Cancer Chemother Pharmacol (1995) 37: 117–124

ORIGINAL ARTICLE

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Stability of the i.v. and oral formulations of etoposide in solution

Received: 27 September 1994/Accepted: 31 March 1995

Abstract Etoposide is a widely used cytotoxic drug that requires complex formulation for both the i.v. and oral preparation to ensure drug stability. Data on the stability of the i.v. formulation when diluted in infusion fluids are contradictory, and there is little information on the stability of the oral preparation in gastric or intestinal fluids. The stability of both i.v. and oral etoposide was therefore evaluated in the present investigation. The stability of the i.v. preparation was investigated across a range of concentrations in infusion fluids, being determined by regular sampling for high-performance liquid chromatography (HPLC) analysis and by visual inspection. The stability of the oral preparation was studied in both artificial gastric and intestinal fluids, again with regular sampling for HPLC analysis, and the influence of pH, concentration and the addition of ethanol and bile salts on oral stability was determined. The i.v. preparation showed a marked decrease in stability with increasing drug concentration, but stability was additionally reduced in i.v. bags regularly sampled with a syringe and needle as compared with bags that were inspected visually only (minimal stability in sampled bags, 24 h at 0.5 mg/ml and 5 h at 1.0 mg/ml, as compared with 10 days and 18 h at the respective concentrations in unsampled bags). Stability was also greater at room temperature, 20–23°C, as compared with 8–12°C. Loss of stability was indicated by a decrease in etoposide concentration (measured by HPLC) and the appearance of a fine white precipitate, shown to be pure etoposide. Importantly, the appearance of precipitate was as sensitive as a specific HPLC assay in detecting loss of stability and was in many cases apparent when the etoposide concentration was within 5% of the starting concentration. The oral formulation also showed a marked concentration-dependent decrease in stability in artificial intestinal fluid at pH 7.5 (percentage of etoposide in solution after 2 h at 0.5, 1.0, 1.5 and 2.0 mg/ml, 94 ± 2%, 80 ± 5%, 68 ± 13% and 41 ± 9%, respectively). There was no concentration effect on stability in gastric fluid at pH 3.0, although stability was much greater at pH 3 and pH 5 as compared with pH 1 or in intestinal fluid at pH 7.5. Stability in artificial intestinal fluid, pH 7.5, was also significantly improved by the addition of the bile salt sodium tauroglycocholate (2 mg/ml) at etoposide concentrations of 1 ($P < 0.0001$) and 2 mg/ml ($P < 0.0001$) and by the addition of ethanol (10%, v/v) at etoposide levels of 1 ($P < 0.001$) and 2 mg/ml ($P < 0.001$). These studies clearly demonstrate the concentration-dependent stability of both the i.v. and the oral formulation of etoposide, that the appearance of precipitate is a sensitive indicator of loss of stability in i.v. fluids, and that stability in artificial intestinal fluid can be modulated by the use of other agents.

Keywords Etoposide · Stability · Oral formulation
Intravenous solution · Stability-modulating agents

Introduction

Etoposide is a semi-synthetic podophyllotoxin used in the treatment of a number of malignancies that has more recently been used in prolonged schedules employing either continuous infusions or oral dosing. However, prolonged dosing with either the i.v. or the oral formulation may be hampered by the poor solubility of etoposide, which requires complex formulation. Each 100-mg i.v. ampoule also contains Tween 80, 400 mg; polyethylene glycol 300, 3.25 g; benzyl alcohol, 150 mg; anhydrous citric acid, 10 mg; and absolute

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alcohol to a volume of 5 ml. The non-ionic surfactant Tween 80, in addition to the organic solvents, increases the miscibility of the i.v. preparation in solution.

The current data on the stability of i.v. etoposide in solution are unclear. Etoposide was stable for 6 h at room temperature in 0.9% saline or 5% dextrose at a concentration of 0.2 mg/ml in one report [3] but was determined to be stable at 0.25 mg/ml for 72 h in another [8]. At a concentration of 0.4 mg/ml the drug is reportedly stable for 3–6 h in 0.9% saline or 5% dextrose [3, 6, 8], whereas other investigators describe that at that concentration in either saline or dextrose, stability is maintained for at least 4 days [5]. The concentration-dependent nature of etoposide stability is clearer at higher concentrations; at 1 mg/ml, etoposide remains completely in solution for only 5–30 min [3, 8]. However, other authors have reported that when diluted in 0.9% saline and stored in 10-ml syringes at 4°C and 20°C, etoposide was stable at 1 mg/ml for at least 14 days [1, 2] (although random precipitation, attributed to particle seeding rather than to chemical degradation, occurred in about 10% of syringes) and at 10 mg/ml for 22 days [17].

Indeed, the contradictory nature of these data extends to the drug information sheets, as the United Kingdom pack insert states that the i.v. preparation must be diluted to a concentration of not more than 0.25 mg/ml and used within 6 h, whereas the United States insert states that the preparation can be diluted to a concentration of 0.2 or 0.4 mg/ml, which is stable for 96 and 48 h, respectively. Stability may also be affected by other drugs, with etoposide being stable at a concentration of 0.4 mg/ml for 72 h in the presence of cytarabine and daunorubicin [21] but showing precipitation after only 24 h at the same concentration in the presence of cisplatin [23].

Little is known of the stability in solution of the oral preparation of etoposide, yet this feature of etoposide pharmacology may be important in view of the variable bioavailability and the known non-linear relationship between oral etoposide dose and absorption [13, 14]. Indeed, the poor and variable bioavailability of oral etoposide has been attributed to its poor aqueous solubility and instability at low pH [22]. The soft gelatin capsule of etoposide contains 100 mg of drug in a 1-ml formulation that also contains citric acid, water, glycerine and polyethylene glycol 400. Data from Bristol-Myers indicate that etoposide is stable for at least 3 h when diluted in orange juice, lemonade or dextrose water [7], but its stability and solubility in gastric and intestinal fluid is unknown. In acidic solution, etoposide has been shown to degrade to its lignan P [10] and aglycone forms [4, 10, 19, 24], whereas in basic solution it degrades to the cis-lactone form [4, 9, 16], which can further degrade to the cis-hydroxy acid form [4, 16].

Investigation of the stability of the i.v. preparation of etoposide in solution was therefore performed to determine its stability over a range of concentrations in the types of i.v. fluids given to patients and in bags sampled by syringe. Studies were also conducted to determine the stability of the oral preparation of etoposide in artificial gastric and small-intestinal fluids to observe the effects of concentration on stability in such fluids and to investigate factors that may improve stability, such as the addition of ethanol or bile salts. The data presented herein to describe the stability of the i.v. preparation have previously been published in abstract form [15].

Materials and methods

Investigation of the stability of the i.v. formulation of etoposide in i.v. fluids

Ampoules of etoposide were obtained from Bristol-Myers (UK); i.v. solutions (250- and 500-ml bags) of 9% saline, 5% dextrose and 4% dextrose in 0.18% saline were supplied by Travenol in Viaflex bags.

Effect of etoposide concentration on drug stability in sampled i.v. solutions

Solutions of etoposide in 500-ml bags of 0.9% saline, 5% dextrose and 4% dextrose in 0.18% saline were prepared at concentrations of 0.25, 0.4, 0.5, 0.6, 0.75 and 1 mg/ml using syringes and needles supplied by Sabre. At each concentration and in each i.v. fluid, solutions were made up in triplicate. Solutions were left at room temperature and were exposed to light during the hours of daylight (12 h light per 24-h cycle). Samples for the measurement of etoposide concentration were withdrawn at regular intervals through the bung of the i.v. bag using a syringe and needle, with the actual interval being dependent on the etoposide concentration. For instance, at a concentration of 1 mg/ml, bags were sampled and inspected hourly; at 0.5 mg/ml, 6-hourly; and at 0.25 mg/ml, 12-hourly.

Etoposide concentrations were measured using a previously described high-performance liquid chromatography (HPLC) assay [12] with separation on a 5-μm ODS column (15 × 4.5 cm), an isocratic mobile phase (47% methanol:53% water) and UV detection at 229 nm. (The methanol content of the mobile phase was reduced to 47% from the 51% published value to ensure resolution of the cis- and trans-etoposide.) Retention times for etoposide and possible or reported breakdown products in this assay system were: cis-hydroxy acid, 1 min; 3′-*O*-demethyl etoposide, 2.5 min; 3′-demethyl 4-dehydro-etoposide, 3 min; aglycone, 4 min; etoposide, 7.5 min; and cis-etoposide, 9 min (all compounds kindly supplied by Bristol-Myers Squibb, Syracuse, N.Y.). Samples were not put through the extraction stage of the published assay but were spun at 13,000 rpm for 5 min and then diluted 1:1 with methanol:water (47%:53%) containing di-phenylhydantoin (Sigma Chemicals, Poole, Dorset) as the internal standard (retention time, 10.5 min). The reproducibility of this system (calculated from multiple dilutions and injections of the same sample) was $< 2\%$. The time of loss of stability at each concentration or in each fluid was taken as either (1) the time of appearance of precipitate in any bag or (2) the time at which the concentration of etoposide fell by more than 10% below the initial two etoposide concentrations in any bag.

Stability of etoposide in unsampled bags of 0.9% saline

Preparations of etoposide in 250-ml bags of 0.9% saline were made up at concentrations of 0.25, 0.5, 1.0 and 2.0 mg/ml in quintuplicate.

The bags were left unsampled in the ward treatment room at 20–23°C and in a cool side-room at 8–12°C. At the time of performing this study it was not clinical practice to leave bags of etoposide in solution in the refrigerator, and these temperatures reflect the range that made-up bags may be exposed to either on a hospital ward or during outpatient infusions. The time of appearance of precipitate in each bag was noted.

Investigation of the stability and solubility of oral etoposide

Etoposide capsules were supplied by Bristol-Myers (UK). Pepsin and pancreatin were purchased from Sigma Chemicals. Artificial gastric and intestinal fluid were prepared according to the United States Pharmacopoeia. Gastric fluid comprised 2 g sodium chloride and 3.2 g pepsin in 1 l of de-ionised water, adjusted to the desired pH with concentrated hydrochloric acid and 0.2 *M* sodium hydroxide. Gastric fluid was prepared at pH 1, 3 and 5. Artificial intestinal fluid consisted of 6.8 g potassium dihydrogen orthophosphate, 10 g pancreatin, 190 ml of 0.2 *M* sodium hydroxide and 700 ml de-ionised water, adjusted to pH 7.5 and then made up to 1 l. Solutions were prepared freshly before each experiment. These fluids were pre-incubated to 37°C before the addition of drug.

Immediately before addition to the artificial gastric or intestinal fluid the solution of etoposide inside the capsule was withdrawn with a syringe and needle. This solution contains 100 mg etoposide/ml. The appropriate amount of drug was added to 25-ml volumes of gastric or intestinal fluid in 30-ml universal plastic containers with airtight screw-caps (Sterilin). These were incubated at 37°C and agitated slowly for the duration of sampling using a Denley Spiramix 5 apparatus.

Samples of 200 µl were withdrawn at 0.15, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6 and 8 h after the addition of etoposide. These specimens were centrifuged at 13,000 rpm for 5 min, and 50 µl supernatant was added to an appropriate volume of mobile phase (47% methanol:53% water) to which di-phenylhydantoin had been added as the internal standard at a concentration of 100 µg/ml. These were then injected directly into the HPLC system. All experiments were performed in quintuplicate. Gastric and intestinal fluid blanks were also run as negative controls and similarly assayed. The effects of various factors on the stability and solubility of etoposide were also investigated.

The pH of artificial gastric and intestinal fluid

The stability of oral etoposide in solution at a concentration of 1 mg/ml was tested in artificial gastric fluid at pH 1, 3 and 5, and in artificial intestinal fluid at pH 7.5. As the pH of gastric fluid in the fasting state is around pH 3, statistical comparisons of stability at pH 1, 5 and 7.5 in intestinal fluid were made against that in gastric fluid at pH 3.

Concentration of etoposide in solution

The stability of oral etoposide in solution at concentrations of 0.5, 1.0, 1.5 and 2.0 mg/ml was examined in intestinal fluid at pH 7.5. The concentrations of 1 and 2 mg/ml were also tested in gastric fluid at pH 3.

The co-addition of sodium tauroglycocholate

The stability of oral etoposide in solution at a concentration of 2 mg/ml in artificial gastric fluid of pH 3 and at concentrations of 1 and 2 mg/ml in intestinal fluid was examined with and without the presence of sodium tauroglycocholate at a concentration of 2 mg/ml.

The co-addition of ethanol

Etoposide in solution at a concentration of 2 mg/ml in artificial gastric fluid of pH 3 and at concentrations of 1 and 2 mg/ml in intestinal fluid was examined with and without the presence of ethanol at a concentration of 0.1 ml/ml (v/v).

Statistical analysis

A time-series median test was used to compare the in vitro stability data on oral etoposide obtained in one incubation against those acquired in another up to specific time points, and two-way analysis of variance (AOV) was used to compare different incubations across all time points.

Results

Investigation of the stability of the i.v. formulation of etoposide in i.v. fluids.

Effect of etoposide concentration on stability in i.v. solutions.

A summary of the results of this study is shown in Table 1. The values shown are the time at which any one of the triplicate bags demonstrated a change in appearance or a > 10% drop in etoposide concentration. These data confirm that the stability of etoposide in solution is clearly dependent on concentration. At a concentration of 1 mg/ml, the minimal stability was only 5 h; at 0.5 mg/ml, 24 h; and at 0.25 mg/ml, at least

Table 1 Effect of concentration on the stability of i.v. etoposide in 0.9% saline, 5% dextrose and 4% dextrose in 0.18% saline. The time of loss of stability is given in hours. Data shown represent the time of loss of stability for any one of triplicate bags at each concentration and for each solution (*S* 9% saline, *D* 5% dextrose, *DS* 4% dextrose in 0.18% saline)

Etoposide (mg/ml)	Time of precipitation (h)			Time of > 10% fall in conc^n (h)			Minimal recommended stability (h)		
	S	D	DS	S	D	DS	S	D	DS
0.25	> 192	> 192	> 192	> 192	> 192	> 192	> 192	> 192	> 192
0.4	> 168	> 168	> 168	> 168	> 168	> 168	> 168	> 168	> 168
0.5	30	48	30	30	48	30	24	30	24
0.6	24	24	24	30	24	24	18	18	18
0.75	24	10	12	24	10	24	12	8	10
1.0	6	6	8	8	8	> 8	5	5	6

8 days. There was little difference in etoposide stability as determined between 0.9% saline, 5% dextrose or 4% dextrose in 0.18% saline. The visual appearance of precipitate frequently preceded a 5% drop in etoposide concentration as measured by HPLC and in no instance did the measured etoposide concentration fall by > 10% before the visual detection of precipitate.

No cis-etoposide was detected in any of the samples taken. In several of the bags in which a precipitate had formed, this precipitate was harvested, re-dissolved in mobile phase and injected into the HPLC system. In every case this re-dissolved precipitate was demonstrated to be pure trans-etoposide.

Effect of concentration of etoposide in unsampled bags

The results are shown in Table 2. These results confirm the effect of concentration on etoposide stability and demonstrate that the stability of etoposide in unsampled bags was greater than that in bags that had been frequently sampled with a syringe and needle as described above. Stability was also greater at higher temperature, such that at 20–23°C, etoposide at a concentration of 0.5 mg/ml was stable for a minimum of 10 days, whereas at 8–12°C, stability was assured for only 6 days.

Investigation of the stability of oral etoposide in solution

Effect of pH in artificial gastric and intestinal fluids

The decrease in etoposide concentration with time from a starting concentration of 1 mg/ml is shown in Fig. 1. Etoposide concentrations for the first 90 min of the study were typically > 90% of the made-up concentration value (1 mg/ml) at pH 1, 3 and 5, but incubation in intestinal fluid at pH 7.5 gave a concentration at 15 min of only just over 0.85 mg/ml, demonstrating an almost immediate loss of stability (versus pH 3 at 0.15 h; $P = 0.002$). Although at pH 3 and pH 5 in gastric fluid the stability was retained for at least the first 4 h, with the measured concentration typically being > 0.90 mg/ml, the initial loss of stability at pH 5 was also significantly different from that at pH 3 (concentration at 0.15 h at pH 3, 0.95 ± 0.02 mg/ml, as compared with 0.90 ± 0.02 at pH 5; $P = 0.002$). There was a marked decline in stability in gastric fluid at pH 1.0 that reached statistical significance as compared with pH 3 at 3 h (pH 3 concentration, 0.90 ± 0.03 mg/ml, versus 0.72 ± 0.03 mg/ml at pH 1; $P = 0.01$). Stability in intestinal fluid also continued to decline with time, giving a 3-h concentration of 0.75 ± 0.09 mg/ml. At pH 1, etoposide degraded to lignan P and the aglycone form, whereas at pH 7.5 no degradation product was detected.

Effect of concentration of etoposide in artificial intestinal fluid at pH 7.5 and in gastric fluid at pH 3

Intestinal fluid at pH 7.5. Recovered etoposide concentration against time at four different starting concentrations is shown in Fig. 2. These results show a clear concentration-dependent loss of stability of etoposide in artificial intestinal fluid, where the difference in stability between 0.5 mg/ml and the three higher concentrations was apparent as early as after only 10 min [percentage of etoposide remaining in solution at 0.5 mg/ml

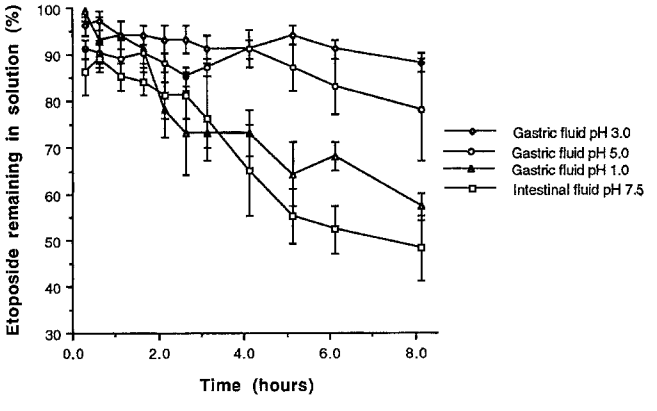

Fig. 1 Effect of pH on the stability of oral etoposide at 1 mg/ml (mean ± SD)

Table 2 Effect of concentration on the stability of i.v. etoposide in 0.9% saline in unsampled bags at 8–12°C and 20–23°C. Data shown represent the time of loss of stability for any one of quintuplicate bags at each concentration and for each storage temperature

Etoposide concentration (mg/ml)	Temperature	Time of precipitation	Minimal recommended stability
0.25	8–12°C	> 3 weeks	> 3 weeks
	20–23°C	–	–
0.5	8–12°C	7 days	6 days
	20–23°C	11 days	10 days
1.0	8–12°C	12 h	10 h
	20–23°C	24 h	18 h
2.0	8–12°C	8 h	6 h
	20–23°C	10 h	8 h

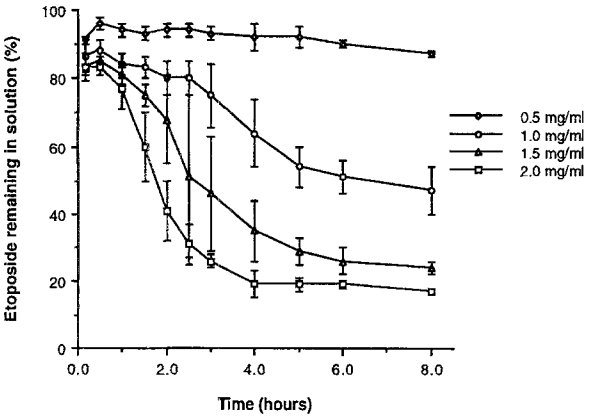

Fig. 2. Effect of concentration of oral etoposide on stability in artificial intestinal fluid at pH 7.5 (mean ± SD)

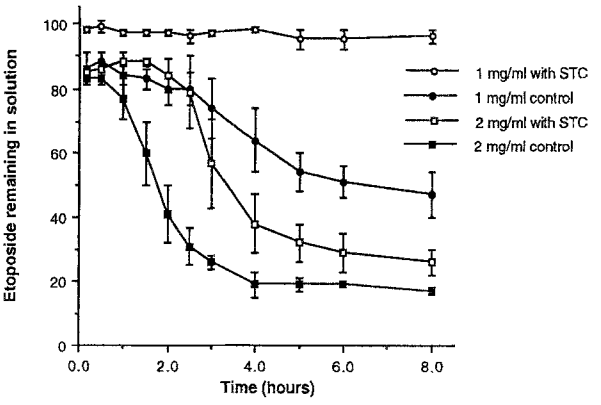

Fig. 3. Effect of sodium tauroglycocholate (*STC*) at 2 mg/ml on the stability of oral etoposide at 1 and 2 mg/ml in intestinal fluid (mean ± SD)

versus 1 mg/ml ($P = 0.04$), 1.5 mg/ml ($P = 0.007$) and 2 mg/ml ($P = 0.007$)]. Stability continued to decline with time such that at 2 h the mean percentage of etoposide remaining in solution at 0.5, 1, 1.5 and 2 mg/ml was 94 ± 2%, 80 ± 5%, 68 ± 13% and 41 ± 9%, respectively, and that at 4 h was 92 ± 4%, 64 ± 10%, 35 ± 9% and 19 ± 4%, respectively. From 4 to 8 h the 2-mg/ml solutions plateaued at around 20% of the starting concentration, whereas from 6 to 8 h the 1.5-mg/ml solutions started to plateau at around 25% of the starting concentration and the 1.0 mg/ml solutions, at 40–50%. In each case this degree of loss of stability would result in a remaining etoposide concentration of around 0.4 mg/ml.

Gastric fluid at pH 3. Stability at 1 and 2 mg/ml in gastric fluid at pH 3 did not show a dependence on concentration, with the percentage of etoposide remaining in solution typically being > 90% at both concentrations across the 8-h study period.

Effect of addition of sodium tauroglycocholate

The results of co-incubation of etoposide with sodium tauroglycocholate in artificial intestinal fluid at pH 7.5 are shown in Fig. 3 and in gastric fluid at pH 3, in Fig. 4. Sodium tauroglycocholate significantly improved the stability of etoposide both at 1 mg/ml ($P < 0.0001$, AOV, versus etoposide with no STC) and at 2 mg/ml ($P < 0.0001$, AOV). At 1 mg/ml this difference was apparent immediately (at 10 min, $P = 0.001$ versus the control with no STC) and at 2 mg/ml it was apparent after 1 h ($P = 0.003$ versus the control). Although incubation of etoposide and bile salt in gastric fluid resulted in an immediate 20% loss of drug (at 10 min, $P = 0.001$), there was no further loss with continued incubation.

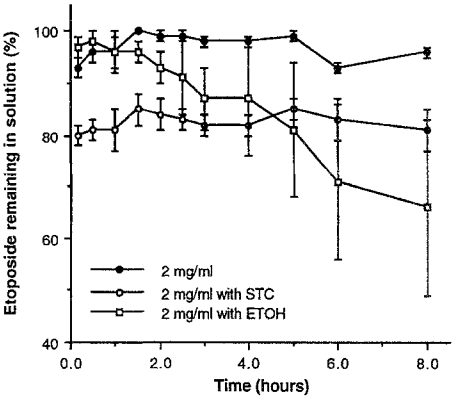

Fig. 4. Effect of STC (2 mg/ml) and ethanol (*ETOH*; 10%, v/v) on the stability of oral etoposide (2 mg/ml) in artificial gastric fluid at pH3 (mean ± SD)

Effect of addition of ethanol

The results of etoposide incubation with ethanol in artificial intestinal fluid are shown in Fig. 5 and those of incubation in gastric fluid at pH 3, in Fig. 4. These show that ethanol significantly improved stability at etoposide concentrations of both 1 mg/ml ($P < 0.001$, AOV, versus the control with no ethanol) and 2 mg/ml ($P < 0.001$, AOV, versus ethanol). This effect was apparent after only 10 min (1 mg/ml versus the control, $P = 0.001$; 2 mg/ml versus the control, $P = 0.001$). After 2 h of incubation at 2 mg/ml the percentage of drug remaining in solution in the presence of ethanol was increased by over 50% as compared with the control (41 ± 9% versus 66 ± 4%). The addition of ethanol to artificial gastric fluid at pH 3 resulted in a 15% loss of etoposide during the first 4 h of incubation and a 25% loss over the full 8-h incubation period. This loss of stability, as compared with control tubes with no added ethanol, became significant at 2.5 h (99 ± 1% versus 91 ± 7%, $P = 0.009$).

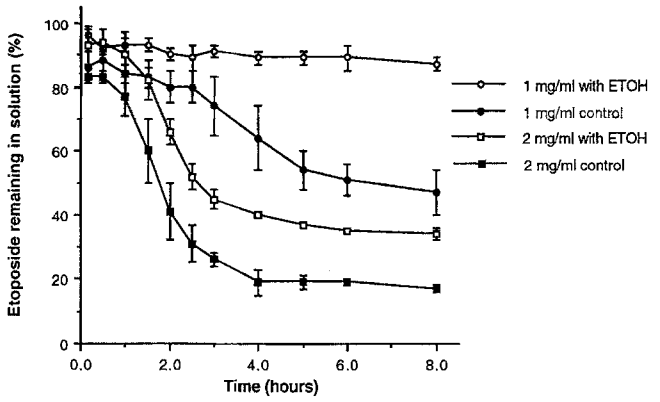

Fig. 5. Effect of ETOH (10%, v/v) on the stability of oral etoposide at 1 and 2 mg/ml in intestinal fluid (mean ± SD)

Discussion

The schedule–dependent nature of the activity of etoposide has led to an increasing number of reports describing prolonged dosing with either oral or, more recently, infusional regimens. It is therefore particularly important that the stability of the i.v. preparation be clearly defined such that both clinicians and pharmacists are aware of the limitations of its use in prolonged infusions, and also that factors that may affect the stability and, thus, the bioavailability of the oral formulation be understood.

The data reported herein have again demonstrated the concentration-dependent nature of etoposide stability, which decreases markedly with increasing drug concentration, but have also suggested that the stability of the i.v. preparation of etoposide in solution is greater than previously described [3, 8]. However, an explanation for differences in etoposide stability at the same concentration between reported studies may lie in the effect of frequent sampling from i.v. fluid bags, as shown by the difference in stability found between the sampled i.v. bags and those that were not sampled in the present study. Another report has described that the unpredictable stability of etoposide in syringes may be due to particulate matter speeding its precipitation [1, 2], and it is possible that the frequent sampling required to permit HPLC analysis of etoposide in the first of our studies may have introduced some particulate matter into the bag or that the disruption to the port surface itself may have been sufficient to provide a locus for particle seeding. This would explain the more prolonged stability observed in the unsampled bags in our second study and would suggest that the results shown in Table 2 are the definitive stability data for practical use when etoposide is made up in i.v. bags. The even more prolonged stability reported for etoposide at 1 mg/ml [1, 2] and 10 mg/ml [17] when the diluted i.v. formulation is stored in syringes may be due to syringes containing less particulate matter than i.v. bags and, therefore, a reduced tendency for particles to form a locus for precipitation.

It should be noted, however, that although the stability time reported herein represents the minimal stability for the five bags at each concentration, there was considerable variability in stability within each concentration. For instance, at 8–12°C, four of five bags showed precipitation between days 6 and 7, whereas the drug in the remaining bag was stable after 21 days. It is therefore possible that if large numbers of bags are prepared some may have a lower stability than that reported herein.

This study also confirms that the loss of stability in i.v. fluids is not accompanied by structural degradation of the molecule, but rather by the formation of a fine white precipitate. Collection, dissolution and analysis of this precipitate showed it to be pure trans-etoposide, the epimer of the drug present in the i.v. formulation and not the considerably less active cis-isomer, as has previously been suggested [18]. Moreover, this study demonstrated that the visual appearance of precipitate is as sensitive, or more so, than the measurement of a fall in etoposide concentration using a sensitive analytical method. There was no instance of the measured etoposide concentration falling by more than 10% of the starting concentration without the appearance of a precipitate, and in many cases precipitation was apparent when the concentration was within 5% of the starting concentration. It is possible that micro-crystals not visible to the naked eye may be present for some time before precipitation is visible and that such microcrystals may be infused into the patient. If so, it is likely that these would dissolve in the greatly increased volume and lower etoposide concentration of the circulation. Additionally, they would be expected to represent less than 5% of the total etoposide dose, as clear precipitation is visible above that point and, thus, would be unlikely to have any clinical consequence.

The daily doses of most standard chemotherapy regimens containing i.v. etoposide vary from 100 to 200 mg, and if given in 500 ml isotonic fluid the final concentration of drug is likely to be between 0.2 and 0.4 mg/ml. At these concentrations etoposide has been found to be stable for at least 10 days at ward temperature. This means that a typical 5-day course of i.v. etoposide could be made up at the start of treatment. If the desired volume of infusion is small or if high-dose etoposide regimens are employed, the etoposide concentration can be raised to 2 mg/ml at the price of prompt use and daily preparation. Etoposide was also shown to be equally stable in 0.9% saline, in 5% dextrose and in 4% dextrose in 0.18% saline. Hence, etoposide in dextrose is an alternative when the administration of saline is contraindicated. The data obtained in this study also show that prepared solutions of

etoposide in i.v. fluids are most stable at room (ward) temperature and should not be refrigerated as this will compromise stability.

The oral capsule of etoposide contains 100 mg of drug in a 1-ml solution as compared with 100 mg in 5 ml in the i.v. ampoule. Since the stability in solution of the i.v. preparation was found to be concentration-dependent, it was suspected that a similar phenomenon would be observed with oral etoposide, but of a possibly higher magnitude in view of the greater starting concentration of the oral formulation. It is difficult to be certain of what the likely intra-gastric and intra-duodenal concentrations of etoposide might be in patients receiving oral etoposide capsules. Most patients swallow their tablets with 100 ml water, and if fasted, the volume of the resting stomach is likely to be 100 ml or less [11]. Thus for doses of 50–400 mg, etoposide concentrations of 0.25 to 2.0 mg/ml would be expected, and etoposide concentrations in artificial intestinal fluid were tested over the range of 0.5–2 mg/ml in this study. However, these concentrations may well be an underestimate unless complete mixing occurs within the gut, and it is possible that local concentrations may be considerably higher than this.

As with the i.v. preparation, etoposide stability in artificial intestinal fluid was distinctly shown to be dependent on the concentration of drug. After 2 h at an etoposide concentration of 2 mg/ml, only 41% of the etoposide remained in solution, whereas at 1 mg/ml the figure was 80%. Doubling the concentration from 1 to 2 mg/ml in artificial gastric fluid at pH 3 did not result in any loss of stability. (The latter pH was chosen because the fasting gastric pH is usually in the pH range of 3–5 [11].) The finding of a concentration-dependent loss of stability offers possible explanations for the variable bioavailability of oral etoposide and the non-linear relation of absorbed drug with increasing dose.

Etoposide has also been shown to lose stability at low pH by both the formation of a precipitate and by degrading to its lignan P and aglycone forms, a phenomenon that has been described elsewhere [4, 10, 24]. The oral preparation of etoposide was shown to be considerably more stable at pH 3 and pH 5 as compared with pH 1 in this study, in line with the pH of 5 reported for pure etoposide in solution [4, 24]. This is in agreement with the data of Shah and colleagues [22], who reported a degradation half-life at 0.1 mg/ml of 2.9 h at pH 1.3 and around 50 days at pH 5–6. At a pH of 7.5 in our study, there was no evidence of epimerisation to the cis-lactone nor any degradation to the cis-hydroxy acid as reported in other studies [4, 24].

As with the i.v. formulation of etoposide, the oral capsule contains organic solvents (polyethylene glycol 400 and glycerine) to keep the drug in solution. When diluted with aqueous solvents, such as gastric and intestinal secretions, the solubility of oral etoposide is inevitably compromised. This suggests that stability may be improved by the addition of an organic solvent, such as ethanol, during incubation in gastric and intestinal fluids. The studies reported herein established that the addition of ethanol (0.1 ml/ml, or 10% final concentration) to artificial intestinal fluid significantly increased the etoposide stability. Although ethanol in gastric fluid of pH 3 appeared to diminish the stability of etoposide, this effect occurred after 4 h, by which time the fasting stomach is likely to have emptied of fluids.

The absorption of a solution containing etoposide in bile salt micelles has been shown to be increased in isolated rat intestinal loops [20]. Although the postulated mechanism for this was by reducing resistance to absorption by affecting the intestinal mucus barrier [20], it is possible that bile salts helped maintain etoposide in solution. The physiological concentration of bile salts in bile lies in the range of 6–24 mg/ml. Since this is diluted with duodenal contents, a concentration of 2 mg/ml was employed in our study. The in vitro studies described herein demonstrated a definite increase in the stability of etoposide in intestinal fluid containing tauroglycocholate, particularly at higher concentrations of etoposide. As sodium tauroglycocholate is insoluble at pH 3, it is not surprising that a 15% loss of etoposide stability occurred in gastric fluid at that pH. Nevertheless, the greater stability produced in intestinal fluid outweighed the loss observed in gastric fluid. This improved stability of etoposide in intestinal fluid containing a bile salt suggests that this may be an important contributory factor to etoposide stability in the gut in vivo.

This somewhat crude method of in vitro assessment of etoposide stability in artificial gastric and intestinal fluids provides pointers to understanding the problems encountered with the absorption of oral etoposide. Etoposide was shown to have limited stability in intestinal fluid, particularly at higher concentrations of drug. Nevertheless, this approach also demonstrates two possible ways of improving the absorption of etoposide by increasing its stability in intestinal fluid with the addition of ethanol or sodium tauroglycocholate. These studies also suggest that as stability is concentration–dependent in intestinal fluid, the rate of gastric emptying may affect the stability of etoposide in both gastric and intestinal fluids by influencing the concentration of drug at those sites. In vivo clinical studies conducted with the stability-modulating agents used in this study, and with agents that modulate gastric emptying may thus improve the bioavailability of oral etoposide and reduce its reported variability.

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