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PRELIMINARY PREFORMULATION INVESTIGATION OF TEBUFELONE, A NOVEL NON-STEROIDAL ANTI-INFLAMMATORY DRUG

G. R. Kelm[1,2] and A. A. Sakr[1]

[1]College of Pharmacy
University of Cincinnati Medical Center
Cincinnati, OH 45267
and
[2]Procter & Gamble Company
Miami Valley Laboratories
Cincinnati, OH 45329

ABSTRACT

A preliminary preformulation investigation of tebufelone, a novel member of the di-*tert*-butyl phenol class of non-steroidal anti-inflammatory compounds was conducted. Radiolabeled tebufelone was found to be very poorly water soluble (~1 μg/mL) with no significant pH effect upon solubility. It is considerably more soluble in both long and medium chain lipids

and very soluble in polar solvents such as ethanol. The calculated apparent log $P_{octanol/water}$ is approximately 5.5 Tebufelone has a melting point of 69°C and an enthalpy of fusion of 20 cal/g. No evidence of polymorphism was detected upon reheating or after precipitation from an ethanol solution. Dissolution of tebufelone in simulated intestinal fluid, USP (without pancreatin) with 2% bile salts (50% sodium cholate/50% sodium deoxycholate) failed to increase proportionally with calculated surface area at a mean particle size of 90 μm suggesting that the hydrophobicity of the drug precludes efficient wetting of the drug particle size is decreased to this level.

INTRODUCTION

Tebufelone is a novel member of the di-*tert*-butyl phenol class of anti-inflammatory compounds.[1] Chemically, it is 1-3, 5-Bis(1, 1-dimethylethyl)-4-hydroxyphenyl-5-hexyn-1-one.

The chemical stability of tebufelone has be determined at 23°, 38°, and 49°C under ambient humidity conditions.[2] No evidence of compound degradation was detected chromatographically after one year of testing.

The efficacy of tebufelone has been shown in classical models of anti-inflammatory activity.[3] In the carrageenan rat paw edema assay, tebufelone has demonstrated a peroral potency significantly than that of ibuprofen.[3] Also, tebufelone has demonstrated significant inhibitory activity in the arachidonic acid induced inflamed mouse ear test. *In vitro* studies suggest that this anti-inflammatory activity probably results from the compound's ability to inhibit the cyclooxygenase and 5-lipoxygenase path-

ways in the arachidonic acid synthetic cascade.[4,5] Other preclinical studies have indicated that tebufelone has the ability to inhibit joint destruction and bone resorption in the classical adjuvant arthritis assay.[3]

This report describes the results of a preliminary investigation of the physical/chemical properties of the potential new drug.

EXPERIMENTAL

Material

Bile salts (50/50 sodium cholate/sodium deoxycholate), monobasic sodium phosphate, and peanut oil were obtained from Sigma Chemical Co. (St. Louis, MO). Hydrochloric acid, sodium chloride, and sodium hydroxide were obtained from Fisher Scientific Co. (Pittsburg, PA). Methanol, methylene chloride, and polyethylene glycol (PEG) 200 were obtained from Baker Chemical Co. (Phillipsburg, NJ). Ethanol was obtained from Aaper Alcohol and Chemical Co. (Shelbyville, KY), medium chain triglyceride (MCT) (Captex® 300) was obtained from Capital City Products Co. (Columbus, OH), and 1-octanol was obtained from Aldrich Chemical Co. (Milwaukee, WI). Optifluor® scintillation cocktail was obtained from Packard Instrument Co. (Downers Groves, IL) and Triton® X scintillation cocktail was obtained from Research Products International (Mount Prospect, IL).

Unlabeled tebufelone was prepared at Procter & Gamble's Miami Valley Laboratories, Cincinnati, OH, or at its subsidiary, Baker Chemical Co., Phillipsburg, NJ, and had a purity of greater than 97%. Radiolabeled tebufelone ^{14}C in carbonyl carbon) was prepared at the Miami Valley

Laboratories and had a specific activity of 46.5 μCi/mg and a radiochemical purity greater than 99%.

Thermal Properties

The melting points of tebufelone from four lots (minimum of two samples per lot) were determined using an Electrothermal Melting Point Apparatus (Fisher Mark II, Fisher Scientific, Inc., Pittsburg, PA). The melting point was recorded as the temperature at which all of the sample had melted using a slow rate of heating.

A Differential Scanning Calorimeter (DSC 4, Perkin Elmer, Inc., Norwalk, CT) was employed to study the thermal behavior of tebufelone. Samples from three lots were investigated. The sample weight was approximately 10 mg and a 50 lambda stainless steel pan was used. The heating rate was 10°C/min and the cooling rate was 320°C/min. The temperature range investigated was 20° to 100°C. One sample was subjected to two heating/cooling cycles to ascertain if tebufelone polymorphism. In addition, the thermal behavior of a sample of tebufelone precipitated from an ethanol solution by evaporation was determined. Enthalpy of fusion was calculated using the Perkin Elmer TADS software ratioing the area under the DSC curve for tebufelone to that of an indium reference.

Aqueous Solubility

Radiolabeled tebufelone was diluted with unlabeled tebufelone for these studies. about 100 mg unlabeled tebufelone was dissolved in 50 mL ethanol and 50 μCi ^{14}C tebufelone (from ethanolic stock solution) added.

The ethanol was evaporated, and the residue passed through a 90 μm screen onto a 60 μm screen, and assayed for radiolabel content via scintillation counting.

The following aqueous media were prepared:

(1) distilled/deionized water;

(2) simulated gastric fluid (SGF), USP (without pepsin) (United States Pharmacopeia XXII, 1990);

(3) simulated intestinal fluid(SIF), USP (without pancreatin) (United States Pharmacopeia XXII, 1990);

(4) SIF with 0.5%$_{w/v}$ bile salts;

(5) SIF with 1.0%$_{w/v}$ bile salts; and

(6) SIF with 2.0%$_{w/v}$ bile salts.

Three 25 mL aliquots of each medium were saturated with the previously prepared radiolabeled tebufelone powder and placed in a water bath shaker (Gyrotory Model G75, New Brunswick Scientific, Edison, NJ) at 37°C. Five milliliter aliquots were withdrawn at 24 ,48, and 72 hours, and filtered through a 0.22 μm syringe filter. One to two milliliters were mixed with about 15 mL scintillation cocktail and counted for two to five minutes using a quench curve corrected program.

Solvent/Lipid Solubility

Two approximately 10 mL aliquots of each of PEG 200, ethanol, 1-octanol, peanut, oil, and MCT were saturated with tebufelone powder and placed in a shaker bath at 25°C for 24 hours. aliquots were filtered using a 0.45 μm syringe filter, diluted with methanol or methylene chloride

and assayed using UV spectroscopy with a photodiode array spectrophotometer at 282 nm (methanol) or 278 nm (methylene chloride).

Apparent Octanol/Water Partition Coefficient

One milligram of radiolabeled tebufelone (prepared as described previously) was dissolved in each of three 5 mL aliquots of 1-octanol. Ten milliliters distilled, deionized water were added to each aliquot and the mixtures were placed in a water bath shaker at 25°C for 24 hours. Two milliliters of each phase were pipetted from the mixtures and analyzed for radiolabel via scintillation counting. the apparent 1-octanol/water partition coefficient was calculated as the ratio of the concentrations of radiolabel in 1-octanol and in water.

Dissolution as a Function of Particle Size

An ASM Sonic Sifter (ATM Corp., Milwaukee, WI) was used to obtain size fractions of tebufelone. The sieve size fractions were 16/20, 35/40, 60/80, and 140/200 mesh, which corresponded to mean particle size diameters of 1016, 460, 141, and 100 μm. Dissolution tests were conducted on each size fraction using the standard USP Apparatus 2 dissolution testing equipment (Vanderkamp 600, Van Kel Industries, Inc., Edison, NJ). The dissolution medium consisted of simulated intestinal fluid (SIF), USP without pancreatin with 2% bile salts maintained at 37°C. A volume of 500 mL dissolution medium and 20 mg tebufelone were employed. Tebufelone was measured via direct UV spectroscopy at 278 nm.

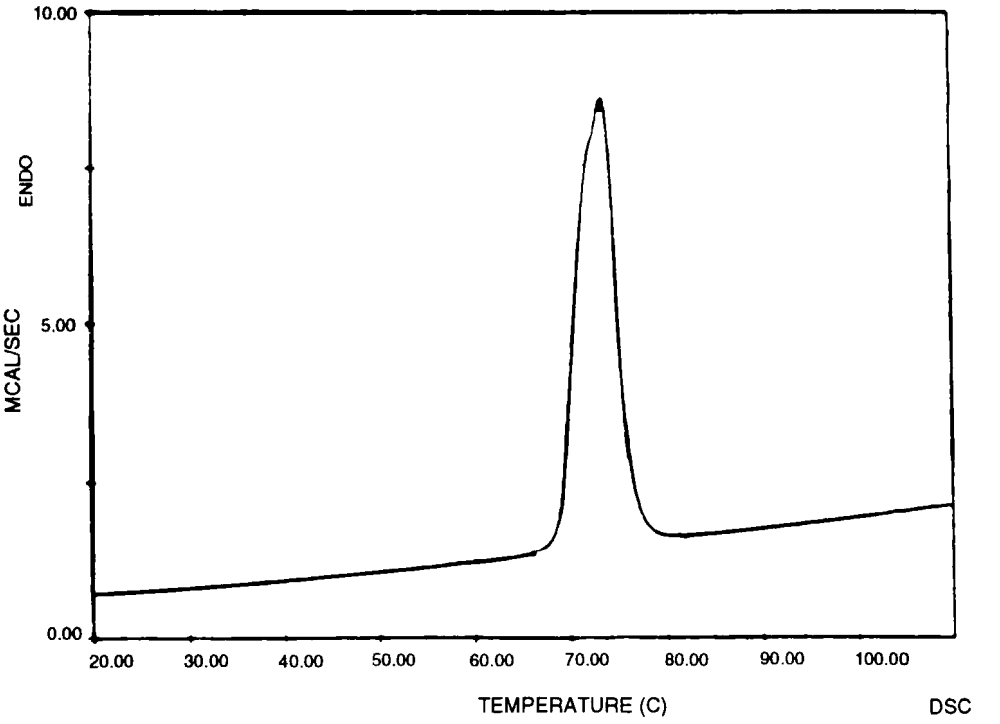

FIGURE 1
Representative DSC thermogram of tebufelone.

RESULTS AND DISCUSSION

Thermal Properties

The average melting point of four lots of tebufelone is 69.1°C (standard deviation [S.D.] of 0.7°C). A sample DSC curve of tebufelone appears in Figure 1. A single endotherm is observed. The average enthalpy of fusion of three lots of drug tested is 20.1 cal/g (S.D. 0.15 cal/g). No evidence of polymorphism is detected with repeat heating after cooling to 4°C (Figure 2), nor after precipitation from an ethanol solution (Figure 3) in that the same endotherm is observed with no change in enthalpy of fusion.

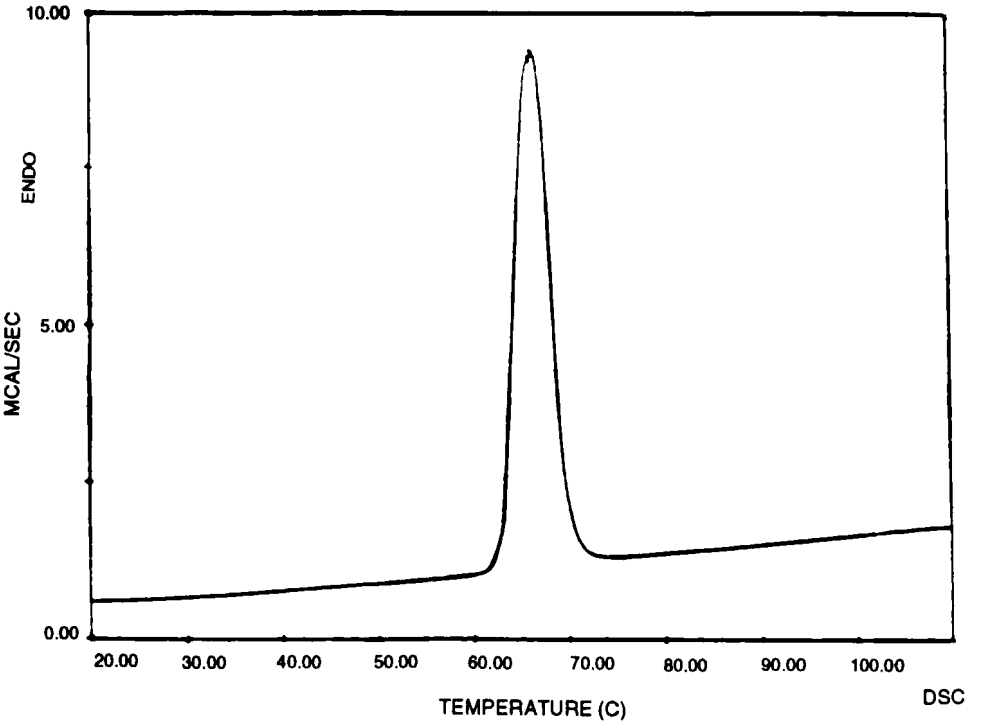

FIGURE 2
DSC thermogram of tebufelone following one heating/cooling cycle.

Aqueous Solubility

The results of the analyses of radiolabel in the various aqueous media, reported as μg equivalents of tebufelone/mL are depicted in Table 1. There are no significant changed in radiolabel concentration at the three time points of 24, 48, and 72 hours suggesting that equilibrium was attained.

There are no significant differences among the overall mean solubilities of radiolabel in distilled deionized water, SGF, and SIF which are 0.89, 1.15, and 1.58 μg-eq./mL, respectively. There is a significant increase in radiolabel solubility in SIF when increasing amounts of bile salt are added. The increase in solubility is over 38 fold at a 2% level of surfactant.

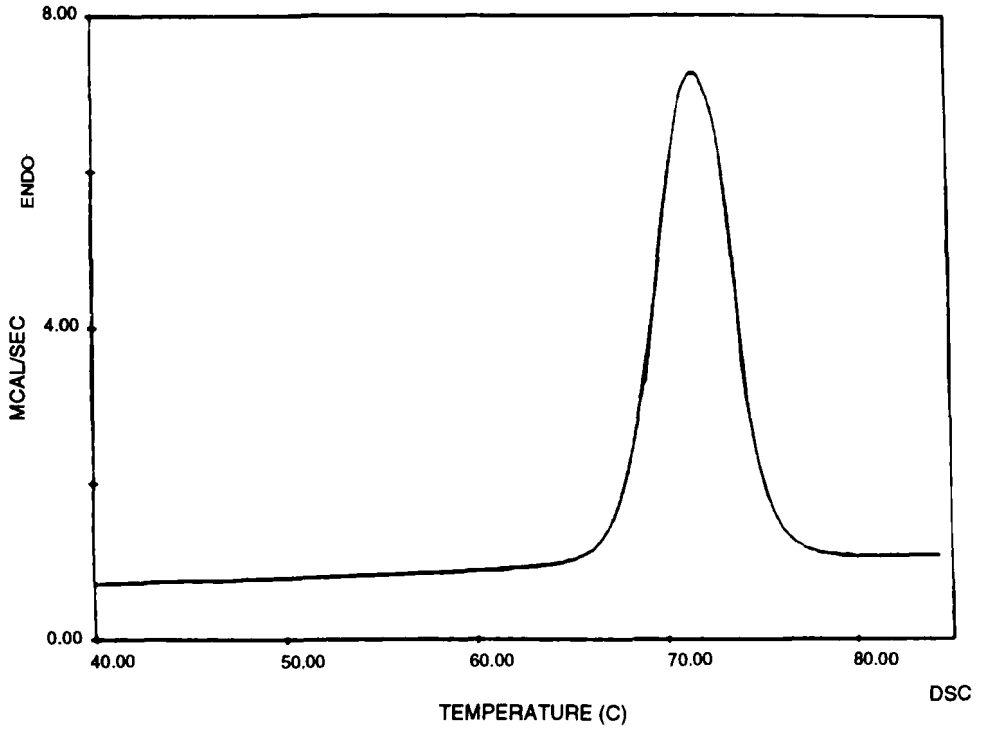

FIGURE 3
DSC thermogram of tebufelone precipitated from an ethanol solution.

The aqueous solubility of radiolabeled tebufelone is sufficiently low so as to indicate potential absorption problems due to insufficient solubilization of the compound in the gastrointestinal tract to permit it to diffuse across the unstirred boundary layer to the absorbing membrane.[6]

Solvent/Lipid Solubility

The solubilities of tebufelone in various solvents and lipids are shown in Table 2. The solubility of tebufelone in ethanol, PEG 200, 1-octanol, and MCT exceeds 10%. The solubility of tebufelone in 1-octanol,

TABLE 1
Aqueous Solubility of Radiolabeled Tebufelone

System	Solubility at 24 hr (ug-eq/mL)		Solubility at 48 hr (ug-eq/mL)		Solubility at 72 hr (ug-eq/mL)		Overall Solubility (ug-eq/mL)	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Distilled Deionized Water	0.88	0.01	0.91	0.03	0.87	0.08	0.89	0.05
Simulated Gastric Fluid, USP (without pepsin)	1.26	0.33	1.10	0.04	1.16	0.05	1.18	0.18
Simulated Intestinal Fluid, USP (without pancreatin)	1.66	0.34	1.55	0.23	1.55	0.50	1.58	0.33
Simulated Intestinal Fluid, USP (with 0.5% Bile Salts)	12.4	0.5	12.7	0.7	13.9	0.8	13.0	0.9
Simulated Intestinal Fluid, USP (with 1.0% Bile Salts)	30.7	5.8	37.6	7.5	41.9	6.2	36.7	7.5
Simulated Intestinal Fluid, USP (with 2.0% Bile Salts)	55.9	13.9	60.9	21.0	64.7	22.8	60.5	17.4

TABLE 2
Solvent and Lipid Solubility of Tebufelone

System	Solubility (Percent)	
	Mean	Std. Dev.
Ethanol	46.2	4.4
Polyethylene Glycol 200	14.2	0.6
1-Octanol	28.6	0.5
Medium Chain Triglyceride	20.8	1.3
Peanut Oil	3.14	0.28

which is often considered a good model for the absorbing mucosal cells[6] suggests that tebufelone should be readily absorbed from an aqueous solution.

The solubility of tebufelone in peanut oil, a model long chain triglyceride, is considerably less, about 3%. However, this is still one thousand fold greater than the aqueous solubility of tebufelone. Nonetheless, the solubility of tebufelone in a long chain triglyceride is not high enough to predispose it to lymphatic absorption.[7]

Apparent Octanol/Water Partition Coefficient

The mean apparent octanol/water partition coefficient determined by the shake flask method is 350 ∓ 11 (S.D.) which corresponds to an apparent log P of 2.54 (Table 3).

The ratio of the solubilities of tebufelone in 1-octanol and water can also provide an estimate of the apparent octanol/water partition coefficient. the solubility of tebufelone in 1-octanol is 236,000 μg/mL (25°C) and

TABLE 3
Apparent Octanol/Water Partition Coefficient of Radiolabeled Tebufelone

Variable	Mean	Std. Dev.
Radiolabel Concentration in Octanol (ug-eq./mL)	257.0	16.5
Radiolabel Concentration in Water (ug-eq./mL)	0.73	0.06
Apparent Partition Coefficient	350.7	11.0
Log (P)	2.54	0.01

its solubility in water is about 0.9 μg/mL (37°C). The ratio of these two values provides an estimated apparent partition coefficient of 263,000, corresponding to a log P of 5.4. Since the aqueous solubility was determined at 37°C and the octanol solubility at 25°C, this estimate is probably somewhat low for a 25°C partition coefficient. Nonetheless, it is about three orders of magnitude higher than the experimentally determined value.

Another estimate of the apparent octanol/water partition coefficient can be obtained from a relationship derived by Yalkowsky and Valvani.[8] This equation relates the aqueous molar solubility of a compound with its entropy of fusion, partition coefficient, and melting point. The melting point of tebufelone (69°C), and estimates of its aqueous molar solubility at 25°C (~0.000003) and entropy of fusion (~13.5 e.u.[8]) can be employed to calculate an estimate of the apparent octanol/water partition coefficient.[8]

$$\log S_w = -1.00[\log P] - \{(1.11)(\Delta S_f)(mp\text{-}25)/(1364)\} + 0.54$$

where: S_w = molar solubility in water at 25°C

ΔS_f = entropy of fusion

mp = melting point

This provides an estimated log P of about 5.6.

The apparent log P of tebufelone as determine by the shake flask method (2.54) is considerably lower than those calculated using the Yalkowsky and Valvani relationship (5.6) or by ratioing the solubilities of tebufelone in 1-octanol and water (5.4). The shake flask method has been reported to provide misleading results for highly lipophilic and sparingly soluble drugs due to the finite solubility of octanol in water.[9] Therefore, the latter values are most likely better estimates of the apparent log P of tebufelone.

This relatively large value confirms the lipophilic nature of tebufelone and indicates that it should readily partition into the lipoidal absorbing membranes of the gastrointestinal tract once it diffuses across the unstirred boundary layer and through the glycocalyx.

Particle Size Dissolution Behavior

The dissolution behavior of tebufelone as a function of particle size is summarized in Figure 4. The dissolution data were evaluated using first-order, cube root, square root, and two-thirds root models for dissolution of multiparticulate systems.[10] The calculated model parameters are shown in Table 4. Correlation coefficients were excellent for all models. The rate constants of each model increased with decreasing particle size. This is as expected since the available surface area increases with decreasing particle size and the number of particles increases with decreasing particle size (the

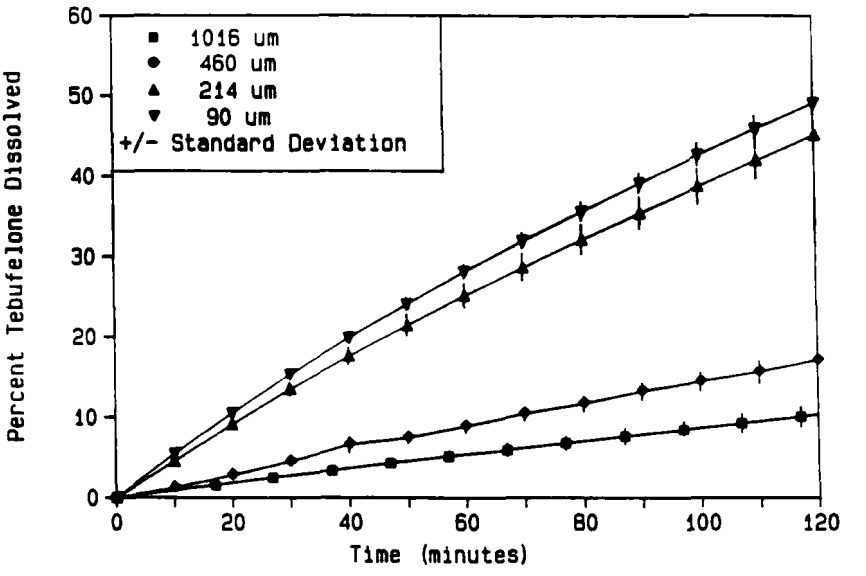

FIGURE 4
Dissolution of tebufelone as a function of particle size. Each symbol represents the mean (± standard deviation) of six replicates.

initial weight of particles was the same for all particle sizes). The cube root model assumes a constant unstirred boundary layer thickness, whereas the square root and two-thirds root models assume that the boundary layer thickness changes as a function of particle size.[10]

Adjustment of the rat constant for the cube root model to compensate for the differing numbers of particles at each particle size results in a reasonably constant adjusted value for particle sizes from 214 to 1016 μm as indicated in Table 5. Similar adjustments of the rate constants for the square root and two-thirds root models produce values that decline with decreasing particle size over this range. Thus, the assumption of a constant unstirred boundary layer thickness inherent in the cube root model appears to valid over this particle size range for tebufelone.

TABLE 4
Dissolution Model Parameters

First Order Model						
Particle Size (um)	R-Squared		Slope (/min)		Intercept (Percent)	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
1016	0.998	0.001	0.0009	0.0001	99.9	1.0
460	0.993	0.005	0.0016	0.0001	100.1	1.0
214	1.000	0.000	0.0050	0.0004	100.5	1.0
90	1.000	0.001	0.0056	0.0003	100.2	1.0

Cube Root Model						
Particle Size (um)	R-Squared		Slope (mg^[1/3]/min)		Intercept (mg^[1/3])	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
1016	1.000	0.000	0.0008	0.0001	0.0011	0.0002
460	0.996	0.006	0.0014	0.0001	0.0001	0.0023
214	1.000	0.000	0.0041	0.0003	0.0031	0.0009
90	0.999	0.001	0.0045	0.0002	0.0078	0.0043

Square Root Model						
Particle Size (um)	R-Squared		Slope (mg^[1/2]/min)		Intercept (mg^[1/2])	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
1016	1.000	0.000	0.0020	0.0002	0.0032	0.0005
460	0.996	0.006	0.0034	0.0003	0.0012	0.0056
214	0.999	0.001	0.0096	0.0006	0.0164	0.0016
90	0.998	0.001	0.0106	0.0004	0.0297	0.0099

Two-Thirds Root Model						
Particle Size (um)	R-Squared		Slope (mg^[2.3]/min)		Intercept (mg^[2/3])	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
1016	1.000	0.000	0.0043	0.0005	0.0079	0.0013
460	0.996	0.007	0.0073	0.0006	0.0046	0.0122
214	0.998	0.001	0.0202	0.0012	0.0533	0.0042
90	0.997	0.002	0.0221	0.0008	0.0861	0.0201

The adjusted cube root model rate constant for the smallest mean particle size evaluated (90 μm) is considerably smaller than the values for the lager mean particle sizes. This suggests that the unstirred boundary layer is either increasing as the particle size is reduced, or that the number

TABLE 5

Normalized Cube Root Dissolution Rate Constants

Particle Size (um)	Mean Rate Constant (mg^[1/3]/min)	Adjusted Rate Constant (mg^[1/3]/min)
1016	0.0008	0.000299
460	0.0014	0.000237
214	0.0041	0.000323
90	0.0045	0.000149

of particles calculated by the apparent mean particle size is an overestimate. The former explanation is contrary tot he observations that led to the development of the square root model.[11]

The latter situation could arise if the effective surface area available for dissolution did not correspond to the theoretical surface area determined from the mean particle size. This may be due to an increase in the surface energy of smaller particles which can result in incomplete wetting of their surfaces.[12]

CONCLUSIONS

Tebufelone was found to be very poorly water soluble with an aqueous solubility of about 0.9 μg/mL. This value is not significantly affected by pH over the physiological range as would be expected by the lack of an ionizable group on the molecule. However, tebufelone solubility is significantly increased by low levels of the endogenous solubilizing agents, sodium cholate and sodium deoxycholate. Tebufelone is very

lipophilic with an estimated apparent octanol/water partition coefficient (log P) of approximately 5.5. These properties suggest that tebufelone absorption should be dissolution rate limited. The low aqueous solubility predicts slow dissolution, but the high lipophilicity of tebufelone suggests that it should readily partition into absorbing membranes when in solution.

The *in vitro* dissolution behavior of tebufelone as a function of particle size indicates that the lipophilicity of the drug could be affecting the amount of effective surface area for dissolution. This suggests that reduction in particle size alone may not be completely effective in significantly increasing the dissolution rate of tebufelone.

Tebufelone has a relatively low melting point of about 69°C with an enthalpy of fusion of about 20 cal/g. No evidence of polymorphism has been detected following heating and cooling cycles. Tebufelone is also very soluble in ethanol, 46%, and no evidence of polymorphism is detected in drug obtained from an evaporated ethanol solution.

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