 17	66 (62–74)
Eulexin/Denmark	92I07 13	63 (61–64)
Eulexin/Finland	92K05 18	67 (63–73)
Fugerel/Germany	92L14 21	56 (54–59)
Drogenil/United Kingdom	93J22 21	62 (61–63)
Euflex/Canada	3XCPA 07	61 (59–63)
Flucinome/Switzerland	93A27 01	57 (53–62)
Flucinome/Switzerland	93B25 04	62 (61–64)
Flucinome/Switzerland	93C10 05	65 (64–65)
Eulexin/Italy	9	66 (63–69)
Flucinome/Switzerland	93F08 11	65 (63–67)
Flucinome/Switzerland	93I10 19	70 (67–74)
Fugerel/Germany	93I10 19	70 (67–72)
Eulexin/Italy	21	73 (72–75)
Eulexin/Italy	28	73 (69–80)

[a] Flutamide release from tablets was determined according to the standard method of Leiras, using 0.1 N HCl with 0.5% of sodium lauryl sulphate as the dissolution medium. The percentages released refer to the declared flutamide content of the tablets (250 mg). Three to six tablets were tested per batch.

Similar results have also been reported in a survey study on immediate release propafenon 300 mg tablets (Rytmonorm®) on the market in Germany but manufactured by a number of different manufacturers in several European countries. Significant differences were found in the in vitro dissolution rates of tablets of different manufacturers, and tablets showing the fastest and slowest dissolution rate were also found not be bioequivalent concerning the active main metabolite (5-hydroxypropafenon) [10,11].

3.3. Bioavailability data and in vitro – in vivo correlation

The AUC and in vitro dissolution data for the tablet batches compared in the four bioequivalence studies are presented in Table 2. The AUC and dissolution data within individual studies were correlated by calculating the ratios 'AUC (test)'/'AUC (reference)' = R_{AUC} and 'amount dissolved in 45 min (test)'/'amount dissolved in 45 min

(reference)' = R_{Diss}, respectively. The numerical values of the ratios R_{AUC} and $R_{Diss.}$ for the individual studies were fairly close to each other suggesting that there is a correlation between in vitro dissolution rates and, on the other hand, relative bioavailability of the active metabolite OH-flutamide within one particular study. So e.g. the calculated R_{AUC} ratio is 1.03 in Study I and the corresponding $R_{Diss.}$ ratio is 1.05. For Study II the ratios were 1.44. and 1.46, respectively. A less satisfactory relationship was found in Study IV where the dissolution data ($R_{Diss} = 1.07$) suggested 'good' bioequivalence of the batches compared, whereas the true bioequivalence calculations ($R_{AUC} = 1.20$ and also other relevant pharmacokinetic parameters not reported here) showed that bioavailability of the test preparation was close to the upper confidence limit of formal bioequivalence conclusion. However, the established correlations are overall very satisfactory considering the span of dissolution performance of tablet batches studied: the dissolution rates ranged from 47 to 90% dissolved in 45 min. This finding of equal, predictive Diss. and AUC ratios within individual studies constitutes the core result of the present report.

As a concluding statement it should be mentioned that tablet batches compared in Study I as well as in Study IV (as explained above) were found to be bioequivalent. Batches compared in Studies II and III were found non-bioequivalent, the reference tablets showing inferior dissolution performance and bioavailability compared to the test tablets.

4. Conclusions

1. A discriminatory in vitro dissolution test method for conventional immediate-release flutamide 250 mg tablets has been developed. The discriminatory power of the method has been established in a number of comparative bioequivalence studies with reference tablet batches showing variable in vitro dissolution performance and, consequently, different relative bioavailabilities.
2. A quantitative correlation could be established between the in vitro dissolution rate (here, the percentage released after 45 min) and relative bioavailability (AUC for the active metabolite OH-flutamide). The ratios of the dissolution percentages and mean AUCs showed generally very similar numerical values for the individual studies. The ratios R_{AUC} and R_{Diss} as used in this study also allow the comparison of bioavailability data obtained from different biostudies. It should also be possible to extend this concept of in vitro – in vivo correlation to other, poorly soluble high-dose drug substances formulated into conventional immediate-release solid oral dosage forms showing dissolution rate limited bioavailability.
3. The pharmaceutical examinations suggest that original flutamide 250 mg tablets of the same outer appearance and the same general pharmaceutical properties are distributed globally. However, marked batch-to-batch variability in the in vitro dissolution performance of the tablets was evident, irrespective of the marketplace. The results hence also suggest differences between batches in their relative bioavailability and, most probably, clinical safety and efficacy.
4. Based on the results discussed above, a manufacturer aiming to perform a bioavailability comparison with the original reference product is facing a problem so far not discussed in scientific literature: how to select a batch of the reference product if this, as may be concluded from the experimental data presented in this report, shows marked batch-to-batch variability and, as may be further concluded, is suggested to be non-bioequivalent within the brand itself! This kind of a rarely occurring situation may present a problem to the generic manufacturer aiming to show bioequivalence of its product with the original reference, and also to the regulatory authorities.

References

[1] K. Parfitt, Martindale, Complete Drug Reference, 32nd ed. Pharmaceutical Press, London, 1999, p. 537.
[2] Information from the Pharmaceutical Development Laboratory of Leiras, Turku, Finland.
[3] E.F. Fiese, T.A. Hagen, Preformulation, in: L. Lachman, H.A. Lieberman, J.L. Kanig (Eds.), The Theory and Practice of Industrial Pharmacy, third ed., Lea and Febiger, Philadelphia, 1986, pp. 185.
[4] Section 3AQ1a Development pharmaceutics and process validation, Volume 3A: Guidelines, Medicinal products for human use, Quality and biotechnology. European Commission (1998 Edition).
[5] Chapter < 1088 > In vitro and in vivo evaluation of dosage forms, The United States Pharmacopeia 23, 1995, pp. 1924-1929.
[6] Erläuterungen des BGA zum Antrag auf Zulassung eines Arzneimittels, 2. Auflage, Bundesgesundheitsamt, 1988, p. 31.
[7] W. Rothe, J. Schellhorn, Bestimmung der Auflösungsrate für das Europäische Arzneibuch, Pharm. Ind. 41 (1979) 785–789.
[8] V.P. Shah, et al., In vitro dissolution of sparingly water-soluble drug dosage forms, Int. J. Pharm. 125 (1995) 99–106.
[9] Section 3AQ19a Quality of prolonged release oral solid dosage forms, Volume 3A: Guidelines, Medicinal products for human use, Efficacy. European Commission (1998 Edition).
[10] H. Blume, J. Krämer, B. Böing, Zur pharmazeutischen Qualität von Rytmonorm-300-Importpräparaten, Pharm. Ztg. 139 (1994) 908–912.
[11] H. Blume, U. Gundert-Remy, B. Schug, M. Elze, A. Staab, Bioverfügbarkeit von Rytmonorm-Importen, Pharm. Ztg. 141 (1996) 4475–4489.