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Investigation of new degradation products arising from the encapsulation of an oil-based suspension formulation of topotecan

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Abstract

Topotecan [Hycamtin®; (*S*)-9-dimethylaminomethyl-10-hydroxycamptothecin hydrochloride; SKF-S-104864-A] is currently marketed as a parenteral product for the treatment of ovarian cancer. Early in the development of an oral dosage form, it was noted that when an oil-based suspension formulation of topotecan, containing 1 mg of drug/g of suspension, was filled into hard-gelatin capsules and placed on stability, two previously undetected degradation products were formed. In contrast, these products were not formed in the non-encapsulated suspension which was stored under the same conditions. This report details the studies which were performed to characterize the new degradation products and to understand the mechanism of their formation. © 1997 Elsevier Science B.V.

Keywords: Topotecan; Anticancer; Stability; Gelatin; Capsules

1. Introduction

Topotecan (Hycamtin®; SKF-S-104864-A; (*S*)-9-dimethylaminomethyl-10-hydroxycamptothecin; shown as the free base form in Fig. 1), which is currently marketed as a parenteral product for the treatment of ovarian cancer, is a semisynthetic, water-soluble analogue of the naturally occurring camptothecin (Kingsbury et al., 1991). Its cyto-

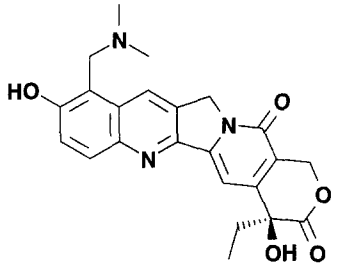

Fig. 1. The structure of topotecan (SKF-S-104864; (*S*)-9-dimethylaminomethyl-10-hydroxycamptothecin).

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Table 1
Stability assessment of topotecan capsule formulation stored at 40°C/75% RH[a,b]

Storage intervals (months)	% of Topotecan remaining[b]	% of 9-AMT present[c]	% of Amine dimer present[c]
1	99.4	0.08	0.08
3	98.5	0.18	0.18
6	96.3	0.34	0.46

[a] About 265 mg of suspension containing 1 mg of topotecan/g of suspension was filled into size 2 white opaque capsules and band sealed with gelatin.
[b] The other hydrolytic products were also detected but not reported here.
[c] The calculated percentage is based on peak area responses.

toxicity results from inhibition of DNA synthesis or, more specifically, the stabilization of the binding of topoisomerase I to DNA which ultimately results in fragmentation of DNA (Hsiang et al., 1985). Currently, an oral formulation of topotecan is being evaluated in the clinic.

Early in the development of an oral dosage form, it was noted that when an oil-based suspension formulation of topotecan, containing 1 mg of drug/g of suspension (5% glyceryl monostearate in fractionated coconut oil), was filled into hard-gelatin capsules and placed on stability, two previously undetected degradation products were formed to a small extent, 0.34 and 0.46% (expressed as a percentage of the topotecan peak area), following storage for 6 months at 40°C and 75% relative humidity (Table 1). Although only small levels of the new degradation products were detected, their characterization is important because current regulations being discussed by the International Conference on Harmonization propose that impurities/degradants present at levels of 0.1% or more should be identified and evaluated for toxicity (Guideline for Impurities in New Drug Substances (Q3A), International Conference on Harmonization).

The formation of these new degradation products results from the interaction of the gelatin shell and the encapsulated drug as evidenced by the absence of these products in the non-encapsulated suspension stored under the same conditions. The chemical degradation of a drug by a capsule shell component appears to be an atypical finding. The scientific literature dealing with this subject is sparse, and most of these references discuss changes in the dissolution rate of encapsulated drugs arising from the interaction of the drug or a formulation component with the gelatin of the capsule shell (e.g. Hom et al., 1973; Ofner and Schott, 1987). Additionally, there has been some discussion of the migration of drugs from oily vehicles into soft-gelatin capsules (Armstrong et al., 1984). This report details the studies which were performed to characterize the new degradation products and to understand the mechanism of their formation.

2. Experimental

2.1. Materials

Topotecan hydrochloride was synthesized by the Synthetic Chemistry department at SmithKline Beecham Pharmaceuticals (King of Prussia, PA). The ammonia measuring kit was obtained from Sigma Chemical Co. (St. Louis, MO), and the white, opaque, size 2, hard-gelatin capsules were obtained from Capsugel (Greenwood, SC). All other chemicals were of reagent grade, and the water was de-ionized then filtered through a Milli-Q Water Purification System prior to use.

2.2. Analysis

The analyses were conducted on a Shimadzu HPLC system equipped with a Shimadzu SPD-6A or an Hitachi L-4000 UV spectrophotometric detector and a Cosmosil 5C18-AR column (4.6 × 250 mm, 5 μm). A stability-indicating gradient elution method was utilized. Mobile Phase A consisted of 0.1% trifluoroacetic acid in a 15:85 (%

v/v) mixture of acetonitrile and water; and Mobile Phase B consisted of 0.1% trifluoroacetic acid in a 40:60 (%v/v) mixture of acetronile and water. An analytical run was performed using 100% of Mobile Phase A for the first 16 min followed by a linear ramp to 100% of Mobile Phase B over the ensuing 25 min. The flow rate was 1.0 ml/min; the detection wavelength was 267 nm; the injection volume was 50 μl; and the total run time was 60 min. Data acquisition and integration were performed with PE Nelson Access*Chrom (version 1.8) Software. A typical HPLC chromatogram is shown in Fig. 2.

The LC/MS analysis was performed by the Analytical Sciences Department at SmithKline Beecham Pharmaceuticals (King of Prussia, PA) using an HP 5989A MS Engine equipped with an HP 59987A Electrospray Interface and an HP 1050 HPLC System.

2.3. Kinetic experiments

The kinetics of degradation of dilute aqueous solutions of topotecan ($\sim$0.1 mg/ml or 2.4×10^{-4} M in free base equivalents) in the presence of 100 mM ammonium chloride were determined over a pH range of 2.0–10.0 and at 40($\pm$0.5)°C. The pH was maintained using an universal buffer (Perrin and Dempsey, 1974) containing boric acid, citric acid and trisodium phosphate. Control studies, in the absence of ammonium chloride, were also carried out under the same conditions. The kinetics of degradation were also followed in the presence of varying concentrations of ammonium chloride (ranging from 0 to 100 mM) at pH 10.0 and 40°C. Additionally, the kinetics of degradation were followed in the presence of varying concentrations of dimethylamine hydrochloride (ranging from 0 to 75 mM) at 40°C and in pH 10.0 buffer containing 100 mM ammonium chloride. The pH of the reaction mixtures were determined at room temperature at the beginning and end of the experiment to ensure that there was no significant change in pH over the course of the study.

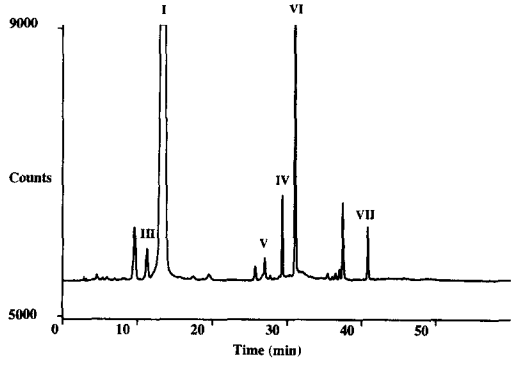
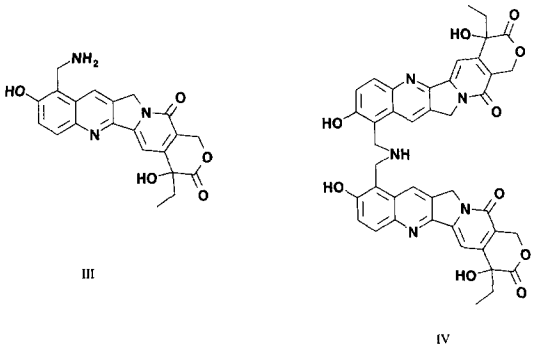


Fig. 2. A typical HPLC chromatogram showing topotecan and its degradation products (Peaks: (I) topotecan; (III) 9-aminomethyl-10-hydroxycamptothecin; (IV) amine dimer; (V) 9-hydroxymethyl-10-hydroxycamptothecin; (VI) 10-hydroxycamptothecin; (VII) hydrolytic dimer). Other peaks are process impurities which are present in the drug substance.

The kinetic experiments were conducted in one of two ways depending on the rate of the reaction. For the relatively fast reactions (pH $\geq$ 5), the experiments were initiated by adding 100 μl of an aqueous stock solution of topotecan to 25 ml glass volumetric flasks of the buffered media that were temperature equilibrated in a circulating water bath. At appropriate time intervals, aliquots were withdrawn and diluted 1–5 with Mobile Phase A; then aliquots of the diluted samples were transfered into HPLC vials. For the slower reactions (pH $\leq$ 4), the experiments were performed by flame-sealing 1-ml aliquots of the buffered media containing topotecan into pre-cleaned 2 ml Flint glass ampules. These ampules were then placed in a temperature-controlled oven. At appropriate time intervals, ampules were withdrawn and cooled in an ice-water bath. For both sets of studies, the samples were stored at 5°C until all of the samples for a given condition were collected; then they were assayed for topotecan and the degradation product peaks by HPLC.

2.4. Determination of ammonia in capsule shells

A dispersion was prepared by heating, with mixing, for 2 h at 60°C, a 1% w/w mixture of gelatin capsules in water. The resulting mixture was then cooled to room temperature. The ammonia content of the dispersion was determined using an ammonia measuring kit. The ammonia determination is based on the reductive amination



III IV

Fig. 3. The structures of the degradation products arising from the encapsulation of a topotecan suspension formulation: 9-aminomethyl-10-hydroxycamptothecin (III) and the associated dimer (IV).

of 2-oxoglutarate in the presence of ammonia, glutamate dehydrogenase, and reduced nicotinamide adenine dinucleotide phosphate (NADPH). The resulting oxidation of NADPH causes a decrease in absorbance at 340 nm which is proportional to the ammonia concentration in the sample (Sigma Diagnostics, 1992).

3. Results and discussion

3.1. Identification of the degradation products

The identity of the degradation products arising from the encapsulation of a topotecan suspension formulation was confirmed by LC/MS and the kinetic studies. The mass and fragmentation pattern of the peak at an apparent molecular weight of 393 Daltons was consistent with 9-aminomethyl-10-hydroxycamptothecin (henceforth called 9-AMT), and that of the peak at an apparent molecular weight of 769 Daltons is consistent with an *N-N* bis-adduct (henceforth called the amine dimer). Fig. 3 shows the structures of these products.

3.2. Evaluation of the reaction kinetics

It was previously shown (Kearney et al., 1996) that topotecan can undergo deamination via formation of the reactive quinone methide intermediate (compound II in Fig. 4). Based on the proposed identity of the degradation products and invoking a mechanism proceeding through a quinone methide intermediate, the involvement of ammonia as a nucleophile becomes apparent. Hence, to more fully characterize the degradation mechanism, the pH-dependency of the reaction was determined in an aqueous environment containing ammonium chloride as the source of ammonia. An aqueous system was chosen due to the complexities associated with working with an oil-based system.

The degradation of topotecan in the presence of 100 mM ammonium chloride was followed for up to 96 h over a pH range of 5–10 at 40°C. As can be seen in Table 2, the rate of formation of the ammonia-dependent products increases as the pH increases. Over the time frame investigated, 9-AMT was detected over the pH range of 6–10, whereas the amine dimer was detected only over the pH range of 8–10. Under acidic conditions ($pH \leq 4$), the reaction was followed for 63 days; however, there was no detectable formation of the ammonia-dependent products. Although it is not feasible to extrapolate results from the aqueous solutions to an oil based suspension, these findings suggest that the degradation pattern observed for topotecan in an alkaline aqueous environment, containing a source of ammonia, approximates the pattern observed in the oil-based suspension formulation.

It should be noted that over the time frame investigated, the previously discussed (Kearney et al., 1996) hydrolytic products (i.e. 9-hydroxymethyl-10-hydroxycamptothecin (compound V in Fig. 4), 10-hydroxycamptothecin (compound VI in Fig. 4), and the corresponding dimer (compound VII in Fig. 4)) were detected over the pH range of 5–10, whereas only 9-hydroxymethyl-10-hydroxycamptothecin and 10-hydroxycamptothecin were detected over the pH range of 2–4. In control studies, conducted in the absence of ammonium chloride, 9-AMT and the amine dimer were not detected over the time frame investigated.

To further characterize the role that ammonia plays in the chemical interaction between topotecan and the hard-gelatin capsule shell, the degradation of topotecan was studied in the presence of

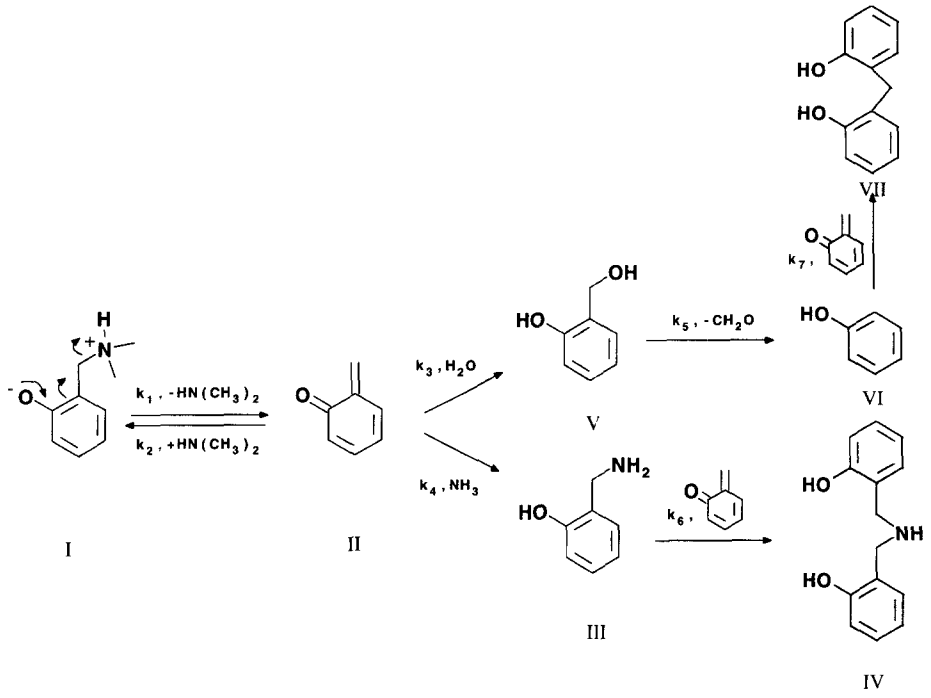


Fig. 4. Postulated mechanism for the degradation of topotecan to form the 9-hydroxymethyl and the 9-aminomethyl analogues.

varying concentrations of ammonium chloride at pH 10.0 and 40°C. Ammonium chloride was used to provide a ready source of ammonia; the pH and temperature conditions were chosen to allow for a relatively fast assessment of the effect; additionally, the temperature condition was chosen because it matches one of the conditions to which the encapsulated oil-based suspension had been exposed.

As can be seen from Fig. 5(a), as the concentration of NH_4Cl was increased so to did the initial rate of formation of 9-AMT. The apparent rate of formation of 9-AMT (represented by the slopes of the lines in Fig. 5(a)) appears to be linearly related to the NH_4Cl concentration, consistent with a bimolecular reaction involving topotecan and ammonia. As expected, the occurrence of a maximum in the 9-AMT plot and the lag time observed in the appearance of the amine dimer (Fig. 5(b)) are mechanistically consistent with the formation of the dimer proceeding through 9-AMT.

3.3. Postulated mechanism of degradation

The postulated degradation mechanism (which also incorporates the hydrolytic degradation pathway (step k_3)) is shown in Fig. 4. The first step involves the formation of the reactive quinone methide intermediate via an elimination reaction where the dimethylamino group is expulsed. Nucleophilic attack by ammonia at the terminal methylene group of this intermediate yields 9-AMT (the k_4 step), and subsequently, nucleophilic attack by 9-AMT on the quinone methide intermediate yields the amine dimer (the k_6 step).

As a final probe of the mechanism, the effect of added dimethylamine (DMA) hydrochloride on the extent of topotecan loss and product formation was studied at pH 10.0 and 40°C in the presence of 100 mM NH_4Cl. As can be seen in Table 3, the extent of loss of topotecan and the associated extent of formation of 9-AMT and the amine dimer, after 32 h, decreased as the

Table 2
The effect of pH on the relative percent of topotecan, 9-aminomethyl-10-hydroxycamptothecin (9-AMT), and amine dimer present after 49 h at 40°C and in the presence of 100 mM NH_4Cl[a]

pH	% of Topotecan remaining	% of 9-AMT present	% of Amine dimer present
10	40.9	17.1	4.4
9	41.7	16.8	0.73
8	38.9	6.5	0.14
7	37.4	1.7	ND
6	60.5	0.34	ND
≤5	>90.5	ND	ND

[a] The relative percentages represent the peak area of a given compound, after 49 h at a given condition, relative to the initial peak area of topotecan (i.e. the area after 0.1 h) under the same conditions.
ND, none detected.

DMA concentration increased. This finding is consistent with the mechanistic involvement of a reactive quinone methide intermediate and the reversibility of its formation as discussed previously for the hydrolytic degradation of topotecan (Kearney et al., 1996).

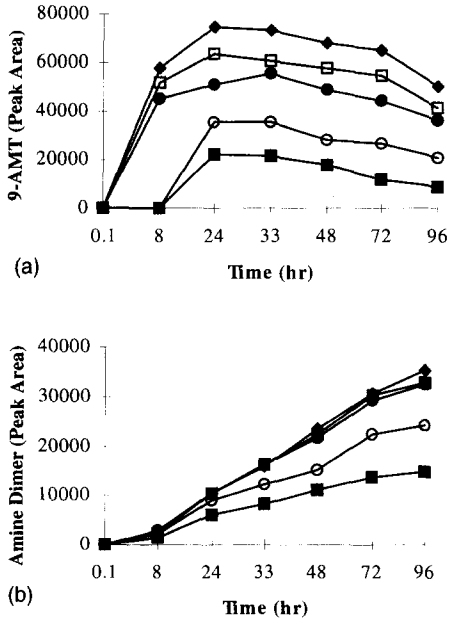


Fig. 5. Effect of ammonium chloride concentration (10 mM, ■; 25 mM, ○; 50 mM, ●; 75 mM, □, 100 mM, ◆) on the rate of formation of the (a) 9-aminomethyl-10-hydroxycamptothecin (9-AMT) peak, and (b) the amine dimer at pH 10.0 and 40°C.

3.4. Ammonia content of hard gelatin capsules

As discussed above, the formation of the degradation products requires topotecan and a source of ammonia. Since the suspension formulation is devoid of ammonia (as evidenced by the absence of the degradation products in the non-encapsulated suspension), the hard-gelatin capsule shell must provide the source of ammonia. The preparation of gelatin generally involves the acid/alkaline hydrolysis of collagen which may result in the amide hydrolysis of asparagine and glutamine residues and the associated liberation of ammonia.

The ammonia content of the 1% w/w aqueous dispersion of capsules was 48.2 μM which translates into an average ammonia content of 0.28 μM per capsule. To put this into more meaningful terms, if a capsule contains 0.25 mg or 0.59 μM of topotecan and if all of the ammonia present in the capsule is available for reaction with topotecan, this would result in a 47% loss of topotecan to the combined products.

4. Conclusions

The chemical degradation of a drug by a component of hard gelatin capsules appears to be an atypical finding. For an encapsulated oil-based suspension formulation of topotecan, only small levels of the new degradation products were detected; however, it is important to remember that

Table 3
The effect of added dimethylamine (DMA) on the relative percent of topotecan, 9-aminomethyl-10-hydroxycamptothecin (9-AMT), and amine dimer present after 32 h at pH 10.0 and 40°C[a]

[DMA] (mM)	% of Topotecan remaining	% of 9-AMT present	% of Amine dimer present
0	53.5	22.0	4.9
25	88.1	5.9	0.53
50	91.5	3.5	0.16
75	95.5	2.7	0.14

[a] The relative percentages represent the peak area of the given compound, after 32 h at the given condition, relative to the initial peak area of topotecan (i.e. the peak area after 0.1 h) under the same conditions.

current regulations being discussed by the ICH propose that impurities present at levels of 0.1% or more should be identified and evaluated for toxicity.

For encapsulated topotecan suspension, the occurrence of a new degradation pathway (i.e. formation of the amine adducts) is favored by at least three factors: (1) the intermediacy in the degradation pathway of a reactive quinone methide which is not overly selective of potential nucleophiles (Turner, 1964); (2) the lipophilic nature of the formulation vehicle which lessens the availability of water as a nucleophile; and (3) the availability of ammonia from the capsule shell.

One way of minimizing the formation of the degradation products is to minimize the ammonia content of the capsule shell. This might be possible by using hard gelatin capsules composed of gelatin obtained from a bovine source. The average ammonia content of these capsules was 0.17 μM per capsule which is 40% less than that of the conventional gelatin capsules.

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