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Sustained-release multiparticulate formulations of Zileuton. I. In vitro and in vivo evaluation

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Abstract

Zileuton is an active 5-lipoxygenase inhibitor which has been shown to be useful in the treatment of asthma. However, it requires dosing four times per day. In order to investigate the feasibility of developing sustained-release formulations of zileuton, two multiparticulate matrix formulations (A and B) with different in vitro release rates were prepared using extrusion/spheronization techniques. Oral absorption from the two formulations was compared with a solution formulation in a three-way crossover study using fed beagle dogs. The results indicated sustained release of zileuton from both multiparticulate formulations with prolonged T_{max} (4.72 h for formulation A and 3.67 h for formulation B vs. 1.21 h for the oral solution) and decreased C_{max} (1.21 μg/ml for formulation A and 4.14 μg/ml for formulation B vs. 9.07 μg/ml for the oral solution). Based on 90% confidence intervals, both matrix formulations were less bioavailable than the solution in terms of the extent of absorption. The estimates of relative bioavailability were $15 \pm 3\%$ and $48 \pm 7\%$ for formulation A and B, respectively. It is possible that these formulations may offer better bioavailability in humans due to inherent physiological differences between humans and animals. In vivo drug release from the sustained-release matrix formulations in dogs was estimated by deconvolution based on data of both formulations and correlated well with in vitro dissolution data.

Keywords: In vitro/in vivo correlation; In vitro release; Multiparticulate; Relative bioavailability; Sustained-release; Zileuton

1. Introduction

5-Lipoxygenase is an enzyme which converts arachidonic acid to 5-hydroperoxy-eicosa-6,8,11, 14-tetraenoic acid (5-HPETE) in the pathway leading to the production of leukotrienes. Inhi-

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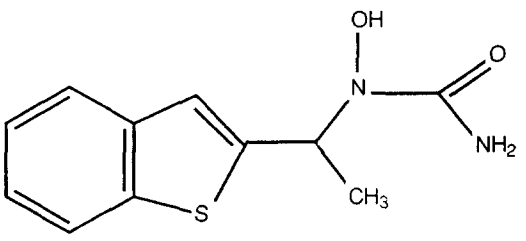
bition of leukotriene synthesis has many potential therapeutic benefits for conditions in which leukotriene synthesis is elevated. Zileuton, N-(1-(benzo[b]-thein-2-yl)ethyl)-N-hydroxyurea (Scheme 1), is a 5-lipoxygenase inhibitor which has been shown to be a potent inhibitor of leukotrienes for alleviating airway constriction in asthma (Hui et al., 1991). The compound is very slightly soluble in aqueous medium (170 μg/ml) with no ionizable functional groups. It is expected to be highly permeable through gastrointestinal membranes due to its high lipophilicity (log PC = 2.1). However, this compound has a relatively short elimination half-life in humans (group mean estimates: 2.8–4.5 h for R- and 1.8–3.1 h for S-enantiomers), requiring a four-times daily dosing regimen (Braeckman et al., 1992).

The present study was undertaken to help in the development of a sustained-release formulation of zileuton with reduced dosing frequency and improved patient compliance. A multiparticulate dosage form was chosen for development because it offers the convenience of administration as a ready-to-use suspension (after suspending in a suitable media), sprinkle, and capsule. Due to the relatively low aqueous solubility of zileuton and high dose (up to 600 mg four-times a day), development of a matrix type of multiparticulate formulation was initiated for sustaining the release rate of the drug. This report summarizes the results of in vitro and in vivo studies on two prototype sustained-release multiparticulate formulations of zileuton. In vitro/in vivo results were correlated to facilitate further product development and/or optimization efforts.



Scheme 1. The structure of zileuton

2. Materials and methods

2.1. Materials and equipment

The following materials were used in the study: Zileuton, lot no. 65-337-AL and Abbott-66649 (internal standard), lot no. 40-036-AL (Pharmaceutical Products Division, Abbott Laboratories). All other chemicals and reagents were either AR or HPLC grades and used as received. A Hobart mixer, a LUMA marumarizer (model Q5-400) and a LUMA extruder (model EXDCS-100) were used for preparation of multiparticulate formulations. A Spectra Physics HPLC pump (model SP8800), a Hitachi auto sampler (model 655A-40), a ABI Spectroflow 783 programmable absorbance detector and a ABI integrator (model 429A) were used for analytical assay of the samples.

2.2. Formulations

Two multiparticulate matrix formulations, A and B, containing 72–78% zileuton were prepared by extrusion/spheronization. The final dosage forms consisted of spherical beads in the size range of 0.42–0.59 mm in diameter filled in a hard gelatin capsule. The release rate was slowed by embedding the drug in insoluble and/or erodible, pH-insensitive matrices. The reference oral product, C, was a 2.5 mg/ml solution of zileuton containing water/ethanol/propylene glycol (4:3:3, v/v/v) as a solvent.

2.3. In vitro release

The in vitro release rates of zileuton from the multiparticulate formulations were investigated using the USP apparatus II, i.e. paddle method. A dissolution medium consisting of 900 ml of 50 mM sodium dodecyl sulfate (SDS) solution, pH 7.5, was used and a temperature of 37 $\pm$ 0.5°C was maintained. Initial results at 50 rpm demonstrated incomplete drug release; hence, in the subsequent testing, the paddle rotation speed was maintained at 100 rpm. SDS was used in the dissolution medium in order to maintain sink

conditions during the release testing. Dissolution samples (5 ml) were withdrawn at predetermined time intervals up to 6 h, and replaced with an equal volume of the fresh medium to maintain the volume constant. Samples were assayed spectrophotometrically at $\lambda = 260$ nm.

2.4. In vivo studies

Nine beagle dogs of either sex, weighing 9.0–13.6 kg, were used for the study. Earlier studies indicated improved absorption of zileuton in the fed state. In addition, fed state was anticipated to provide slightly longer GI residence time. Hence, all dogs were fasted overnight and then fed with a half can of dog food before dosing. A randomized crossover design with 1-week dosing intervals was used to test the formulations. Drug was orally administered at a dose of 100 mg with 40 ml of water. Water was provided ad libitum throughout the study. Serial blood samples were collected from a jugular vein for up to 24 h after single dosing. Plasma samples were immediately frozen at -20°C until assayed for drug concentration by a validated HPLC method briefly described below.

2.5. Sample processing and analytical procedure

2.5.1. Liquid-liquid extraction

For liquid-liquid extraction 0.5 ml of plasma sample was combined with 50 μl of the internal standard solution (2 μg/ml Abbott-66649 in 0.2M phosphate buffer, pH 7.2) and then mixed with 6 ml of methylene chloride:ethanol (9:1). The mixture was shaken at low speed for about 5 min and centrifuged at 2500 rpm for 5 min. Then the aqueous layer was aspirated to waste and 3.5 ml of the organic phase was transferred to a test-tube. Thereafter, the sample was evaporated to dryness with a gentle stream of dry air over low heat (< 40°C) and reconstituted with 0.3 ml of methanol:water (1:1) for HPLC analysis.

2.5.2. HPLC method

A reversed-phase HPLC assay was used to determine the total concentration of zileuton in the plasma. A Waters Nova-Pak® C-18 column (150×3.9, 4 μm) and a Guard-Pak® precolumn insert were used for the assay. Buffer:acetonitrile:tetrahydrofuran:methanol (14:3:2:1, v/v/v/v) at a flow rate of 1.0 ml/min was used as the mobile phase. The buffer contained 40 mM NaH_2PO_4, 7.5 mM H_3PO_4 and 5.0 mM NAHA (acetohydroxamic acid). The UV detection wavelength was set at 260 nm. The limit of quantitation and detection were 0.2 and 0.05 μg/ml, respectively. The method was validated per internal guidelines for sensitivity, specificity and reproducibility prior to implementation. In order to minimize the day-to-day variability, a calibration curve was constructed everyday when the samples were assayed.

2.6. Data analysis

The area under the blood concentration-time curve (AUC) from time zero to the last sampling time point 't' (AUC_t) was calculated by the trapezoidal rule. The AUC's were normalized on the basis of dog weights. Peak drug concentration (C_{max}) and the time to peak drug concentration (T_{max}) were obtained directly from the data without interpolation. Analysis of variance (ANOVA) was carried out on the parameter AUC using JMP 2.0.5 (SAS Institute, Cary, NC). Formulation, subjects, sequence and time period were included as the sources of variation in the model. Bioequivalence between the test and reference formulations was assessed by a two one-sided test procedure via 90% confidence intervals (Westlake) from analysis of AUC values.

3. Results and discussion

3.1. In vitro release

The release of a dispersed drug from an insoluble and/or erodible matrix system is controlled by relative contributions of surface erosion and diffusion mechanisms. The fractional release (F) can generally be related to time according to the equation: $M_t/M_\infty = kt^n$, where 'k' is a constant that incorporates characteristics of the matrix, the drug and the geometry of the dosage form

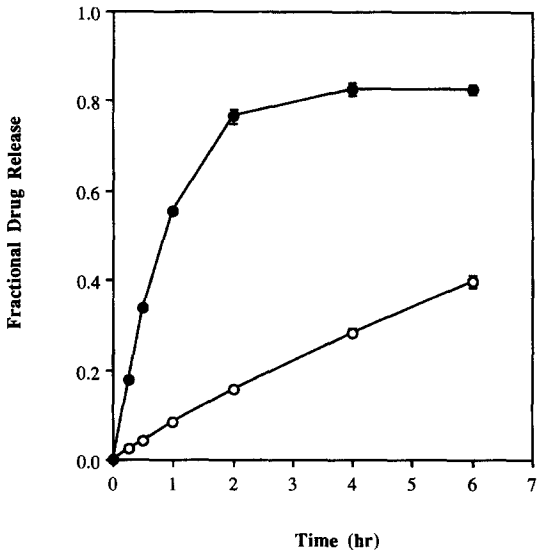


Fig. 1. In vitro release of zileuton from sustained-release multiparticulate formulation A (○) and multiparticulate formulation B (●) using USP apparatus II, 100 rpm. Bar represent S.D. of six experiments.

(Hogan, 1989). Depending upon the predominant release mechanisms, the drug release profiles generally fit well to $F = kt^n$ where $n = 0.5-1.0$. The in vitro drug release profiles from the two sustained-release formulations tested in this study are shown in Fig. 1. About 16% of the drug was released in vitro from formulation A in 2 h as opposed to 76% release from formulation B over the same time period. In 6 h, the formulation A released only about 40% drug, and as displayed in the figure, the release appeared to follow zero-order kinetics. Indeed, the visual observation of the particles from formulation A in dissolution media indicated slow matrix erosion and mass loss. In addition, no burst drug release was observed with this formulation. These observations are indicative of matrix erosion as the main mechanism of drug release from formulation A. On the other hand, the drug release profile from formulation B was better described by a square-root-of-time relationship, suggesting diffusion as the primary release mechanism (Table 1). The difference in the drug release mechanisms is believed to be a result of different rate controlling materials used in matrix formulations A and B. Since a limited number

Table 1
Comparison of the in vitro release rates of zileuton from two formulations

Formulation[a]	Zero-order model		Square-root-of-time model	
	K_o (%/h)	R^2	K (%$\sqrt{}$/h)	R^2
A	6.5	0.997	19.3	0.985
B	32.1	0.946	64.6	0.991

[a]Formulation identification: A, slower-release multiparticulate formulation; B, faster-release multiparticulate formulation.

of data points were available for model fitting in this study, no attempt was made to further investigate the drug release mechanisms.

3.2. In vivo absorption

The plasma concentration profiles of zileuton following single dose administration of the three formulations to beagle dogs are shown in Fig. 2. The mean bioavailability parameter values for the three treatments are summarized in Table 2. For formulations A and B, the estimates of bioavailability relative to C were $16 \pm 3\%$ and $48 \pm 7\%$, respectively. Both multiparticulate formulations demonstrated prolonged drug absorption with extended T_{max} (4.72 h for formulation A and 3.67 h for formulation B vs. 1.21 h for

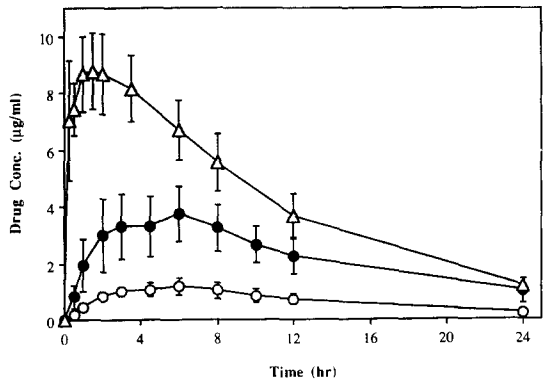


Fig. 2. Mean plasma concentration-time profiles after a single oral dose of zileuton multiparticulate formulation A (○), multiparticulate formulation B (●), and a solution formulation (△) to Beagle Dogs. Bar represents S.D. of nine dogs.

Table 2
Average bioavailability parameters after a single oral dose of zileuton formulations to dogs ($n = 9$)

Parameter (units)	AUC[b] (h* μg/ml)			C_{max} (μg/ml)			T_{max} (h)		
Formulation[a]	A	B	C	A	B	C	A	B	C
Mean	19.06	62.31	130.42	1.21	4.14	9.07	4.72	3.67	1.21
SD	4.56	11.14	14.28	0.28	0.79	1.34	1.64	1.80	0.76
CV	0.24	0.18	0.11	0.23	0.19	0.15	0.35	0.49	0.62

[a]Formulation identification: A, slower-release multiparticulate formulation; B, faster-release multiparticulate formulation; C, oral solution.
[b]AUC through 24 h dosing, normalized to 10 mg/kg dog body weight.

formulation C) and decreased C_{max} (1.21 μg/ml for formulation A and 4.14 μg/ml for formulation B vs. 9.07 μg/ml for formulation C). The analysis of variance on the AUC indicated significant effects of individual subject ($P = 0.031$) and formulation ($P = 0.001$) while the effects of sequence and period were found to be insignificant. Based on classical t-based 90% confidence interval with an acceptable range of $\pm 20\%$, both multiparticulate matrix formulations were found to be less bioavailable than the reference formulation in terms of the extent of drug absorption.

Results from animal models can help design and guide preliminary development of sustained-release dosage forms. However, differences between human and animal anatomy and physiology, as well as variances in biological and physicochemical properties of compounds in human and animal gastrointestinal tract, can often have appreciable impact on data interpretation (Dressman and Yamada, 1991). For example, due to the shorter intestinal residence time and/or altered metabolism, sustained-release dosage forms have been shown to yield incomplete drug absorption in dogs (Dressman and Yamada, 1991). Relatively short residence time in the dog GI tract could be responsible for significantly lower relative drug bioavailability from two multiparticulate formulations tested in this study. Other factors contributing to this observation may include incomplete drug release from dosage form (40–80% in 24 h, in vitro), either due to formulation characteristics and/or low fluid volume for dissolution in lower GI tract, and decreased absorption in lower GI tract due to comparatively smaller surface area. While the rank order of the rate of drug absorption among formulations may be similar between dogs and humans, it is quite possible that sustained-release formulations exhibiting low relative bioavailability in dogs may demonstrate higher bioavailability in humans. Indeed, this phenomenon has been observed with other sustained-release zileuton formulations (data on file, Abbott Laboratories). Therefore, the dog model may be more useful in estimating control of the rate of drug absorption rather than the extent of drug absorption from sustained release formulations.

In order to evaluate the rate of in vivo release from the two multiparticulate formulations tested in this study, deconvolution based on linear system analysis was carried out. The latter is a model independent method useful in the initial design of sustained-release formulations and evaluation of drug absorption processes (Cutler, 1978). Plasma concentration data obtained following oral administration of the solution were fitted to polyexponentials (RSTRIP, Micromath®, Salt Lake City) and used as the unit impulse response. Drug plasma data for the two sustained-release multiparticulate formulations were then deconvoluted using the Program PCDCON (W.R. Gillespie, FDA) to estimate in vivo drug release. The rank order of in vivo release rate of the drug was found to be comparable to the in vitro drug release profiles. However, the release rate of the drug in vitro was found to be higher than the in vivo release rate (see Fig. 3). This observation could be attributed, in part, to higher hydrodynamic flow in vitro compared to the conditions of low hydrodynamic flow in the GI tract (Katori et al., 1995).

It should be noted that the effect of hydrodynamic conditions on drug release can be more pronounced on formulations with large surface area, such as particles tested in this study, as opposed to that on a single unit of a non-disintegrating tablet. Higher in vitro drug release observed in this study could also be a result of the use of sodium dodecyl sulfate in the dissolution medium which may potentially interact with certain formulation components. The 'GI relative bioavailability' of both multiparticulate formulations, as estimated from the plateau values of the respective drug release profiles in vivo, was found to closely match the relative bioavailability estimated based on AUC's, i.e. 16% vs. 16% for formulation A, and 49% vs. 48% for formulation B (see Fig. 3).

3.3. *In vitro/in vivo correlation*

Exploration and development of correlation between in vitro drug release and in vivo drug release/absorption are important during the development of a sustained-release dosage form. An established in vitro/in vivo correlation can be useful in final development and optimization of sustained release formulations (Leeson, 1995; Shah and Lesko, 1995). In this study, deconvolution results were used to establish a correlation between in vitro and in vivo drug release profiles (see Fig. 4). For the two multiparticulate sustained-release formulations tested in this study using USP apparatus II at 100 rpm, the correlation between in vivo drug release and in vitro dissolution could be described using the following linear relationship ($R^2 = 0.994$):

$$F_{\text{in vivo}} = 0.412 * F_{\text{in vitro}} + 0.021 \quad (1)$$

where $F_{\text{in vivo}}$ and $F_{\text{in vitro}}$ are fractional drug release in vivo and in vitro, respectively. The initial slight curvature observed in the data is a result of non-linear drug release observed in vivo for the formulation A. A good correlation between in vivo and in vitro drug release was obtained in the present study; however, a slope of less than one in the above equation suggests a higher in vitro drug release rate than that in vivo. Although the dissolution method used in this study was found to be effective in discriminating the release performance of formulations with minor changes in composition, size and other characteristics (data not included), the correlation described by Eq. (1) and displayed in Fig. 4 suggests a need for modification in this method so that the in vitro and in vivo drug release rates are comparable.

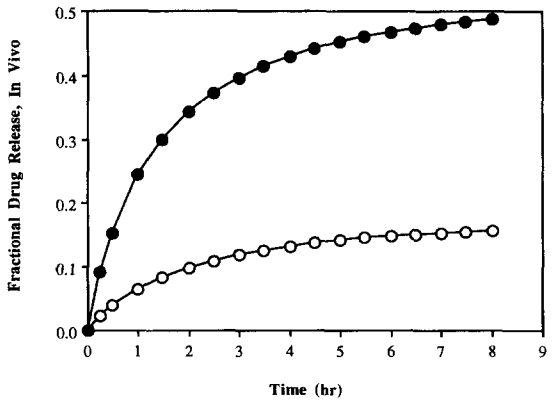


Fig. 3. In vivo drug release in dogs following administration of a single oral dose of multiparticulate formulation A (○) and multiparticulate formulation B (●) estimated by deconvolution.

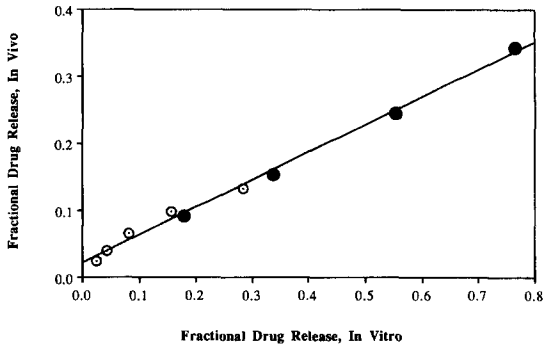


Fig. 4. In vitro/in vivo correlation based on pooled results from multiparticulate formulations A (○) and B (●).

4. Conclusions

The results of this study demonstrate that the release rate of zileuton can be controlled using

small multiparticulate matrix systems. The in vivo study in dogs demonstrated prolonged drug absorption from the two test formulations with good in vitro/in vivo correlation. However, the in vitro release method overestimated the in vivo release of drug from the matrix formulations. The results demonstrate potential utility of in vitro/in vivo correlation in the development of sustained-release multiparticulate formulations.

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