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Oral bioavailability and in situ absorption of etoposide in rat

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Summary

Etoposide, an anticancer drug, has low oral bioavailability in humans which is due to low equilibrium aqueous solubility, slow dissolution rate, and instability at pH 1.3. Etoposide coprecipitate with PEG 8000 in a ratio of 1:10 (PEG weight fraction of 0.91) increased the solubility 2-fold and dissolution rate 42-fold. The oral bioavailability study of etoposide formulations was conducted in male Sprague-Dawley rats using a balanced crossover design. The absolute oral bioavailability of etoposide from coprecipitate, solution and powder was 5.56 ± 2.32, 10.16 ± 7.11 and $1.29 \pm 0.62\%$, respectively. No significant difference in oral bioavailability between solution and coprecipitate formulation was found but both were significantly higher than that of etoposide powder. The variability in serum concentrations and oral bioavailability of etoposide from coprecipitate formulation was much smaller than that from solution formulation. The very low extent of etoposide absorption even from a solution formulation (10.16%), in contrast to that in humans, suggested that factors other than the physicochemical properties may be responsible for the poor oral absorption of etoposide in rat. The in situ absorption studies in rats demonstrated very poor absorption of etoposide from stomach and the whole intestine by both Doluisio and Levine techniques. Bile salt (sodium taurocholate at 10 mM) reduced etoposide absorption. Thus, results from the in situ and in vivo absorption studies suggested that etoposide absorption is permeation rate limiting in rat. Therefore, rat may not be a good animal model for studying etoposide absorption from oral formulations with improved dissolution rate of etoposide.

Introduction

Etoposide is an anticancer drug for the treatment of small cell lung cancer and testicular carcinoma (Issell et al. 1979). Etoposide oral formulations exhibit low and erratic bioavailability in humans (Clark et al., 1987). The marketed formulation (Vepesid, Table 1) was reported to have an oral bioavailability of 50% with absorption ranging from 25 to 75% (Clark et al., 1987; Vepesid, 1991). In our previous study (Shah et al., 1989), the low aqueous equilibrium solubility, slow dissolution rate and instability at pH 1.3 were identified as probable factors responsible for the poor absorption of etoposide in man. In an attempt to improve the oral bioavailability, etoposide coprecipitates with PEGs were prepared and found to enhance the solubility and dissolution

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TABLE I

Composition of etoposide injectable formulation (Vepesid)

Component	Concentration (mg/ml)
Etoposide	20
Citric acid	2
Benzyl alcohol	30
Polysorbate 80	80
PEG 300	650
Ethyl alcohol	30.5 (% v/v)

rate of etoposide (Shah et al., 1992). Etoposide coprecipitate with PEG 8000 in a ratio of 1:10 increased the equilibrium aqueous solubility 2-fold and dissolution rate 42-fold (190.7 μg/ml and 0.42 mg/min per cm^2 vs 93.8 μg/ml and 0.01 mg/min per cm^2 of etoposide powder, respectively).

The study objective was to determine whether the increased dissolution rate of etoposide from the coprecipitate results in increased oral bioavailability of the drug in male Sprague-Dawley rats using a balanced crossover design. Etoposide is available as an injectable solution and a soft-gelatin capsule for oral use. The composition of the injectable formulation of etoposide (Vepesid) is described in Table 1. It is a non-aqueous solution containing ethyl alcohol, PEG 300 and benzyl alcohol to solubilize etoposide. Hence, it must be diluted with various infusion fluids prior to administration. The diluted solution has been shown to be physically unstable and results in precipitation of etoposide. The time of precipitation being dependent on the concentration of the diluted solution, 0.4 mg/ml solution is stable for 48 h, while a solution with a higher concentration may precipitate spontaneously (Clark et al., 1987; Vepesid, 1991). In this study, a solution formulation of etoposide after dilution with water, was used orally as a control formulation. In our study, etoposide did not precipitate on dilution before dosing. Etoposide powder suspended in water was used as another control formulation. In addition, in situ absorption studies were conducted to determine the absorption kinetics from various segments of the gastrointestinal tract.

Based on published reports, it appears that etoposide has not been studied as extensively in animals as in humans. The intravenous pharmacokinetics of etoposide has been studied in mice (Colombo et al., 1981, 1983), rat (Colombo et al., 1983), rhesus monkey (Sinkule, 1984), and dog (Savaraj et al., 1987), but there is not a single report on the oral pharmacokinetics of etoposide in an animal model (Clark et al., 1987). Schurgers et al. (1985) had demonstrated mucus as the rate-limiting barrier for in situ absorption of etoposide in rat, but no data for in vivo oral absorption were presented. For a preliminary investigation of oral bioavailability, we selected rat as the animal model.

Materials and Methods

Etoposide was used as supplied by Bristol Myers (Syracuse, NY). Methanol and acetonitrile were of HPLC grade. PEG 8000 from Aldrich Chemical Co. and salicylic acid and [^{14}C]inulin from Sigma Chemical Co. were used.

Assay

A stability indicating HPLC assay previously developed for etoposide was used (Chow et al., 1987). The HPLC assay was a reversed phase separation on an octyl column (5 μm, 15 cm $\times$ 4.6 mm i.d.) with a mobile phase consisting of acetonitrile-acetic acid-water (27:1:72), pH 4. Flow rate was 1.5 ml/min and drug detected at 230 nm. The assay could be used to analyze both aqueous and plasma samples after extraction. The sensitivity of the assay was 1 ng/injection. The plasma samples were concentrated 15–20-fold during extraction to facilitate quantitation of the drug.

The same HPLC assay was also used for quantitation of salicylic acid in the in situ absorption experiment samples, since it was baseline resolved from the etoposide and internal standard peaks.

Preparation of coprecipitate of etoposide with PEG 8000

The coprecipitate was prepared by dissolving weighed amounts of etoposide and PEG 8000 in 5 ml of methanol using sonication. Methanol was

evaporated for 8–12 h, until a dry cake was obtained. Etoposide coprecipitate contained 91% w/w PEG 8000. The coprecipitate so obtained was stored in a tightly capped glass vial at room temperature. Etoposide was stable during the preparation as demonstrated by the absence of any degradation product's peak in the HPLC chromatogram.

Bioavailability study

Study design

The oral bioavailability study of etoposide formulations was conducted in male Sprague-Dawley rats weighing 275–325 g. Three formulations were evaluated (etoposide oral dose of 20 mg/kg): (a) etoposide powder suspended in 400 μl of water, immediately prior to administration (< 3 min); (b) parenteral solution diluted with 400 μl of water immediately prior to administration (< 3 min); and (c) coprecipitate suspended in 400 μl of water immediately prior to dosing (< 3 min). The rats were divided into three groups of six rats each. The study design was balanced crossover between intravenous and each oral formulation with a 7 day washout period. The order of administration by i.v. or oral route was random and all the rats were fasted with ad libitum access to water for 12 h before dosing. All the rats received intravenous etoposide by administration of the solution into the penal vein at a dose of 10 mg/kg. Rats also received 20 mg/kg etoposide as different oral formulations with 400 μl of water by oral gavage. After oral administration, the syringe and the microtube used for the formulations were flushed with an additional 400 μl of water and the rinse was orally administered. Rats were allowed ad libitum access to water during the study and food was given 3 h after dosing. Blood samples of 100–300 μl were collected from the tail vein by bleeding at various time intervals.

Sample pretreatment

Blood samples were allowed to clot for 15 min, and centrifuged at $18000 \times g$ for 3 min to separate the serum. The serum samples were frozen at -20°C until HPLC analysis. The serum samples were extracted with 6–10 volumes of chloroform after thawing and the addition of internal standard, methoxypsoralen. The chloroform extracts were evaporated to dryness and the residue was dissolved in 15–25 μl of methanol and assayed by HPLC.

Data analysis

The serum concentration vs time data obtained after i.v. and oral administrations were analyzed by noncompartmental pharmacokinetics. The terminal elimination rate constant, K_e, was estimated by ESTRIP (Brown et al., 1978), a nonlinear curve stripping program. The area under the plasma concentration time curve (AUC) till the last determined point was estimated by the trapezoidal rule. AUC_{inf} was obtained by adding the area from the last determined point to infinity (C_{last}/K_e) to the previous value of AUC. The AUC_{inf} values after i.v. and oral administrations of etoposide were used to calculate the absolute oral bioavailability of each formulation. The time to peak (T_{max}) and the peak concentrations (C_{max}) after oral administration were determined from the respective serum concentration-time profiles.

In situ absorption study

Male Sprague-Dawley rats, weighing 275–325 g, were used for the in situ absorption studies. Two procedures, referred to as the Doluisio (Doluisio et al., 1969) and Levine (Levine et al., 1955) techniques, were used to study the rate and extent of etoposide absorption from various segments of gastrointestinal tract.

The Doluisio technique (Doluisio et al., 1969)

The perfusion solution used was Krebs-Henseleit Original Ringer bicarbonate buffer, pH 7.4 at 37°C. Etoposide solution (100 μg/ml) was prepared by diluting 0.1 ml of etoposide i.v. solution (20 mg/ml) with 20 ml of Krebs buffer. 200 μl of [^{14}C]inulin (12 μCi) were added to 20 ml of the drug solution to obtain 5000 dpm in 0.1 ml of the perfusion solutions. For the preparations in which salicylic acid was used, 2 mg of salicylic acid was dissolved in 20 ml of Krebs buffer containing etoposide and [^{14}C]inulin to obtain 100

μg/ml of salicylic acid. All the drug solutions were freshly prepared before perfusion and maintained at 37°C in a water bath during the experiment.

Procedure for absorption study

The rats were fasted with ad libitum access to water for 18 h, and anesthetized by intra-peritoneal administration of sodium pentobarbital at 50 mg/kg 30 min prior to surgery. The surgical procedure described in the original publication (Doluisio et al., 1969) was used. After the setting up of the cannula and cleaning of the segment under study, 10 ml of drug solution was introduced into the intestine through the proximal cannula. The drug solution was pumped once through the intestine all the way to the distal cannula and drained back into the intestine to ensure uniform drug solution concentration throughout the gut segment. The stopwatch was started at this time. To further ensure uniform drug solution concentration throughout the gut segments, samples were taken alternately from the two ends of the segment. The drug solution was pumped alternately into either the proximal or the distal syringe, and two 0.1 ml aliquots were withdrawn. The remaining perfusion solution was introduced back into the intestine within 30–60 s. Samples of 0.1 ml were analyzed for etoposide and salicylic acid by HPLC, and another 0.1 ml aliquot was added to 5 ml of Aquasol-2 to monitor the radioactivity of inulin using liquid scintillation counting.

Three different sets of absorption experiments were carried out using the following segments: (i) the whole small intestine; segment from duodenum, which was identified as 1 cm distal to the pylorus of the stomach, to ileum; (ii) the upper small intestine; segment from duodenum to jejunum, approx. 15 cm in length, and extending from duodenum to the middle of the small intestine; and (iii) the lower small intestine; segment from jejunum to ileum, approx. 15 cm in length and extending from middle of the small intestine to ileum. The study was conducted for 90 and 60 min, for the whole small, and the upper and lower small intestinal preparations, respectively. The drug concentrations were corrected for water absorption (estimated by the increase in inulin concentration) and the rates and extents of absorptions of etoposide, salicylic acid and water estimated.

The Levine technique (Levine et al., 1955)

The perfusion and the drug solutions used for the Levine technique were similar to those used for the Doluisio technique. The surgery and cleaning of the segments with perfusion solution were also conducted similarly. The stomach and small intestine were ligated at the distal end, filled with 5 and 10 ml of drug solution, respectively, and then ligated at the proximal ends. Immediately, both segments were returned to the abdominal cavity, and the incision was sutured and covered with a wet gauge pad. The abdomen was reopened at the end of 2 h and the solutions from stomach and the small intestine were recovered and analyzed for etoposide and salicylic acid by HPLC, and for inulin by liquid scintillation counting.

Effect of bile salts on the in situ absorption of etoposide

The effect of bile salt, sodium taurocholate, at normal physiological concentrations, 10 mM (Gibaldi et al. 1970), on the in situ etoposide absorption was studied in male Sprague-Dawley rats with the Doluisio technique. The bile salt was dissolved in the perfusion solution along with etoposide and salicylic acid and the duration of the study was 120 min.

Statistical analysis

All the present data are reported throughout as means $\pm$ one standard deviation in the text and tables. All the pharmacokinetic parameters were compared by unbalanced one-way ANOVA at $p \leq 0.05$. The absolute oral bioavailabilities of various formulations were compared by unbalanced one-way ANOVA after testing for the homogeneity of variance by Bartlett's test. The oral bioavailabilities of various formulations were ranked by one-tailed Newman Keuls test at $p \leq 0.05$. The pharmacokinetic parameters between two groups were compared by Student's two sam-

ple t-test, and those within groups were compared by Paired t-test at $p \leq 0.05$.

Results

Bioavailability study

The mean serum concentrations of etoposide following i.v. and various oral formulation administrations were plotted vs time in Fig. 1. The serum concentrations of etoposide after i.v. administration exhibited a biexponential decline with a very short distribution half-life and the terminal half-life of elimination was 57.75 ± 17.82 min (Table 2). The serum concentrations after oral administration of etoposide powder suspension were very low, scattered, and did not exhibit a typical serum concentration-time profile for absorption (Fig. 1). The coefficient and exponents, and hence the terminal half-life, could not be accurately estimated using ESTRIP. The absolute oral bioavailability of etoposide from the powder suspension estimated from AUC_{inf} was 1.29 ± 0.62%, indicating extremely poor absorption from powder suspension. The second subgroup of three rats to have received powder suspension following i.v. administration were not studied because of the consistently poor absorption of etoposide observed in the first three rats.

The serum etoposide concentrations following oral solution administration were low but 10–15-fold higher than that obtained after powder suspension administration (Fig. 1). There was very high variability in serum concentrations, 35–140%, among rats after oral solution administration. The T_{max} and C_{max} could not be accurately identified due to the wide variability in serum concentrations. The absolute oral bioavailability estimated from AUC_{inf} was also highly variable, 10.16 ± 7.11%, ranging from 2.29 to 24.39%. The absolute oral bioavailability from solution was significantly higher than that from powder suspension.

The serum concentrations after oral administration of etoposide coprecipitate with 91% w/w PEG 8000 were 5–7-fold higher than that after powder suspension administration but lower than that after oral solution dosing (Fig. 1). Neverthe-

TABLE 2

Pharmacokinetic parameters of etoposide following intravenous (10 mg/kg) and oral (20 mg/kg) administration of various formulations

Treatment	$t_{1/2}$ (min)	AUC_{inf} (μg min ml^{-1})	Cl (ml min^{-1} kg^{-1})	V (l kg^{-1})	F (%)	T_{max} (min)	C_{max} (μg ml^{-1})
Group 1, i.v.	50.94 ± 4.10 [a]	160.68 ± 54.14	66.51 ± 19.12	4.87 ± 1.40	–	–	–
Group 2, i.v.	73.57 ± 22.61	163.41 ± 53.12	69.76 ± 27.13	7.86 ± 4.35	–	–	–
Group 3, i.v.	47.99 ± 6.08	144.22 ± 32.18	73.68 ± 12.71	5.16 ± 1.31	–	–	–
Pooled i.v. data	57.75 ± 17.82	151.18 ± 46.51	73.04 ± 23.37	6.27 ± 3.28			
Group 1, powder suspension	–	4.07 ± 1.93	–	–	1.29 ± 0.62	–	–
Group 2, solution	143.91 ± 110.10	38.51 ± 36.73	–	–	10.16 ± 7.11	–	–
Group 3, coprecipitate	105.07 ± 44.49	15.30 ± 5.78	–	–	5.56 ± 2.32	39.00 ± 18.00	0.12 ± 0.06

[a] All values are reported as means ± SD with $n = 6$ in groups 2 and 3, while $n = 3$ for group 1.

All the pharmacokinetic parameters of etoposide after i.v. administration in each group were similar by one-way ANOVA at $p \leq 0.05$. Values of F, the absolute oral bioavailability from different formulations, were similar by one-way ANOVA ($p = 0.06$), at $p \leq 0.05$. The F values from solution and coprecipitate were higher than that from etoposide powder by Newman Keuls test at $p \leq 0.05$.

less, the variability in serum concentrations after oral coprecipitate administration was much lower than those after powder and solution dosings. The absolute oral bioavailability from coprecipitate was $5.56 \pm 2.32\%$ as estimated from AUC_{inf}. The mean oral bioavailabilities from the three oral formulations were not different ($p = 0.06$) by one-way ANOVA at $p \leq 0.05$ but the variability in oral bioavailabilities of the different formulations was significantly different by Bartlett's test for homogeneity of variances. The oral bioavailability of etoposide from coprecipitate was similar to that from solution, and both were significantly higher than that from powder suspension by Newman Keuls test at $p \leq 0.05$. The individual and mean values of the absolute oral bioavailability of etoposide from powder suspension, solution and coprecipitate formulations were compared in Fig. 2. The individual values of oral bioavailability

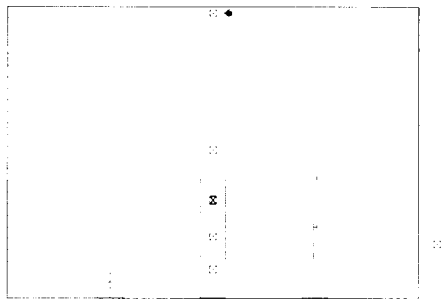

Fig. 2. Individual and mean values (bar) of absolute oral bioavailabilities of etoposide from various formulations.

of etoposide from coprecipitate were in the same range as those from solution formulation for all rats except for one which had a much higher oral bioavailability from solution formulation, 24.39%, as indicated by the arrow in Fig. 2.

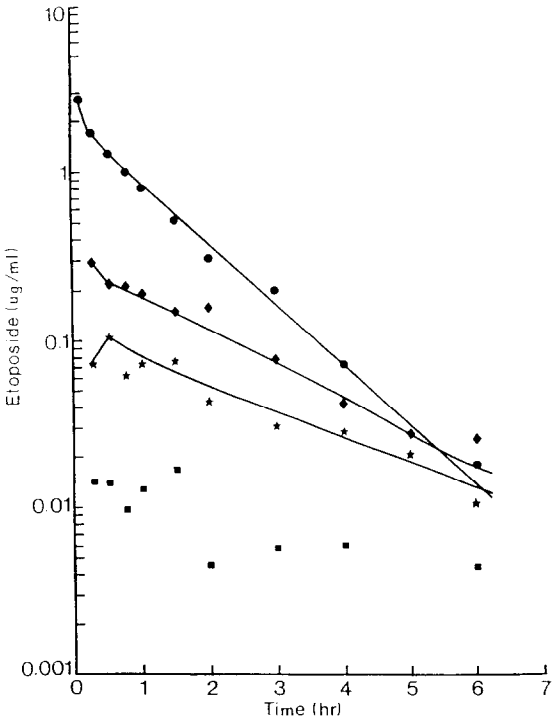

Fig. 1. Mean serum etoposide concentration time profiles after 10 mg/kg, i.v. (●) and 20 mg/kg, oral administrations of solution (◆), coprecipitate (★), and powder suspension (■) formulations of etoposide. $n = 6$ for each formulation except for powder suspension where $n = 3$.

In situ absorption study of etoposide

The in situ absorption studies of etoposide were conducted using the two above-mentioned techniques (Doluisio and Levine). The volume corrected concentrations of etoposide and salicylic acid were reported as percentage of the zero time concentration in Fig. 3. Etoposide concentrations in the perfusate remained around 100% throughout the duration of the experiment, while the concentration of salicylic acid declined with time (Fig. 3). The dpm count due to radioactive inulin in the perfusate increased with time indicating water absorption. Salicylic acid was absorbed to the extent of 62.74%, while water was absorbed to the extent of $42.62 \pm 20.37\%$ in the 2 h period. Water was absorbed to a greater extent in the lower small intestine (44.5%) as compared to that in upper small intestine (12.4%). Water and salicylic acid absorptions confirmed the validity of the in situ technique and the animal model for absorption studies. However, no significant absorption of etoposide was demonstrated in rat with the Doluisio technique.

Etoposide absorption from stomach and whole small intestine was also studied by the Levine technique. Etoposide was not absorbed from stomach, but 7.91% of the drug was absorbed

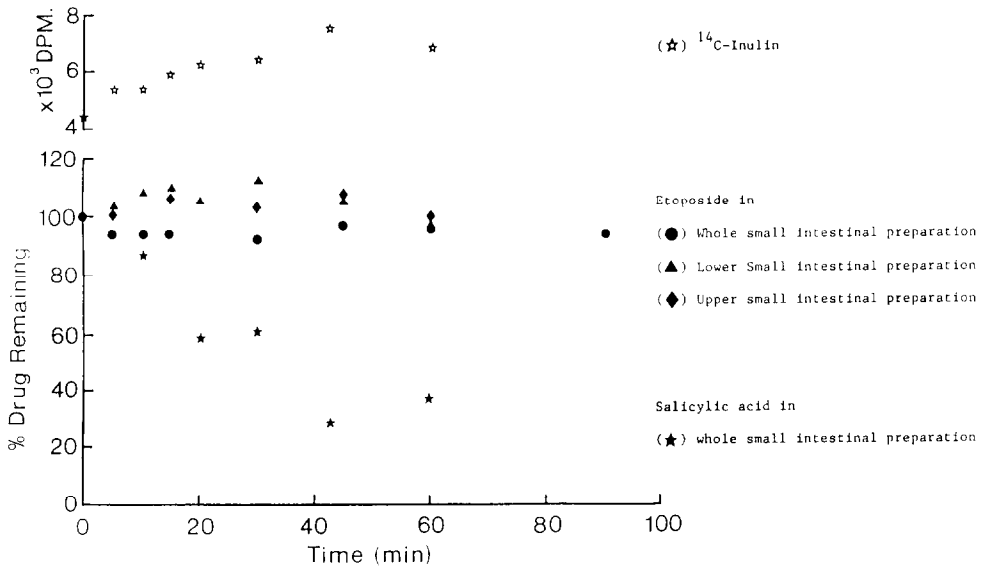

Fig. 3. Percentage of drug remaining in the perfusate vs time profile in the in situ absorption experiments by the Doluisio Technique. $n = 3$.

from whole small intestine. On the other hand, both salicylic acid and water were significantly absorbed, demonstrating the validity of the in situ technique. Salicylic acid was absorbed to a greater extent from small intestine (92.69%) as compared to that from stomach (35.48%, Table 3). Water was also absorbed to a greater extent from the small intestine (44.92%) as compared to 25.54%

TABLE 3

Extents of absorption of etoposide, salicylic acid and water from different segments of gastrointestinal tract by the in situ absorption experiments

Technique used	% etoposide absorbed	% salicylic acid absorbed	% water absorbed
(1) Doluisio technique ($n = 3$)			
(a) Whole small intestine	7.89 ± 3.45 [a]	62.74	42.62 ± 20.37
(b) Upper small intestine	1.58	–	12.42
(c) Lower small intestine	2.38	–	44.50
(2) Levine technique ($n = 1$)			
(a) Stomach	0.00	35.48	25.54
(b) Whole small intestine	7.91	92.69	44.92
(3) Effect of 10 mM sodium taurocholate (Doluisio technique, whole small intestine, $n = 3$)	0.00	98.38 ± 0.26	39.12 ± 3.77

[a] Mean ± SD.

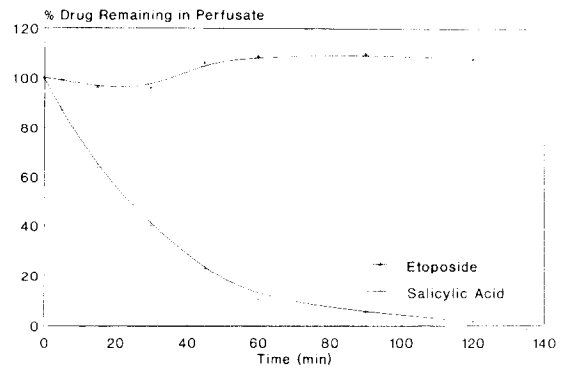

Fig. 4. Effect of 10 mM sodium taurocholate on in situ absorption of etoposide and salicylic acid. $n = 3$.

from stomach. Therefore, the Levine technique also failed to demonstrate any significant absorption of etoposide from stomach and small intestine.

Effect of bile salt on etoposide absorption

The percentage of etoposide remaining in the perfusate at various time intervals following perfusion of the whole small intestine with 10 mM sodium taurocholate containing Krebs buffer is reported in Table 3, and plotted vs time in Fig. 4. Etoposide concentrations in the perfusate remained around 100% throughout the duration of the study, while salicylic acid concentration decreased exponentially with time. The apparent absorption rate constant of salicylic acid was estimated from the slope of the logarithm of % drug remaining in the perfusate vs time profile. The absorption half-life of salicylic acid was 19.75 ± 1.39 min, and $98.38 \pm 0.26\%$ of perfused salicylic acid was absorbed in 2 h. Water was absorbed to the extent of $39.12 \pm 3.77\%$ in 2 h. Thus, sodium taurocholate at 10 mM concentration reduced etoposide absorption from 7.9 to 0%, but salicylic acid and water were absorbed rapidly.

Discussion

Etoposide exhibited two-compartmental pharmacokinetic characteristics in rat. There was less variability in elimination half-life and plasma clearance than the volume of distribution, within and between groups (Table 2). Etoposide has a high plasma clearance in rat, 73.04 ml/min per kg, with a very short elimination half-life, 57.75 min, in contrast to the plasma clearance and elimination half-life in humans of 0.5–0.65 ml/min per kg and 3–11 h, respectively, (Clark et al., 1987). Colombo et al. (1981, 1983) reported the plasma clearance and elimination half-life of etoposide in rat to be 100 ml/min per m^2 and 0.6 h, respectively, which agree with the values determined in this study.

The oral pharmacokinetics of etoposide in rat has not yet been reported in the literature. This study showed that etoposide absorption from powder suspension was very poor, erratic, and almost negligible in rat. Unlike these findings in rats, 30% etoposide was absorbed in humans, from a powder suspension in lipophilic solvent, although absorption was erratic (Clark et al., 1987). The solution formulation involved no dissolution step and should result in maximum bioavailability of all the oral formulations tested. However, the results of this study indicate that bioavailability from solution formulation, although significantly higher than that of powder suspension, was low and highly variable, $10.16 \pm 7.11\%$.

The bioavailability of etoposide from the coprecipitate was significantly higher, 4–5-fold, than that from powder suspension, indicating that enhancement of aqueous solubility and dissolution rate did improve oral absorption of etoposide. In addition, bioavailability from the coprecipitate was less variable than that from solution (Figs 1 and 2; Table 2), and there was no significant difference in bioavailabilities from both of these formulations.

The solution formulation of etoposide has been reported to have physical instability, characterized by the concentration-dependent precipitation of etoposide on dilution (Clark et al., 1987; Vepesid, PDR 1991). The rate of precipitation will depend on the extent of dilution, rate of agitation, gastrointestinal motility, the effect of cosolvents, and the rate of redissolution. The variability of the above factors could all contribute to the variable oral bioavailability from

the solution formulation. Since coprecipitate increased the dissolution rate 42-fold without subsequent precipitation of etoposide, the abovementioned factors cannot affect the bioavailability from the coprecipitate. Hence, the bioavailability from the coprecipitate formulation was much less variable than that from solution. In humans, administration of oral solution results in unpredictable and highly variable bioavailability ranging from 25 to 75% (Clark et al., 1987). Thus, the finding of highly variable absorption from an oral solution formulation in rat is somewhat similar to that in humans. The extent of etoposide absorption from all the formulations was very low, even the solution formulation had only 10.16% bioavailability. The poor absorption of etoposide in rat in contrast to that in humans cannot be adequately explained by low aqueous solubility, slow dissolution rate and instability at acidic pH values of the drug.

In addition, the terminal half-lives of etoposide estimated by ESTRIP, after oral administrations of solution and coprecipitate formulations were higher than that after i.v. administration (Table 2). The apparent absorption half-lives, estimated by ESTRIP, in many rats were similar to the elimination half-life after i.v. dosing. These differences in terminal apparent elimination half-lives can be due to several reasons: a flip-flop model and/or a prolonged absorption phase, or a change in clearance. The change in clearance after one dose of etoposide is unlikely, since acute renal or hepatic dysfunction has not been observed with the clinically equivalent doses of etoposide (Clark et al., 1987). In addition, there was a 7 day washout period between i.v. and oral dosings, long enough time to allow all the drug to be eliminated from the body in view of the very rapid elimination of etoposide in rat. Etoposide was absorbed to the extent of only 5–10% from different oral formulations and hence there is a possibility of prolonged continuous absorption of etoposide in the terminal apparent elimination phase. Moreover, etoposide is also possibly absorbed at a slower rate than when being eliminated which could result in a flip-flop model of absorption. Thus, poor absorption characteristics of etoposide may be more responsible for the observed difference in terminal half-lives than a change in clearance.

The in situ absorption studies showed that etoposide was absorbed poorly in the entire gastrointestinal tract of rat from solution. Etoposide concentration in the perfusate was below the saturation solubility and hence no precipitation of drug could have occurred. Etoposide was stable throughout the duration of the in situ experiment as no degradation product's peak was observed in the stability indicating HPLC assay (Chow et al., 1987). There was no presystemic metabolism as the concentration of etoposide in the perfusate remained nearly around 90–100%. The in situ preparations were physiologically normal as evident by the absorptions of water and salicylic acid (Figs 3 and 4, Table 3). However, the physicochemical properties of etoposide are quite different from those of water and salicylic acid and hence, similar rates and extents of absorptions should not be expected. The Levine technique is more physiological than the Doluisio technique because the gastrointestinal segments are placed back into the abdominal cavity and the incision is sutured without a significant alteration of circulation to the segments. Nevertheless, even experiments with the Levine technique failed to demonstrate significant etoposide absorption. In the true physiological situation, free and conjugated bile salts such as sodium taurocholate are present in the gastrointestinal fluids at 5–10 mM concentrations and facilitate the absorption of dietary lipids and lipid-soluble drugs (Gibaldi et al., 1970). But the absorption of etoposide was reduced rather than enhanced by sodium taurocholate at 10 mM concentration (Fig. 4). The extent of etoposide absorption in the in situ Doluisio experiment (7.89%) agrees well with the absolute oral bioavailability of 10.2% from solution formulation. Thus, the in situ absorption experiments confirmed that etoposide was poorly permeable through the entire gastrointestinal tract of rat. This also explained the very low oral bioavailability of etoposide from solution formulation (Table 2) and the extremely poor and erratic absorption from powder suspension, from which absorption would be further limited by poor dissolution rate.

Unfortunately, there is not a single detailed report on oral absorption and pharmacokinetics of etoposide in rat (Clark et al., 1987). In previous i.v. pharmacokinetic studies in rat (Colombo et al., 1981, 1983), large amounts (50–90%) of etoposide were found in bile and intestine, and eliminated in the feces after i.v. administration to rat. No secondary peaks were observed in the plasma concentration-time profile in the same study. The plasma clearance of etoposide in rat is very high which may be due to the elimination via bile into feces. All these observations point to the absence of enterohepatic recirculation, i.e., no reabsorption of etoposide from intestine.

To determine the role of mucus in etoposide absorption, the appearance of etoposide in the efferent jejunal vein was studied following drug perfusion in the intestine of rat (Schurgers et al., 1985). The extent of etoposide absorption was poor, only 2% of the perfused drug being absorbed in 1 h. The flux of etoposide from the lumen of intestine into the blood was 3.5 ng/min per cm, equivalent to 2.79 ng/min per cm^2 of intestinal wall (Schurgers et al., 1985). The intrinsic dissolution rate of etoposide was 0.01 mg/min per cm^2 (Shah et al., 1989), far greater than the flux or the intestinal permeability reported above. Therefore, etoposide absorption in rat is intestinal permeability limited.

Etoposide bioavailability in humans is dissolution rate limited but not permeation rate limited as evident based on up to 75% bioavailability from solution (Clark et al., 1987). Hence, rat may not be a good animal model to evaluate oral bioavailability of etoposide formulations with improved dissolution rate, followed by extrapolation of the results to humans.

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References

Brown, R.D. and Manno, J.E., ESTRIP, a basic computer program for obtaining initial polyexponential parameter estimates. *J. Pharm. Sci.*, 67 (1978) 1687–1691.

Chow, D., Shah, J. and Chen, J.R., Stability-indicating high-performance liquid chromatography of etoposide at various pH conditions using a reversed phase octyl column. *J. Chromatogr.*, 396 (1987) 217–223.

Clark, P.I. and Slevin, M.L., The clinical pharmacology of etoposide and teniposide. *Clin. Pharmacokinet.*, 12 (1987) 223–252.

Colombo, T., Broggini, M., Torti, L., Erba, E. and D'Incalci, M., Pharmacokinetics of VP-16-213 in lewis lung carcinoma bearing mice. *Cancer Chem. Pharmacol.*, 7 (1981) 127–131.

Colombo, T., D'Incalci, M., Farina, P., Rossi, C., Guaitani, A., Bartosek, I., Benfenati, E., Fanelli, R. and Garattini, S., Comparison of pharmacokinetics of VP-16 in mice, rats, and humans. *Proc. Am. Assoc. Cancer Res.*, 24 (1983) 292.

Doluisio, J.T., Billups, N.F., Dittert, L.W., Sugita, T.E. and Swintowksy, J.V., Drug absorption. I: An in-situ rat gut technique yielding realistic absorption rates. *J. Pharm. Sci.*, 58 (1969) 1196–1200.

Gibaldi, M. and Feldman, S., Mechanism of surfactant effects on drug absorption. *J. Pharm. Sci.*, 59 (1970) 579–589.

Issell, B.F. and Crooke, S., Etoposide (VP-16-213). *Cancer Treat. Rev.*, 6 (1979) 107–124.

Levine, R.M., Blair, M.R. and Clark, B.B., Factors influencing the intestinal absorption of certain monoquarternery anticholinergic compounds with special reference to benzomethamine. *J. Pharmacol. Exp. Ther.*, 114 (1955) 78–85.

Savaraj, N., Lu, K., Feun, L.G., Burgess, M.A. and Loo, T.L., Comparison of CNS penetration, tissue distribution and pharmacology of VP-16-213 by intracarotid and intravenous administration in dogs. *Cancer Invest.*, 5 (1987) 11–16.

Schurgers, N., de Blaey, C.J. and Crommelin, D.J.A., Absorption of etoposide (VP-16-213) from the small intestine of the rat: The potential role of mucus as an absorption rate limiting barrier. *Pharm. Res.*, 2 (1985) 162–165.

Shah, J., Chen, J.R. and Chow, D., Preformulation study of etoposide: Identification of physicochemical characteristics responsible for the low and erratic oral bioavailability of etoposide. *Pharm. Res.*, 6 (1989) 408–413.

Shah, J., Chen, J.R. and Chow, D., Preformulation study of etoposide-II: Increased solubility and dissolution rate by solid dispersions and polymorph formation. *J. Pharm. Sci.*, (1992) submitted.

Sinkule, J.A., Etoposide: A semisynthetic epipodophyllotoxin. *Pharmacotherapy*, 4 (1984) 61–73.

Vepesid, Physicians Desk Reference, Medical Economics Co. Inc., Oradell, NJ, 45th Edn, 1991, pp. 737.