ARTICLE

Simulation of Y-Site Compatibility of Irinotecan and Leucovorin at Room Temperature in 5% Dextrose in Water in 3 Different Containers

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ABSTRACT

Background: No data are available on the physical compatibility and chemical stability of irinotecan and leucovorin when these 2 drugs are mixed.

Objective: The objective of this study was to evaluate the physical compatibility and chemical stability of irinotecan diluted in 5% dextrose in water (D5W) and combined with the racemic form of leucovorin when stored at 23°C, unprotected from light, conditions that simulate Y-site administration of these drugs.

Methods: Six combinations of irinotecan and leucovorin were prepared (0.56 mg/mL and 0.94 mg/mL, 0.53 mg/mL and 0.74 mg/mL, 0.59 mg/mL and 0.66 mg/mL, 0.56 mg/mL and 0.27 mg/mL, 0.32 mg/mL and 3.60 mg/mL, and 0.30 mg/mL and 0.68 mg/mL, respectively), representing the concentrations (dose, volume, and infusion rate) most commonly administered in clinical practice. The stability of each solution was evaluated at room temperature (23°C) in 3 different types of containers: polyvinyl chloride (PVC), polypropylene–polyethylene copolymer, and glass (control). Each solution was visually inspected for precipitate, colour change, and evolution of gas, and the concentration of each drug was measured by high-performance liquid chromatography at time 0 (immediately after mixing) and at 0.5, 1, and 24 h. Each concentration measurement was completed in triplicate.

Results: All solutions remained clear and colourless throughout the 24-h study period. More than 96% of the initial concentration of leucovorin and more than 91% of the initial concentration of irinotecan remained after 0.5 h. Rapid degradation of irinotecan was observed in one mixture: irinotecan 0.30 mg/mL and leucovorin 3.60 mg/mL. In this mixture, the concentrations of irinotecan were between 91.57% and 95.09% of the original concentration at 0.5 h, but declined rapidly to between 76.30% and 78.34% by 24 h. This rapid degradation was likely due to the higher pH of the solution created by the high concentration of leucovorin (3.60 mg/mL, equivalent to a dose of 400 mg/m^2 body area, in 100 mL, for a 60-min infusion). For all mixtures, the mean

RÉSUMÉ

Historique : On ne dispose d'aucune donnée sur la compatibilité physique et la stabilité chimique de l'irinotécan et de la leucovorine lorsque ces deux médicaments sont mélangés ensemble.

Objectif : Évaluer la compatibilité physique et la stabilité chimique de l'irinotécan dilué dans une solution de dextrose à 5 % dans l'eau (D5W) et mélangé au composé racémique de leucovorine, puis entreposé à 23 ºC, non protégé de la lumière, dans des conditions simulant l'administration de ces médicaments dans un raccord en Y.

Méthodes : Six mélanges d'irinotécan et de leucovorine ont été préparés (0,56 mg/mL et 0,94 mg/mL; 0,53 mg/mL et 0,74 mg/mL; 0,59 mg/mL et 0,66 mg/mL; 0,56 mg/mL et 0,27 mg/mL; 0,32 mg/mL et 3,60 mg/mL; et 0,30 mg/mL et 0,68 mg/mL, respectivement), représentant les concentrations (dose, volume et vitesse de perfusion) les plus fréquemment administrées en pratique clinique. On a évalué la stabilité de chaque solution à la température ambiante (23 ºC) et dans trois différents types de contenants : polychlorure de vinyle (PVC), copolymère de polypropylène et polyéthylène, et verre (témoin). Chaque solution a été inspectée visuellement pour la présence d'un précipité, un changement de couleur et le dégagement de gaz, et la concentration de chaque médicament a été mesurée par chromatographie liquide à haute pression au temps 0 (immédiatement après le mélange) puis à 0,5, 1 et 24 h. La concentration de chaque médicament a été mesurée en triple.

Résultats : Toutes les solutions sont demeurées limpides et incolores au cours des 24 heures qu'a duré l'étude. Les solutions ont conservé plus de 96 % de la concentration initiale de leucovorine et plus de 91 % de la concentration initiale d'irinotécan après 0,5 h. On a observé une dégradation rapide de l'irinotécan dans un des mélanges : irinotécan à 0,30 mg/mL et leucovorine à 3,60 mg/mL. Dans ce mélange, les concentrations d'irinotécan étaient entre 91,57 % et 95,09 % des concentrations initiales à 0,5 h, et elles ont rapidement chuté entre 76,30 % et 78,34 % à 24 h. Cette dégradation rapide semblait être attribuable au pH

concentration of leucovorin at 24 h was greater than 96% of the initial concentration. There was no effect of container type on the rate of degradation of either drug.

Conclusions: Given that contact times are likely less than 3 min when standard IV tubing sets are used, it is concluded that irinotecan and leucovorin are physically compatible and chemically stable for a sufficient period of time to allow concurrent infusion via a Y site.

Key words: irinotecan, leucovorin, stability, compatibility

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plus élevé de la solution, causé par la forte concentration de leucovorine (3,60 mg/mL, équivalant à une dose de 400 mg/m² de surface corporelle, dans 100 mL, administrée par perfusion d'une durée de 60 minutes). Tous les mélanges ont retenu plus de 96 % de la concentration moyenne initiale de leucovorine à 24 h. Le type de contenant n'a pas eu d'effet sur le taux de dégradation de l'un ou l'autre médicament.

Conclusions : Étant donné que la durée de contact est probablement inférieure à trois minutes avec les tubulures IV standard, on peut conclure que l'irinotécan et la leucovorine sont physiquement compatibles et chimiquement stables durant une période de temps suffisante pour permettre la perfusion concomitante dans un raccord en Y.

Mots clés : irinotécan, leucovorine, stabilité, compatibilité

INTRODUCTION

Irinotecan (IR; Camptosar; 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyl oxycamptothecin, or CPT-11) was introduced in Canada in late 1997 for the treatment of relapsed or refractory colorectal cancer. The product monograph indicates that IR may be diluted with a variety of IV solutions, including 0.9% sodium chloride (normal saline; NS) and 5% dextrose in water (D5W), and that such solutions should be used within 12 h when stored at room temperature or 48 h when refrigerated.[1]

Previous evaluations of IR degradation have indicated that the first step involves hydrolysis of the lactone ring to form a ring-opened carboxylate derivative,[2] a reversible, pH-dependent process.[2,3] At a pH of less than 5 equilibrium favours IR, whereas at a pH of greater than 8, virtually all IR is present as the ring-opened carboxylate.[3] As the pH increases from 5 to 8, an increasing proportion of IR exists as the carboxylate at equilibrium. The rate of conversion from IR to the carboxylate is also pH-dependent, increasing 4-fold between pH 5 and pH 8,[2,3] such that equilibrium is established more rapidly at higher pH. The carboxylate derivative also appears to be more photosensitive than IR,[3] and at least 5 degradation products are produced under fluorescent light when the ring-opened carboxylate form is present.[3]

The period for which leucovorin (LV) is considered stable in solution ranges from 12 to 72 h.[4-8] The Novopharm product monograph indicates that when LV is diluted with D5W or dextrose 10% in water (D10W), infusion solutions of 0.06 to 1.0 mg/mL are stable for 12 h, whereas LV diluted with dextrose 10% in saline is stable for only 6 h.[4] The Lederle, Cyanamid Canada product monograph for LV states that the drug is stable for 24 h when diluted in D5W, D10W, or dextrose 10% in saline and stored at room temperature.[5] Lauper[6] and Benvenuto and others[7] reported that LV is stable for 24 h after mixing in D5W, whereas Smith and others[8] demonstrated stability with floxuridine over 72 h. Although these data may be limited to studies of short duration, they suggest that LV should be stable for at least 24 to 72 h when diluted in D5W and stored at room temperature.

Current protocols for treatment of metastatic colorectal cancer call for sequential administration of 180 mg/m² of IR and either 200 mg/m² of L-LV or 400 mg/m² of the racemic mixture of LV, followed by infusion of 5-fluorouracil (5FU). Approximately 3.5 h is required to sequentially administer the IR (90-min infusion) and LV (120-min infusion). Patients would spend much less time in the clinic, and more patients could potentially be treated in a given period of time, if IR and LV could be administered concurrently.

European data have indicated that IR and LV are stable when administered concurrently.[9] Although that study concluded that IR and LV were physically and chemically compatible (because both precipitate and degradation products were absent from the IR–LV mixture after a 2-h incubation) the L-isomer of LV was used in these experiments. Thus, the stability achieved with IR and racemic LV may not be accurately represented by these results.

The objective of this study was to evaluate the compatibility of IR diluted in D5W with the racemic mixture of LV also diluted in D5W when mixed together at concentrations typically encountered during Y-site administration at 23°C, in 3 types of containers, unprotected from light.

METHODS

Assay Development and Validation

HPLC Method for Simultaneous Analysis of Irinotecan and Leucovorin

A high-performance liquid chromatography (HPLC) method was developed that allowed simultaneous ana lyses of IR and LV and ensured separation of the 2 drugs from each other and their degradation products. The mobile phase consisted of a mixture of 10% acetonitrile and 90% 0.05 mol/L potassium phosphate monobasic (pH adjusted to 4.1), which was gradually changed to 35% acetonitrile and 65% 0.05 mol/L potassium phosphate monobasic (pH adjusted to 4.1) over 10 min. The pH of the mixed solution was adjusted to 4.1 with 1 mol/L phosphoric acid. Each sample was analyzed for 30 min. The mobile phase was pumped at 1 mL/min through a 15 cm × 4.6 mm reverse-phase C_{18}, 3-µm column (Supelcosil ABZ Plus, Supelco, Mississauga, Ontario) using a 600E system controller and pump (Waters Corp, Mississauga, Ontario). IR and LV were detected at 244 nm and 240 nm, respectively, using a scanning variable-wavelength detector (Spectra System UV6000LP, Thermo Separation Products, Freemont, California); the chromatograms were recorded directly into a computer database using ChromQuest software (Thermo Separation Products). The assay was developed using samples of IR, LV, and their respective degradation products to ensure accurate measurement of IR and LV stability. The UV spectral purity of the IR and LV peaks was compared between degraded and undegraded samples to demonstrate the specificity of the method. This method is unique and differs from previously published methods of IR analysis because of differences in type of column,[3,10] mobile-phase constituents,[2,3,10,11] temperature,[2] method or wavelength of detection,[2,3,10-12] and ability to separate IR from LV.

Accelerated Degradation of Irinotecan

During development of the HPLC method to separate IR and LV from their degradation products, the pH dependency of IR degradation and formation of the ring-opened carboxylate was evaluated, as was the formation of other irreversible degradation products. The ring-opened carboxylate product of IR was generated by the addition of sodium hydroxide to solutions of IR. A stock solution of IR was prepared by dissolving an accurately weighed quantity of approximately 10 mg of IR hydrochloride trihydrate powder (CPT-11, class A primary standard, Pharmacia Corporation, Mississauga, Ontario; lot C, expiry September 1, 2004) in 25 mL of distilled water to achieve a final IR concentration of 0.4 mg/mL. Eight separate samples with pH ranging from 4.12 to 10.14 were prepared by mixing 1 mL of the 0.4 mg/mL IR solution and 1 mL of sodium hydroxide ranging from 0.002 mol/L to 0.016 mol/L. The pH was determined immediately after mixing, and the concentration of IR was determined 30 min after addition of the sodium hydroxide. HPLC was used to monitor separation of IR and the primary degradation product, the ring-opened carboxylate. To the sample of pH 10.14, 1 mL of 0.016 mol/L hydrochloric acid was added. The pH was determined immediately after mixing and the concentration of IR was determined 30 min later.

Given that sodium hydroxide produces only the ring-opened product, a second set of experiments was performed, in which 10-µL aliquots of various sodium hypochlorite solutions ranging in concentration from 0.25% to 1% were added to 1-mL samples of 0.2 mg/mL IR (prepared by further dilution of the 0.4 mg/mL stock solution). These samples were immediately chromatographed, and the concentration of IR was determined.

Chromatograms from all solutions prepared above were inspected for the appearance of additional peaks, changes in retention time, and changes in peak shape. The ultraviolet (UV) spectral purity (200–365 nm, 6-nm bandwidth, determined with a UV3000LP deuterium lamp, Thermo Separation Products) of the leading edge, middle, and tail of the IR peak in a chromatogram of an authentic undegraded sample and the sample taken at time 0 were also compared. Samples of degraded IR were used to develop an HPLC method for simultaneous analysis of IR, LV, and their degradation products.

Degradation of Leucovorin

A degraded sample from an expired 10 mg/mL vial of LV calcium for injection USP (David Bull Laboratories; Vaudreuil, Quebec; lot 2024022, expiry December 1994) was analyzed by the HPLC method to separate LV from its degradation products. Chromatograms were inspected for the appearance of additional peaks, and the LV peak was compared to the LV peak obtained from HPLC analyses of a fresh sample of LV for changes in concentration, retention time, and peak shape.

Ultraviolet spectral purity (200–365 nm, 6-nm bandwidth, determined with a UV6000LP deuterium lamp, Thermo Separation Products) of the leading edge, middle, and tail of the LV peak in both the fresh and expired samples were compared. The expired LV was used to develop an HPLC method for separation of IR, LV, and their degradation products.

Accuracy and Reproducibility of the HPLC Assay

The accuracy and reproducibility of the HPLC method for simultaneous analysis of IR and LV was tested across 5 standard curves. Each sample containing both IR and LV standards was chromatographed in duplicate. Interday and intraday reproducibility were assessed using the coefficient of variation of the peak area for each compound determined in duplicate, and accuracy was determined on the basis of deviations from the known concentration.

Because the standard curve had an upper limit of 0.4 mg/mL for both IR and LV, all samples required dilution of either 1:2, 1:4, or 1:10. To evaluate the accuracy and reproducibility of an experimental solution that may need to be diluted, 5 replicates of samples containing 0.45 mg/mL of IR and 3.6 mg/mL of LV were prepared and diluted, and the concentrations of IR and LV were measured. All experimental solutions assessed by HPLC had concentrations of IR and LV above 0.00625 mg/mL, the lower limit of the standard curve for both drugs.

HPLC Analyses of Solutions for Compatibility Study

On each study day, fresh standards of IR and LV were prepared and chromatographed separately to construct standard curves. A stock solution of IR was prepared by dissolving an accurately weighed quantity of approximately 10 mg of IR hydrochloride trihydrate powder (CPT-11, class A primary standard, Pharmacia Corporation; lot C, expiry September 1, 2004) in 25 mL of distilled water. This stock solution (0.40 mg/mL) was then diluted to prepare 7 concentrations of IR: 0.00625, 0.0125, 0.025, 0.050, 0.100, 0.250, and 0.400 mg/mL. Four-microlitre aliquots of each of these 7 standards and a blank were directly chromatographed in duplicate to allow construction of the standard curve.

A stock solution of LV was prepared by diluting various volumes of a 10 mg/mL solution (leucovorin calcium for injection USP, Novopharm, Toronto, Ontario; lot 0271202001, expiry December 2004) in 10 mL of distilled water to prepare 7 concentrations of LV: 0.00625, 0.0125, 0.025, 0.050, 0.100, 0.250, and 0.400 mg/mL. Four-microlitre aliquots of each of these 7 standards and a blank were used to construct a standard curve.

IR and LV were quantified simultaneously each day using the newly developed HPLC method described above. The average peak area of 2 replicates from each sample of IR and LV was subjected to least-squares linear regression; the concentration of experimental solutions was interpolated from standard curves and

Table 1. Irinotecan–Leucovorin Treatment Scenarios and Calculation of Final Concentrations

Key Scenario Characteristic	Scenario Designation	Drug	Dose (mg/m^2)	Average Body Size (m^2)	Diluent Volume (mL)	Concentration* (mg/mL)	Infusion Duration (min)	Projected Mixed Final Concentration† (mg/mL)
Standard infusion times for both irinotecan (90 min) and leucovorin (120 min)	1a	IR	180	1.8	500	0.65	90	0.56
		LV	400	1.8	100	7.20	120	0.94
	1b	IR	180	1.7	500	0.61	90	0.53
		LV	400	1.7	250	2.72	120	0.74
Leucovorin infusion twice as long as irinotecan infusion	2a	IR	180	1.8	500	0.65	60	0.59
		LV	400	1.8	100	7.20	120	0.66
	2b	IR	180	1.7	500	0.61	60	0.56
		LV	200	1.7	250	1.36	120	0.27
Equal flow rates	3a	IR	180	1.8	500	0.65	300	0.32
		LV	400	1.8	100	7.20	60	3.60
	3b	IR	180	1.7	500	0.61	120	0.30
		LV	200	1.7	250	1.36	60	0.68

IR = irinotecan, LV = racemic leucovorin.

*This concentration represents the concentration in the bag. It considers dose and nominal bag volume but does not consider bag overfill or the volume of drug added to the bag and is calculated as dose/nominal volume.

†This concentration estimates the concentration after mixing of the 2 solutions based on the nominal bag volume and the infusion duration. For example, the IR concentration is calculated as follows: $\{[Conc_{IR}(Vol_{IR}/ID_{IR})]/[(Vol_{IR}/ID_{IR})+(Vol_{LV}/ID_{LV})]\}$ where Vol = nominal bag volume, ID = infusion duration, and Conc = concentration.

recorded. Concentrations were recorded to the nearest 0.001 mg/mL.

Study Design

This study was designed to simulate concurrent infusion of separate solutions of IR and LV using a Y-site connection and to evaluate the stability of these compounds under such conditions. Six treatment scenarios were designed, including those most frequently used in practice, as well as those representing potential extremes of practice (Table 1). In each scenario, IR at a concentration of 0.61 mg/mL was mixed with LV at concentrations of 1.36 mg/mL, 2.72 mg/mL, or 7.20 mg/mL. A concentration between 0.61 and 0.65 mg/mL of IR would be used for an average person (1.7 m^2 to 1.8 m^2 body surface area) receiving a dose of IR of 180 mg/m^2 diluted in D5W (concentrations of 300 to 324 mg/500 mL). Similarly, the concentrations of LV (1.36, 2.72, and 7.20 mg/mL) would be used for an average person (1.7 m^2 to 1.8 m^2) receiving a dose of LV of either 200 or 400 mg/m^2 diluted in 100-mL or 250-mL bags of D5W.

The final concentration of both drugs in a Y-site line after mixing depends not only on the concentration of the drugs in solution but also the flow rates of each solution. A more complete disclosure of volumes and rates used in the estimates of the final concentrations can be found in Table 1. These concentrations do not consider bag-overfill volumes or the volume of the drug solution added to the bag and so are slightly higher than might actually be encountered in practice under similar scenarios.

Each treatment scenario was implemented by combining IR hydrochloride trihydrate (Camptosar, 20 mg/mL, Pharmacia & UpJohn, Mississauga, Ontario; lot 18JAK, expiry June 2005) and LV calcium for injection USP (10 mg/mL, Novopharm, Toronto, Ontario; lot 027120201, expiry December 2004), each diluted in D5W. Each of the 6 treatment scenarios was evaluated in each of 3 different container types, to simulate different types of infusion tubing: glass test tubes (containing D5W from a PVC bag, Baxter Corporation, lot W2L12A1, expiry June 2004), which served as control; PVC bags (Baxter Corporation, lot W2L12A1, expiry June 2004); and polypropylene–polyethylene copolymer bags (partial additive bags [PAB®]; B. Braun Medical Inc, Irvine, California, lot J2D954, expiry July 2003). Thus, 18 separate experiments were designed, and each experiment was conducted in triplicate (n = 54 experimental solutions). All experiments were conducted at room temperature (23°C) under ambient fluorescent room lighting without protection from light.

The concentration of IR and LV in each solution was determined by HPLC at time 0 (immediately after mixing) and at 0.5 h, 1 h, and 24 h.

Determination of pH

Immediately before mixing, the pH of the IR solution (nominal concentrations of 0.61 mg/mL and 0.65 mg/mL, as listed in Table 1) and each of the 3 LV solutions (7.20, 2.72, and 1.36 mg/mL, as listed in Table 1) was measured. Immediately after the solution for each treatment scenario was mixed (in a glass container), its pH was measured. The pH meter (Accumet model 925, Fisher Scientific, Nepean, Ontario) was standardized on each day on which pH was to be measured using commercially available buffer solutions (Fisher Scientific). The pH was recorded to the nearest 0.001 unit but is reported to 2 decimal places.

Visual Inspection

Before and immediately after mixing (time 0) and after 0.5 h, 1 h, and 24 h, each of the 54 experimental solutions was visually inspected against both a black background and a white background for precipitate, colour changes, and clarity. The samples for physical inspection were removed from the respective containers and placed in glass test tubes before inspection to avoid misinterpretations related to the opacity of the container. After inspection, solutions were centrifuged (3000 rpm, 1500*g*) for 10 min to pellet any precipitate that might have formed. The supernatant was also visually inspected.

Data Reduction and Statistical Analysis

The data for each analysis are presented as mean ± standard deviation. Reproducibility was assessed by coefficient of variation. Mean concentrations of IR and LV in each solution were analyzed by least-squares linear regression to determine the percentage of the initial concentration remaining at each time point during the 24-h study period. Multiple linear regression and analysis of variance (SPSS for Windows, release 10.0.5, 1999) were used to determine whether container type (glass, PVC, or PAB) or concentration of IR or LV in the mixture at time 0 had any effect on rate of degradation of either drug. The commonly accepted cut-off for statistical significance of 5% was used for all analyses.

The concentration of IR or LV at any time point was considered within acceptable limits for degradation if the mean concentration had not declined below 90% of the final mixed concentration measured at time 0. A mixture

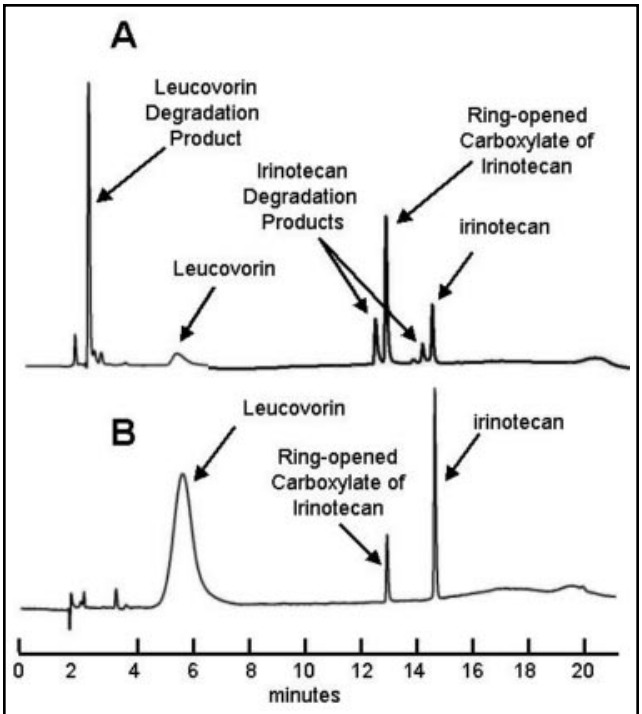

Figure 1. A: Chromatogram of a mixture of expired 10 mg/mL leucovorin and a sample of 0.2 mg/mL irinotecan with 10 µL of 0.5% sodium hypochlorite added. B: Chromatogram of a sample from the stability study (3.60 mg/mL leucovorin and 0.32 mg/mL irinotecan) 24 h after mixing, showing only undegraded leucovorin and irinotecan with about 22% as the ring-opened carboxylate.

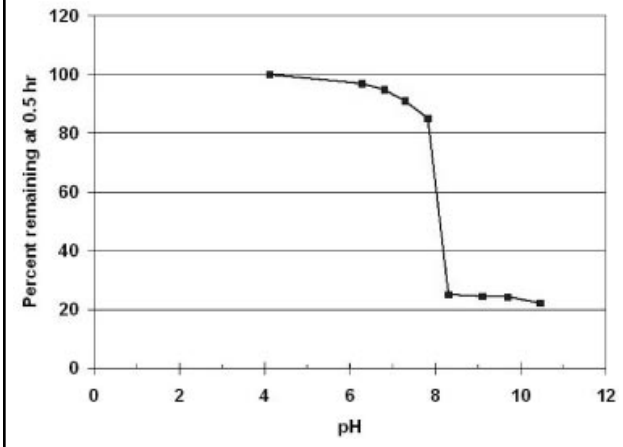

Figure 2. The pH dependency of irinotecan degradation. Eight samples ranging in pH from 4.12 to 10.14 (using concentrations of sodium hydroxide) were prepared, and the percentage of irinotecan remaining after 30 min was measured. Above pH 7, irinotecan degrades quickly. At pH 6.27, only 3% of the initial irinotecan concentration was lost, and at pH 6.79 only 5% of the irinotecan was lost at 30 min. These results are similar to those of Fassberg and Stell[2] and Dodds and others.[3]

was judged to be physically compatible if there was no visual change in the colour or clarity of the mixture, and no precipitate or other particulate formation was visually apparent.

RESULTS

Assay Development and Validation

Figure 1 is a typical chromatogram obtained using the newly developed HPLC method to simultaneously analyse IR and LV. With this separation method, IR eluted at 14.7 min and the ring-opened carboxylate, produced by the addition of either sodium hydroxide or sodium hypochlorite (pH 10), eluted at 13.0 min. Sodium hypochlorite also produces other degradation products (Figure 1A). The addition of increasing amounts of sodium hydroxide to an IR sample increases the formation of the ring-opened carboxylate as the pH increases from 4 to 8 (Figure 2). Above a pH of 8, the ring-opened carboxylate product is the predominate species in solution. Addition of hydrochloric acid to such a sample (e.g., a sample at pH 10.14 through the addition of sodium hydroxide), results in the re-formation of IR within 30 min (when the pH dropped to less than 5). LV and its primary degradation product eluted at 5.7 min and 2.5 min, respectively. The separation of the degradation products from both IR and LV, as well as the similarity of the UV spectra for peaks associated with IR and LV to authentic standards of each, indicates that the method is specific for both IR and LV.

Duplicate analysis of a 0.05 mg/mL IR quality control sample containing LV demonstrated that concentrations were estimated with less than 3% deviation between the observed and known concentrations of IR. The coefficient of variation on duplicate analysis of standards averaged less than 1.5% within days and less than 3% between days. These analyses indicated that the IR concentrations were measured accurately and reproducibly and that differences of 10% or more could be confidently detected with acceptable error rates.[13,14]

Duplicate analysis of a 0.20 mg/mL LV quality control sample containing IR demonstrated that concentrations were estimated with less than 0.7% deviation between the observed and known concentrations of LV. The coefficient of variation on duplicate analysis of standards averaged less than 1.6% within days and less than 0.9% between days.

Based on the separation of IR and LV from each other and from their degradation products and the

Table 2. Observed Mean Concentration (± Standard Deviation) of Irinotecan and Leucovorin in 5% Dextrose in Water Solutions Stored in Glass*

Characteristic	Scenario 1a (IR 0.56, LV 0.94)	Scenario 1b (IR 0.53, LV 0.74)	Scenario 2a (IR 0.59, LV 0.66)	Scenario 2b (IR 0.56, LV 0.27)	Scenario 3a (IR 0.32, LV 3.60)	Scenario 3b (IR 0.30, LV 0.68)
Irinotecan						
Initial concentration (mg/mL)	0.56±0.01	0.56±0.01	0.59±0.02	0.59±0.02	0.35±0.01	0.30±0.01
pH before mixing	3.12	3.12	3.06	3.06	4.02	4.02
% remaining						
At 0.5 h	99.40±2.50	98.67±0.84	99.68±0.04	99.89±0.97	95.09±3.14	98.54±1.95
At 1 h	98.12±1.56	97.84±1.64	99.69±0.20	100.10±0.76	89.08±4.83	98.00±1.36
At 24 h	92.48±0.99	92.78±0.66	96.47±1.10	97.56±2.04	77.23±4.59	95.52±1.03
Leucovorin						
Initial concentration (mg/mL)	0.91±0.02	0.73±0.00	0.68±0.01	0.26±0.00	3.65±0.11	0.62±0.05
pH before mixing	6.08	5.88	5.55	5.41	7.01	5.65
% remaining						
At 0.5 h	100.33±2.51	100.79±0.68	99.19±1.27	100.05±2.96	101.30±1.87	99.44±0.48
At 1 h	100.50±1.34	100.43±0.58	98.99±0.52	98.95±2.45	101.55±1.80	100.27±0.73
At 24 h	101.26±0.89	99.78±0.97	98.84±0.19	96.31±0.83	100.81±0.84	99.57±1.75
Mixture						
pH after mixing	5.56	5.42	5.33	4.76	6.50	5.52

*Scenario designations in column headings represent nominal concentration of each drug (mg/mL).

demonstration that concentrations of both IR and LV could be measured accurately and reproducibly (as indicated by within-day and between-day coefficients of variation), this method can be considered stability-indicating for both IR and LV.[15-17]

Physical Compatibility and Chemical Stability Studies

Physical Inspection and pH Measurements

Each individual solution of IR (nominal concentrations of 0.61 mg/mL and 0.65 mg/mL) and LV (7.20, 2.72, and 1.36 mg/mL) was initially clear and colourless. Mixing these individual solutions did not result in generation of gas, change in colour, or development of a precipitate in any of the 54 experimental solutions created for the 18 individual experiments.

The pH of each IR and LV solution used in the study (various concentrations) before mixing and the pH of each mixture (in glass) is reported in Table 2. These data show that before mixing, the pH of the LV solution was always greater than that of the IR solution. The pH of each mixture fell between the observed pH for the separate solutions, but was often driven by the component with the highest concentration. As a result, the solution containing 0.32 mg/mL IR and 3.60 mg/mL LV (treatment scenario 3a in Table 1) had the highest pH (6.50) of all mixtures.

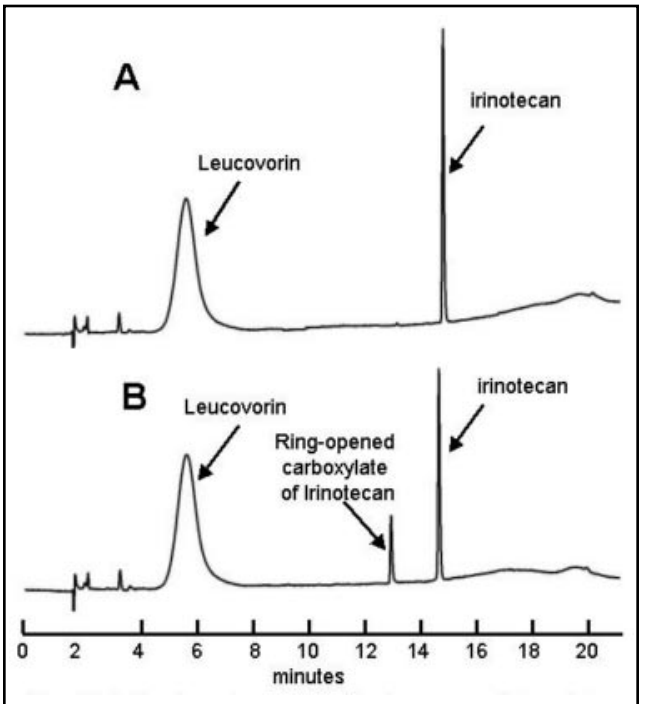

Figure 3. A: Chromatogram of a freshly prepared mixture of leucovorin (3.60 mg/mL) and irinotecan (0.32 mg/mL). B: Chromatogram of the same sample, 24 h later. The retention times were 5.7 min for leucovorin, 14.7 min for irinotecan, and 13 min for the irinotecan degradation product.

Table 3. Observed Mean Concentration (± Standard Deviation) of Irinotecan and Leucovorin in 5% Dextrose in Water Solutions stored in Polyvinyl Chloride*

Characteristic	Scenario 1a (IR 0.56, LV 0.94)	Scenario 1b (IR 0.53, LV 0.74)	Scenario 2a (IR 0.59, LV 0.66)	Scenario 2b (IR 0.56, LV 0.27)	Scenario 3a (IR 0.32, LV 3.60)	Scenario 3b (IR 0.30, LV 0.68)
Irinotecan						
Initial concentration (mg/mL)	0.65±0.03	0.55±0.01	0.47±0.12	0.54±0.00	0.31±0.00	0.34±0.00
% remaining						
At 0.5 h	99.08±0.41	98.70±0.18	99.15±0.78	99.40±0.25	91.76±0.28	99.45±1.26
At 1 h	97.50±0.27	98.10±0.19	99.42±0.89	98.82±0.04	88.69±0.67	97.03±0.34
At 24 h	92.00±0.50	92.58±0.32	96.17±0.23	97.33±0.26	78.34±0.81	95.08±0.32
Leucovorin						
Initial concentration (mg/mL)	0.87±0.03	0.70±0.02	0.68±0.02	0.26±0.00	3.55±0.01	0.66±0.01
% remaining						
At 0.5 h	101.02±1.79	99.67±2.25	99.65±1.21	99.77±0.10	103.39±2.87	98.98±1.32
At 1 h	100.52±0.86	100.94±0.69	100.62±0.79	100.05±0.19	101.83±0.39	99.78±0.58
At 24 h	100.36±1.32	100.03±1.49	99.42±1.06	99.14±0.26	101.57±0.51	100.69±5.20

*Scenario designations in column headings represent nominal concentration of each drug (mg/mL).

Table 4. Observed Mean Concentration (± Standard Deviation) of Irinotecan and Leucovorin in 5% Dextrose in Water Solutions Stored in Polypropylene–Polyethylene Copolymer Bags*

Characteristic	Scenario 1a (IR 0.56, LV 0.94)	Scenario 1b (IR 0.53, LV 0.74)	Scenario 2a (IR 0.59, LV 0.66)	Scenario 2b (IR 0.56, LV 0.27)	Scenario 3a (IR 0.32, LV 3.60)	Scenario 3b (IR 0.30, LV 0.68)
Irinotecan						
Initial concentration (mg/mL)	0.65±0.03	0.55±0.01	0.47±0.12	0.54±0.00	0.31±0.00	0.34±0.00
% remaining						
At 0.5 h	99.16±0.56	99.08±0.13	99.74±0.78	99.52±0.15	91.57±1.22	98.92±0.12
At 1 h	97.18±0.56	98.15±0.47	99.09±0.31	98.63±0.54	88.56±0.79	97.10±0.71
At 24 h	91.88±0.41	92.28±0.42	96.61±1.14	97.21±0.60	76.30±0.69	94.55±0.59
Leucovorin						
Initial concentration (mg/mL)	0.95±0.01	0.72±0.01	0.65±0.02	0.23±0.01	3.67±0.03	0.67±0.01
% remaining						
At 0.5 h	99.81±0.37	99.56±0.86	100.16±2.46	106.81±5.77	96.82±2.80	100.50±1.37
At 1 h	100.27±0.24	101.90±0.69	100.45±2.06	107.08±7.19	99.67±2.34	100.12±0.56
At 24 h	101.17±1.28	100.30±0.88	102.38±2.00	97.66±1.66	99.86±0.62	100.24±0.03

*Scenario designations in column headings represent nominal concentration of each drug (mg/mL).

Chemical Stability of Irinotecan and Leucovorin

To specifically evaluate the stability of IR and LV, HPLC was used to determine the concentrations in each experimental solution at time 0 and at 0.5 h, 1 h, and 24 h after mixing. Mean concentrations (3 determinations at each observation time) of IR and LV for solutions stored in glass, PVC, and PABs are reported in Tables 2, 3, and 4, respectively. These data show that the concentration of IR declined in all 18 experiments over the 24-h experimental period. In 5 of the 6 treatment scenarios (1a, 1b, 2a, 2b, and 3b), the mean concentration of IR was greater than 98% of the original concentration at 0.5 h and was greater than 91% of the original concentration at 24 h. The sixth treatment scenario (3a), with initial projected concentrations of IR 0.32 mg/mL and LV 3.60 mg/mL, had a more rapid degradation of IR. At 0.5 h, the mean concentration of IR was between 91.57% and 95.09%, but continued to decline (to 76.30% to 78.34% of the initial concentration) until 24 h. In these solutions, the only degradation product that was evident was the ring-opened carboxylate (Figure 3).

Analyses of these data by multiple linear regression demonstrated that there was no effect of container type ($p = 0.62$) or initial IR concentration ($p = 0.80$) on the rate of IR degradation, but there was a significant effect of initial LV concentration ($p < 0.0001$) and time ($p < 0.001$) on the rate of IR degradation. The significant effects of both time and initial LV concentration were entirely due to the loss of IR in the experimental solution containing

0.32 mg/mL IR and 3.60 mg/mL LV, in which the IR concentration degraded to 76.30% to 78.34% of the initial concentration at 24 h. It is noteworthy that the degradation of IR was not linear; IR concentration declined by approximately 12% on average in the first hour of incubation and then by a further approximately 12% over the next 23 h.

Analysis of LV concentrations by multiple linear regression demonstrated that there was no trend for a decrease in concentration and so there was no significant effect of container type ($p = 0.12$), initial IR concentration ($p = 0.80$), initial LV concentration ($p = 0.54$), or time ($p = 0.34$) over the 24-h period. The mean concentration of LV at 24 h was greater than 96% of the original concentration in all treatment scenarios.

The rapid degradation of IR observed in treatment scenario 3a was likely due to the higher pH of the solution created by high concentration of LV (Table 2). The pH dependency of IR stability was evaluated in a separate experiment undertaken as part of developing the new HPLC method. These data (Figure 2) indicate that at a pH above 7.82, IR converts rapidly to the ring-opened carboxylate. At pH 7.82, 14.9% of the initial IR concentration was lost in 30 min, whereas at pH 8.31, 74.9% of the initial IR concentration was lost in 30 min. The highest pH for any of the experimental solutions was observed for treatment scenario 3a (pH 6.50). In the pH dependency study, 3.0% of the initial IR concentration was lost in 30 min at pH 6.27 and 5.2% of the initial IR concentration was lost at pH 6.79. These results are in agreement with the percent remaining in all experiments conducted with the mixture of 0.32 mg/mL IR and 3.60 mg/L LV, in which between 4.9% and 8.4% of the initial IR concentration was lost in 0.5 h at pH 6.50.

DISCUSSION AND CONCLUSIONS

The studies reported here simulated the administration of IR and LV in most patient care areas. That is, all experimental solutions were continuously exposed to ambient fluorescent light, 3 types of containers simulating different types of tubing (PVC, PAB, and glass [control]) were used, and 6 concentration combinations representing 6 treatment scenarios (Table 1) were evaluated. PABs are made of a polypropylene–polyethylene copolymer and simulate the more rigid non-diethylhexyl phthalate PVC and polyethylene tubing sets used for administration of some drugs.

The final concentration of both drugs in the Y-site line after mixing depends not only on the concentration of the drugs in solution but also the flow rates of each solution. The concentration estimates in Table 1 do not consider bag-overfill volumes or the volume of the drug solution added to the bag and so are slightly higher than might actually be encountered in practice under similar scenarios.

Actual concentrations in clinical practice will also vary because of dose adjustments related to patients' body weight and because the infusion rate varies with calibration errors. However, the aim was to ensure that the estimated concentrations in Table 1 were closer to the concentrations likely encountered in clinical practice than those produced by the more common method of mixing equal volumes of each solution (scenarios 3a and 3b). Mixing equal volumes would yield LV concentrations 2- to 7-fold higher than those generally seen in clinical practice because of the longer infusion times and smaller bag volumes. To avoid this pitfall, numerous potential concentrations of mixtures were calculated, and concentration scenarios representing and/or encompassing concentrations likely to be observed in clinical practice were selected.

In all treatment scenarios, IR and LV were physically compatible for 24 h. The HPLC analyses of IR and LV concentrations at 0.5 h support these physical compatibility data. The mean concentrations of LV and IR at 0.5 h, as a percentage of the concentrations at time 0, were greater than 96% for LV and greater than 91% for IR in all treatment scenarios, which indicates that, based on a 10% threshold for degradation, both IR and LV were chemically stable for 0.5 h. In other words, concentrations of IR ranging from 0.30 to 0.59 mg/mL were chemically compatible with LV concentrations from 0.27 to 3.60 mg/mL for 0.5 h. Other treatment scenarios that might be used in clinical practice should have concentrations of IR and LV that fall within the ranges evaluated here. For example, if equal infusion times are used for treatment scenarios 2a and 2b, a concentration of 0.51 mg/mL for IR and 0.45 or 1.20 mg/mL for LV would be produced in the final mixuture. It can therefore be expected that the final mixed solution of IR and LV will be chemically stable at 0.5 h.

The stability of both IR and LV for 0.5 h supports the hypothesis that these 2 compounds can be administered simultaneously via Y-site connection because the contact time in the Y-site will be far less than 0.5 h. In fact, the period of contact will be less than 2 min with standard IV tubing set 81 cm (32 inches) long. Standard IV tubing 165 cm (65 inches) in length with an internal diameter of about 2.5 mm holds 9 mL of fluid (equivalent to 0.135 mL/inch or 0.055 mL/cm). Therefore, even if the Y-site is maximally separated from the site of infusion (32 inches or 81 cm) and the lowest flow rates for IR and LV are

employed (Table 1, scenario 3a), the mixing time will be limited to less than 2 min. These data also indicate that container type had no impact on stability, so either PVC or polyethylene-lined tubing could be used for Y-site infusion with IR and LV.

Data collected after 1 h and 24 h of incubation indicated that with each treatment scenario, there was a consistent and measurable decline in IR concentration. However, in 5 of the 6 treatment scenarios (1a, 1b, 2a, 2b, 3b), the concentration of IR remaining after 24 h was greater than 91% of that present at time 0. Therefore, IR remained within acceptable limits of degradation and was considered chemically stable for this time period. Treatment scenario 3a, which contained the highest concentration of LV (IR, 0.32 mg/mL; LV, 3.60 mg/mL) exhibited a higher rate of decline in IR concentration than the other 5 treatment scenarios. The mean concentration (± standard deviation [SD]) of IR remaining at 1 h, expressed as a percentage of the concentration at time 0, was between 88.56% ± 0.79% and 89.08% ± 4.83% (Tables 2–4). At 24 h the mean concentration (± SD) had further declined to 76.30% ± 0.69% to 78.34% ± 0.81% of that present at time 0. In addition to having the highest concentration of LV, treatment scenario 3a also had the highest pH (6.50) (Table 2). This relatively high pH contributed to the accelerated conversion of IR to the ring-opened carboxylate observed in treatment scenario 3a. The conversion of IR to the ring-opened carboxylate was pH-dependent (Figure 2); IR concentrations declined slowly between pH 4 and pH 6 and more rapidly above pH 6. The observation that treatment scenario 3a exhibited the most rapid decline in IR concentration should have been expected, because this was the only treatment scenario in which the pH of the mixture was greater than 6.

These data also show that the quantitative decline in IR observed in the pH dependency experiment (Figure 2) was similar to the decline observed at 0.5 h for each treatment scenario, supporting the notion that pH, possibly in combination with continuous exposure to fluorescent light,[8] is the only factor contributing to an accelerated decline in IR concentration. The decline in IR concentration in scenario 3a (Tables 2–4) resulted in mean concentrations (± SD) after 0.5 h that were between 91.57% ± 1.22% and 95.09% ± 3.14%, values that are in close agreement with concentrations remaining after 0.5 h in the pH dependency experiment (Figure 2). In the latter experiment, 96.79% and 94.82% of the IR remained at pH 6.27 and 6.79, respectively. Since these experiments did not control for exposure to light, it is impossible to separate the contribution of continuous exposure to fluorescent light from the contribution of pH to the decline in IR concentration. However, previous work by Dodds and others[3] demonstrated that the ring-opened carboxylate is a photolabile species and that photodegradation requires the presence of the ring-opened carboxylate. Nonetheless, during the stability study, analyses of IR exposed to both increasing pH and continuous light revealed only the ring-opened carboxylate product, which is produced by hydrolysis of the lactone ring that is in equilibrium with IR.[2,3] Other degradation products, produced by sodium hypochlorite oxidation or photodegradation[3] during continuous light exposure, were not observed.

The rates and proportion of the pH-dependent conversion of IR to the ring-opened carboxylate observed in this study were similar to those previously reported.[3] Both Fassberg and Stell[2] and Dodds and others[3] have demonstrated that IR is more stable in acidic solution (pH 5) than in neutral or basic solutions. Dodds and others[3] found that the photodegradation rate of IR in saline was 0.0245/h, which corresponds to a half-life of 28.28 h and a time of 4 h, 15 min to achieve 90% of the initial concentration. Commercially available IR solutions contain lactic acid, which lowers the pH and makes the product more stable[2,3] by preventing conversion to the ring-opened carboxylate (which undergoes photodegradation). The photodegradation rate of IR in 0.05 mol/L phosphate buffer at pH 5 was 0.0022/h,[3] which corresponds to a half-life of 315 h and a time of about 48 h to achieve 90% of the initial concentration. The dilution of commercial product in saline yields a solution with pH of about 3.[9] As a result, further investigations by Rivory and others[18] did not reveal significant degradation during infusions of 60 to 90 min.

Chamorey and Milano[9] have also evaluated the stability and compatibility of IR (0.36, 1.44, and 2.8 mg/mL in D5W) mixed with the L-isomer of LV (Elvorin, 0.4 and 4.0 mg/mL in D5W [Elvorin is the commercially available form of the L-isomer of LV in Europe]). The current study and the study conducted by Chamorey and Milano[9] evaluated similar concentrations of LV and IR in solutions of similar pH, and the 2 studies had similar results. However, Chamorey and Milano[9] reported a 32% loss in IR concentration immediately after mixing of a solution containing 0.36 mg/mL IR and 4 mg/mL L-LV. In contrast, in the study reported here there was little difference in the nominal and initial concentrations of IR at time 0 in any solution, including the solution containing 3.60 mg/mL LV. However, after

1.0 h the mean IR concentration had declined to between 88.56% ± 0.79% and 89.08% ± 4.83% of the concentration at time 0, and after 24 h the concentration had declined to between 76.30% ± 0.69% and 78.34% ± 0.81%. This difference in results may be explained by the fact that Chamorey and Milano[9] stored solutions at –20°C until "all samples were taken". Thus, conversion of IR to the ring-opened carboxylate might have occurred during storage at –20°C for at least 2 h or during the time required to allow the samples to thaw.

The studies reported here demonstrated conversion of IR to the ring-opened carboxylate following mixing with racemic LV. The 24-h stability data also suggest that IR (0.30 to 0.56 mg/mL) and LV (0.27 to 0.94 mg/mL) could be mixed in the same diluent bag and infused together, as suggested by Chamorey and Milano.[9] Future research should focus on evaluating additional concentrations of racemic LV between 0.94 and 3.6 mg/mL, to more precisely define the limits of LV concentration that can be used while maintaining IR stability.

In conclusion, IR and LV solutions, even those containing 3.60 mg/mL of LV, are physically compatible and chemically stable for a sufficient period of time to allow Y-site infusion, provided the period of contact (time from mixing to entry into the body) is short (less than 30 min).

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