Pharmaceutical Research, Vol. 6, No. 5, 1989

Report

Preformulation Study of Etoposide: Identification of Physicochemical Characteristics Responsible for the Low and Erratic Oral Bioavailability of Etoposide

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Received December 22, 1987; accepted December 29, 1988

Preformulation studies of etoposide, including pH–solubility profile, partition coefficient, pH–stability profile, and *in vitro* dissolution kinetics, were conducted to identify the responsible factor(s) for the low and erratic oral bioavailability of etoposide. A stability-indicating high-performance liquid chromatographic (HPLC) assay was used for drug monitoring. The equilibrium aqueous solubility of etoposide at 37°C was low, 148.5–167.25 μg/ml, and did not vary over the pH range of 2 to 6. The pH–stability profile indicated rapid degradation of etoposide at pH 1.3 and 10, with degradation half-lives of 2.88 and 3.83 hr, respectively, at 25°C. The half-life at pH 7.30 was 27.72 days. Maximum stability at 25°C was reached at pH 5 to 6.15, with half-lives of 63 and 49.5 days, respectively. The intrinsic dissolution rate, determined on a Wood's apparatus, was slow, 0.0094 mg/min/cm^2, while the etoposide partition coefficient between *n*-octanol and water was 9.94. Therefore, etoposide absorption appears to be dissolution rate limited rather than permeation rate limited. The low equilibrium aqueous solubility, slow intrinsic dissolution rate, and chemical instability at pH 1.3 could account for the low oral bioavailability.

KEY WORDS: etoposide; preformulation; pH–solubility; pH–stability; dissolution; partition coefficient.

INTRODUCTION

Etoposide, also known as VP-16-213, is a semisynthetic epipodophyllotoxin derivative (Fig. 1), active against a variety of malignancies (1). Etoposide is the most active single agent for the treatment of small-cell lung cancer and testicular carcinoma (2). The agent is given intravenously in a dose of 300–600 mg/m^2 (450–900 mg for an adult weighing 70 kg) over a period of 3–5 days. The treatment is repeated every alternate week until a beneficial effect is observed (3). The currently available dosage forms are nonaqueous i.v. parenteral solutions and oral soft gelatin capsules containing etoposide solution in a mixed solvent system. The i.v. administration of etoposide on a chronic basis is inconvenient for outpatients. In addition, etoposide precipitates from the parenteral solution as diluted with other i.v. fluids for infusion (4), and too rapid an infusion of etoposide precipitates hypotension of the patient (3). Therefore, an oral formulation is desired. However, the capsule formulation has a reported oral bioavailability of 50% (5). Several investigational oral formulations have been evaluated, namely, (a) hydrophilic, soft gelatin capsules containing etoposide solution (6), (b) lipophilic capsules of etoposide suspension (7), and (c) drinking ampoules (8). However, all these formulations yielded poor oral bioavailabilities (25–74%) with high intra- and interpatient variabilities in the rate and extent of etoposide absorption (9). Therefore, the development of a stable oral formulation with a higher and more reproducible oral bioavailability than the current one is desirable.

This study was intended to identify the possible physicochemical characteristics responsible for the low and erratic oral bioavailability of etoposide, for the purpose of establishing the basis for logical and effective approaches to modify the dosage form. The pH–solubility profile and pH–stability profile of etoposide were established, with the pH range encountered in the gastrointestinal tract (pH 1.3–8). The pH dependence of the solubility and chemical stability of the drug was determined. In addition, the *in vitro* dissolution kinetics of etoposide was evaluated using a Wood's apparatus (10). The possibility of dissolution rate-limiting absorption of etoposide was verified (11). The *n*-octanol/water partition coefficient of etoposide was also determined.

MATERIALS AND METHODS

Chemicals

Etoposide was used as received from Bristol Myers

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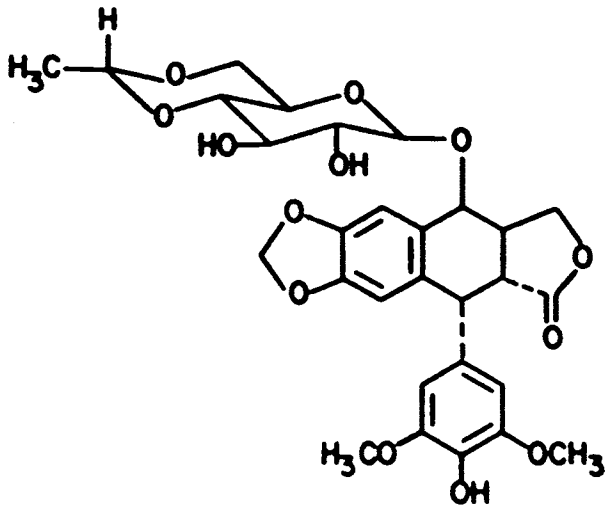

Fig. 1. The chemical structure of etoposide.

(Syracuse, N.Y.). Hydrochloric acid, potassium chloride, sodium citrate, citric acid, acetic acid, sodium acetate, potassium monobasic phosphate, potassium dibasic phosphate, sodium hydroxide, and boric acid were all analytical grade. Acetonitrile of high-performance liquid chromatographic (HPLC) grade was used.

HPLC Assay

A stability-indicating HPLC assay was developed for etoposide (12) with a reversed-phase C8 column (5 μm, 15 cm × 4.6-mm i.d., Custom LC Inc., Houston, Tex.) and acetonitrile–acetic acid–water (27:1:72, pH 4.0) at a flow rate of 1.5 ml/min as the mobile phase. Etoposide was monitored at 230 nm and the detection limit was 0.05 μg/ml. Methoxypsoralen was used as the internal standard.

Preparation of Buffers

All buffers used, pH 1.3–10, as listed in Tables I and II, had concentrations of 0.1 *M*. The buffers used for the pH–stability study had ionic strengths adjusted to 0.5 with KCl.

pH–Solubility Profile

An excessive amount (about 20 mg) of etoposide was agitated with 10 ml of each buffer for 48 hr at 37°C in a water bath. One-milliliter samples were taken at 24 and 48 hr, respectively, filtered through 45-μm membrane filters (Gellman), and subjected to HPLC assay.

pH–Stability Profile

Etoposide solutions of 100 μg/ml prepared in the buffers were maintained at 25°C in a water bath. The samples were taken at various time intervals and analyzed by HPLC until the remaining etoposide level was negligible. The log concentration of etoposide versus time profile was plotted to determine the degradation rate constants at all pH values.

Table I. Solubilities of Etoposide at 37°C in Buffers of Various pH Values

Buffer	pH	Solubility (μg/ml), Mean ± SD[a]
0.1 *M* HCl	1.30	Extensive degradation of etoposide was observed.
0.1 *M* HCl/KCl	2.00	151.31 ± 16.64*
0.1 *M* Na citrate/ citric acid	3.00	167.25 ± 16.67
Distilled water	4.50	147.50 ± 1.75
0.1 *M* Na acetate/ acetic acid	5.00	153.22 ± 10.18
0.1 *M* $KHPO_4/KH_2PO_4$	6.00	149.58 ± 9.73
0.1 *M* $KHPO_4/KH_2PO_4$	7.40	125.93 ± 19.41**
0.1 *M* $KHPO_4/KH_2PO_4$	8.00	116.44 ± 11.95**
		Etoposide degradation was observed after 48 hr
0.1 *M* Na borate/ boric acid	10.00	Extensive degradation of etoposide was observed

[a] N = 3, 24-hr data.
* Statistically no significant difference in solubilities from pH 2 to pH 6 at $P = 0.05$ by ANOVA.
** Statistically significant difference in solubilities from pH 6 to pH 8 at $P = 0.05$ by ANOVA.

The pH–stability profile was constructed by plotting rate constant versus pH.

Drug Dissolution Kinetics

About 25 mg of etoposide was compressed on a Carver press (Model C, Fred S. Carver Inc.) into a disk 6 mm in diameter and then mounted on a rotating shaft of a Wood's apparatus. The disk was rotated at 100 rpm in 30 ml of distilled water at room temperature. The distance of the disk from the bottom of the beaker was kept constant at 2 cm. Samples (100 μl) were taken at various time intervals up to

Table II. First-Order Degradation Half-Life ($t_{1/2}$) of Etoposide at 25°C in Buffers of Various pH Values

Buffer[a]	pH	$t_{1/2}$ (days), mean ± SD[b]
0.1 *M* HCl	1.30	0.12 ± 0.002
0.1 *M* HCl/KCl	2.03	1.19 ± 0.127
0.1 *M* Na citrate/ citric acid	3.05	8.15 ± 0.192
0.1 *M* Na acetate/ acetic acid	5.00	63.00 ± 5.730*
0.1 *M* $KHPO_4/KH_2PO_4$	6.15	49.50 ± 3.536
0.1 *M* $KHPO_4/KH_2PO_4$	7.30	27.72 ± 2.218
0.1 *M* $KHPO_4/KH_2PO_4$	8.00	5.97 ± 0.257
0.1 *M* Na borate/ boric acid	10.00	0.16 ± 0.011

[a] All buffers had concentrations of 0.1 *M*, and the ionic strengths had been adjusted to 0.5 with KCl.
[b] N = 3.
* Statistically no significant difference in half-lives at pH 5 and 6.15 by Student's *t* test at $P = 0.05$.

70 hr, filtered through 45-μm membrane filters, and analyzed by HPLC.

The data obtained were analyzed using the Noyes–Whitney equation (13) as shown below.

$$\frac{dc}{dt} = \frac{D * A * (C_s - C)}{h * V} \quad (1)$$

where dc/dt is the dissolution rate, D is the diffusion coefficient (cm^2/min), A is the surface area of the disk (cm^2), C_s is the aqueous solubility (mg/ml), C is the concentration of etoposide (mg/ml), h is the thickness of the diffusion layer (cm), and V is the volume of the dissolution medium (ml).

Under sink conditions (C is less than 20% of C_s), Eq. (1) is simplified as follows (13):

$$\frac{dc}{dt} = \frac{D * A * C_s}{h * V} \quad (2)$$

which, on rearrangement, leads to

$$\frac{dc * V}{dt * A} = \frac{D * C_s}{h} = \text{intrinsic dissolution rate} \quad (3)$$

Partition Coefficient

The *n*-octanol and water were presaturated with each other in amber-colored bottles for 24 hr. Five milliliters each of the two presaturated solvents was mixed together with 10 mg of etoposide in a screw-capped tube on a rotatory mixer at 25°C. Samples were taken at 6, 12, and 24 hr. The *n*-octanol and water layers of the samples were analyzed separately for etoposide by HPLC.

Statistical Analysis

The effects of pH on the solubility of etoposide were analyzed by one-way ANOVA at $P = 0.05$. To determine the pH of maximum stability, the degradation constants at pH 5 and 6.15 were compared by Student's t test at the $P = 0.05$ level.

RESULTS

pH–Solubility Profile

The solubilities of etoposide at 37°C in various buffers of pH's ranging from 1.30 to 10 are reported in Table I. Extensive degradation of etoposide was observed at pH 1.30 and 10, which precluded the measurement of equilibrium solubilities. The pH–solubility profile of etoposide is shown in Fig. 2. The difference in solubilities from pH 2 to pH 6 was insignificant but those at pH 7.4 and 8 were significantly lower than the rest as determined by ANOVA at $P = 0.05$. The apparent solubility decreased with increasing pH above pH 6, along with increasing etoposide degradation, as reflected by the increasing peak heights of the degradation products in the chromatograms (Fig. 3).

pH–Stability Profile

The log etoposide concentration versus time profiles were constructed. The linear curves of the plots at all pH's indicated first-order degradation. Degradation rate constants obtained from the slopes of the curves were used to determine the half-lives at various pH's (Table II). The pH–stability profile of etoposide is shown in Fig. 4. The degradations were extremely rapid under highly acidic and alkaline conditions. The degradation half-lives were 2.88 and 3.83 hr at pH 1.30 and pH 10, respectively, while pH 5–6.15 was the pH range of maximal stability, with degradation half-lives of 63 and 49.5 days, respectively. The slopes of the pH–rate profile on the acidic and basic sides were −0.70 and 0.68, respectively; therefore, the degradation of etoposide is not specific acid or base catalyzed (14).

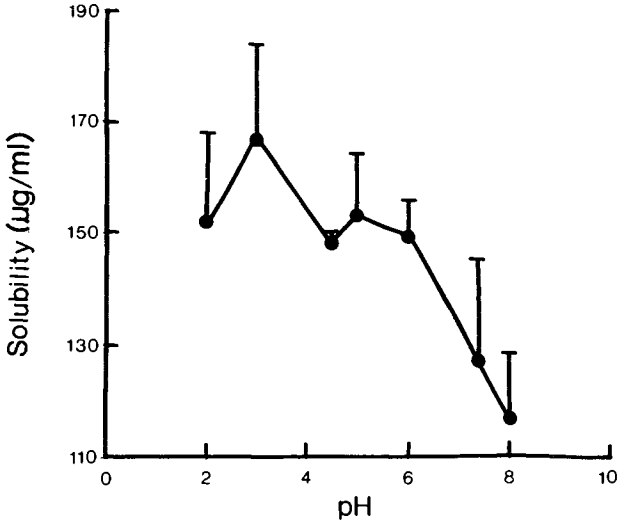

Fig. 2. The pH–solubility profile of etoposide at 37°C. Each point represents the mean of three observations with standard deviation bar.

Drug Dissolution Kinetics

Complete dissolution profiles in three separate runs

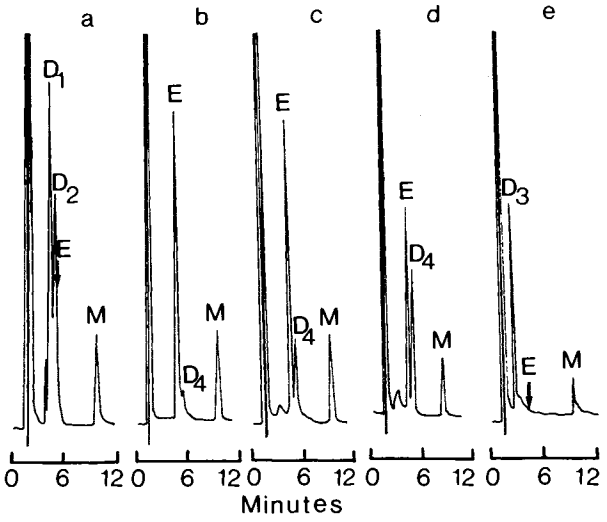

Fig. 3. Typical chromatograms of etoposide (E) solubility samples after 48 hr at (a) pH 1.3, (b) pH 6.0, (c) pH 7.4, (d) pH 8.0, and (e) pH 10.0. Methoxypsoralen (M) is the internal standard; D1, D2, D3, and D4 are different degradation products of etoposide.

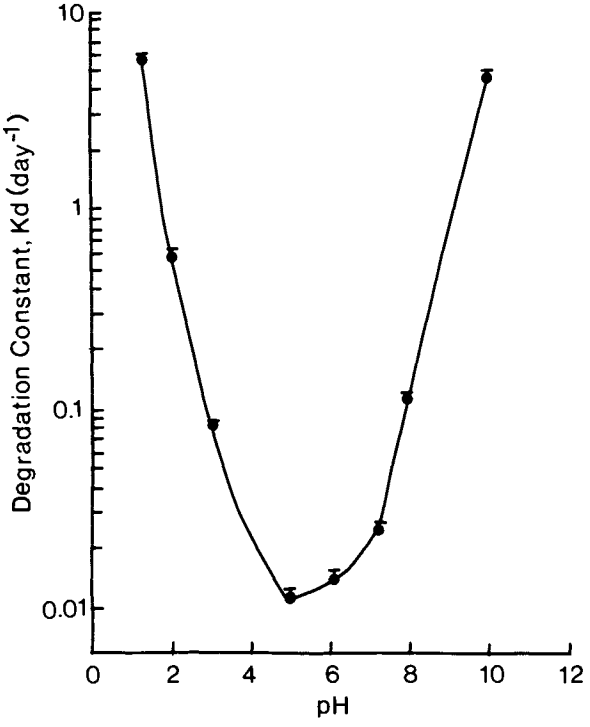

Fig. 4. The pH–stability profile of etoposide at room temperature. Each point represents the mean value of three observations with standard deviation bar.

were constructed until saturation of etoposide (0.1 mg/ml) was achieved as shown in Fig. 5. The dissolution kinetics of etoposide can be described by the Noyes–Whitney equation [Eq. (1)]. The curves were linear when etoposide concentrations were less than 20% of the equilibrium solubility of etoposide (Fig. 5, inset). The slope of the linear portion determined the dissolution rate as described in Eq. (2). The intrinsic dissolution rates were calculated according to Eq. (3) and are listed in Table III.

Partition Coefficient

The equilibrium partition of etoposide between *n*-octanol and water phases was achieved in 12 hr. The partition coefficient (o/w) was 9.94 ± 0.095 at 25°C (N = 3). No degradation products of etoposide were observed by HPLC during the partition coefficient study.

DISCUSSION

Etoposide solubilities ranged from 116.44 to 167.25 μg/ml over the pH range 1.3 to 8. Insufficient aqueous solubility of a drug has been known to yield poor or erratic absorption with large inter- and intrasubject variations in blood levels. Kaplan (11) found that potential bioavailability problems are often present when the aqueous solubility of a drug is less than 10 mg/ml (1%). The extremely low aqueous solubility of etoposide may be responsible for its poor and erratic oral absorption.

In addition, the orally administered drug needs to be stable during its transit through the gastrointestinal tract of various pH's ranging from 1 to 8. Etoposide is most stable in the pH range of 5–6.15 and rapidly degrades at pH <2.03 and pH >8. The half-life of etoposide at pH 1.30 was 2.85 hr. The rapid degradation of etoposide in gastric fluid could also account for its low oral bioavailability. An enteric coating of etoposide may prevent the acidic degradation and effectively improve the oral bioavailability.

Significant statistical correlations between drug absorption and dissolution rate have been reported for many drugs, such as digoxin, prednisone, and acetaminophen (15). Drugs having intrinsic dissolution rates less than 1.0 mg/min/cm^2 at 37°C frequently have bioavailability problems, because the absorption is limited by the dissolution rate (11). Digoxin,

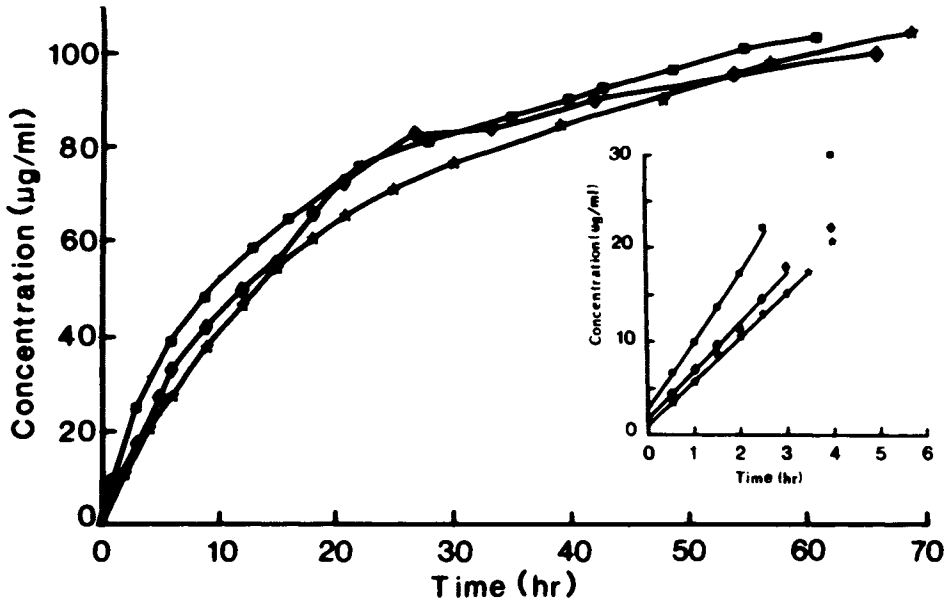

Fig. 5. Intrinsic dissolution profiles of etoposide in three separate dissolution experiments. The inset depicts the dissolution profile of etoposide under sink conditions (concentrations of etoposide less than 20% of aqueous solubility).

Table III. Dissolution Rates of Etoposide at 25°C

Dissolution experiment No.	Dissolution rate, dc/dt (μg/ml/hr)	Intrinsic dissolution rate, $(D/h) \cdot C_s$ (mg/min/cm^2)
1	5.94	0.0105
2	5.27	0.0093
3	4.80	0.0085
Mean	5.34	0.0094
(SD)[a]	(0.57)	(0.0010)

[a] $N = 3$.

various erythromycin esters, and different hydrates of ampicillin are examples of drugs with dissolution rate-limiting absorption (16). The intrinsic dissolution rate of etoposide was 0.0094 mg/min/cm^2 at 25°C, and although it increases with temperature, its magnitude is far less than 1.0 mg/min/cm^2 at 37°C. Therefore, dissolution rate-limited absorption of etoposide may also contribute to the observed low oral bioavailability.

The correlation between the partition coefficient and the rate and extent of absorption of a drug has been reported (15). However, the absorption of etoposide may not be permeation rate limited, because the partition coefficient of etoposide was 9.94 at 25°C, reflecting its high lipophilicity.

In conclusion, the low aqueous solubility, slow intrinsic dissolution rate, and rapid degradation at pH 1.30 of etoposide may all account for the low and erratic bioavailability of the drug. Therefore, approaches to increase the aqueous solubility and dissolution rate of etoposide and to employ an enteric coating to prevent acidic degradation in gastric fluid may effectively improve the oral bioavailability of etoposide.

ACKNOWLEDGMENTS

This work was partially supported by a Sigma Xi Graduate Research Award (J.C.S.). It was presented in part at the 1st National Meeting of the American Association of Pharmaceutical Scientists, Washington, D.C., November 1986.

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