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ORIGINAL ARTICLE

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Spectrophotometric investigation of the chemical compatibility of the anticancer drugs irinotecan-HCl and epirubicin-HCl in the same infusion solution

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Abstract The use of infusional chemotherapy, especially in an ambulatory setting, absolutely requires that the individual agents remain stable in solution at room temperature and that the drugs be compatible. Because of this, investigation of the chemical compatibilities of chemotherapeutic drug combinations given in the same infusion solution is quite important especially if the drugs are to remain in solution for long periods. Thus, the visual and chemical compatibility of irinotecan and epirubicin in the same infusion solution were investigated using both reference standards and pharmaceutical dosage forms. No sign of incompatibility was observed upon visual examination by means of effervescence, pH change, precipitation and colour change. But a chemical incompatibility was observed using a spectrophotometric method in the spectra of irinotecan-HCl and epirubicin-HCl. The molar ratio of epirubicin-HCl/irinotecan-HCl at which the interaction reached a maximum was found to be 2:1. The chemical interaction occurred immediately after admixing and no visual or spectral change was noticed for 24 h after the interaction had occurred. It is concluded that these drugs are chemically incompatible. While the applicability of these two drugs in combination is investigated in further pharmacological studies, their chemical interaction should also be a consideration. The positive or negative contribution of this interaction to the pharmacological effect of the combination might be of importance, and therefore should be investigated in further clinical trials.

Keywords Irinotecan · Epirubicin · Chemical compatibility · Interaction · Spectrophotometry · Visual compatibility

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Introduction

The use of drugs in combination is widespread in cancer chemotherapy. Combination therapy is usually more toxic than single-agent therapy, but occasionally, combinations of drugs with different mechanisms of actions and side-effect profile can be as or less toxic than single-agent therapy. So, it is sometimes possible to decrease serious dose-limiting side effects through combined drug therapy. Also combination chemotherapy can decrease the resistance of tumor cells.

Technological advances are on the horizon that will provide the capacity to administer multiple drugs through a single access site to allow the use of a single delivery source, thus obviating the need for admixtures of drugs [18]. Because the administration of infusions sometimes needs a long period of time, it would appear that the best and easiest method is to give more than one drug in the same infusion solution. From a practical point of view, the administration of infusional chemotherapy, especially in an ambulatory setting, absolutely requires that the individual agents remain stable in solution at room temperature and that the drugs be compatible [18] Because of this, investigation of the chemical compatibility of chemotherapeutic drugs given in the same infusion solution, before clinical studies, is quite important. Thus, the visual and chemical compatibility of the anticancer drugs irinotecan and epirubicin in the same infusion solution was investigated.

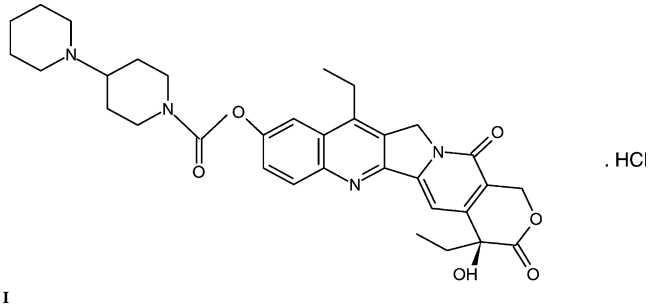
Irinotecan-HCl (**I**; Fig. 1) is a new drug which has antitumour activity in a wide range of malignancies, such as metastatic colorectal cancer, upper gastrointestinal, pancreatic, lung and breast cancer, and gynaecological malignancies.[2, 5, 9]. Camptothecin derivatives are inhibitors of the enzyme topoisomerase I, which inhibits nucleic acid synthesis by interfering with the coiling and recoiling of DNA during replication. Irinotecan exists in an active lactone form and an inactive hydroxy acid anion form [2]. A closed-ring lactone configuration is necessary for topoisomerase I inhibition. Irinotecan-HCl

is infused into blood for 90 min after mixing with 0.9% NaCl or 5% dextrose solution in the infusion bag [13, 15].

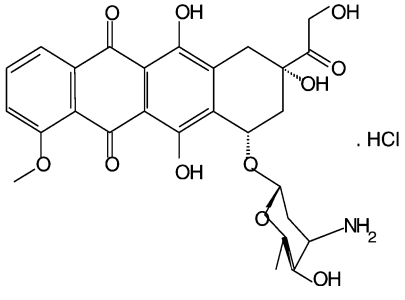
Epirubicin-HCl (**II**; Fig. 1), an anthracycline derivative, is used in colorectal cancers, in breast, lung, stomach and ovary tumours, and in Hodgkin's lymphomas and non-Hodgkin's lymphomas [10, 12–15]. When administered as long-duration infusions, it may be given in 0.9% NaCl or 5% dextrose solutions [19]. It inhibits nucleic acid and protein synthesis through complexing with DNA. This chelation causes the destruction of DNA by topoisomerase II. The drug interferes with the replication and transcription [6]. Epirubicin is also involved in oxidation/reduction reactions by generating cytotoxic free radicals. There is a cardiotoxicity risk in the use of epirubicin [4]. Life-threatening congestive heart failure-like symptoms are mostly associated with high-dose administrations. Although irinotecan shows similar side effects, it lacks the cardiotoxic effect, and seems suitable for use in combination with epirubicin-HCl.

Secondary leukemia may develop when anthracyclines are given in combination with DNA-damaging antineoplastic agents, when patients have been heavily pretreated with cytotoxic drugs, or when doses of the anthracyclines have been escalated [4].

One of the major dose-limiting toxicities of irinotecan and epirubicin is myelosuppression [4, 6, 11, 16, 17]. Irinotecan may cause severe myelosuppression in patients with previous pelvic and abdominal radiation [11, 17]. The concurrent administration of irinotecan-HCl with irradiation has not been adequately studied and is not recommended [7]. It is likely that the use of epirubicin with radiotherapy may sensitize tissues to the cytotoxic actions of irradiation [4]. Administration of epirubicin after previous radiation therapy may induce an inflammatory recall reaction at the site of the irradiation. Concurrent treatment with irradiation and the combination of these two drugs may be associated with an increased risk of secondary tumour.



I



II

Fig. 1 The molecular formulas of irinotecan-HCl (**I**) and epirubicin-HCl (**II**)

Hwang et al. [5] suggest, following a clinical trial of the combination of epirubicin and irinotecan, that each agent works through the inhibition of topoisomerases and inhibition of topoisomerases I and II may show significant clinical synergy, because these proteins play complementary but necessary and non-overlapping roles in DNA replication. There are only two pharmacological studies of this combination, both phase I trials (no phase II trial has been performed to date). In the study by Hwang et al. patients with histologically proven advanced solid malignancies and no beneficial standard therapy were treated with the irinotecan and epirubicin combination. They had various tumour types, including breast, pancreaticobiliary, lung, ovarian, and cervical cancer, gastroesophageal junction adenocarcinoma, hepatocellular carcinoma, soft-tissue sarcoma, and metastatic carcinoma of uncertain primary. Initially, patients were treated with irinotecan at 100 mg/m^2 and epirubicin 50 mg/m^2 After substantial toxicity at this level, the doses to be evaluated were changed to irinotecan 50, 50, 75 and 75 mg/m^2 and epirubicin: 20, 25, 25 and 30 mg/m^2. Irinotecan was administered as an IV infusion over 90 min, followed by epirubicin as an IV bolus. The primary toxicity was myelosuppression. Gastrointestinal toxicities are a prominent part of the toxicity profile of irinotecan, but in this study, the combination was well tolerated. No cardiac dysfunction was noted. In this heavily pretreated population, limited antitumour efficacy was noted; no objective responses occurred. Stabilization of disease was noted in patients with metastatic carcinoma of unknown primary, gastroesophageal junction adenocarcinoma and liposarcoma. The toxicity, primarily myelosuppression, exceeded that was expected for the doses of irinotecan and epirubicin alone, suggesting the possibility of a synergistic interaction of the agents on this schedule, at least in the bone marrow. Other toxicities were acceptible and not dose-limiting.

Hwang et al. indicate that the optimal manner of combining these agents in terms of dose and schedule remains to be defined. Results with sequential administration of topoisomerase I and II inhibitors have generally been disappointing with increased toxicities and minimal activity [1]. Interestingly, concurrent administration of topoisomerases I and II inhibitors have been found to be active in clinical trials of small-cell lung cancer, non-small-cell lung cancer and non-Hodgkin's lymphoma.

The second study of this combination is that of Chen et al. [1] in which irinotecan and epirubicin were administered concurrently in the same combination with cisplatin. This combination regimen had promising broad antitumour activity. The study included patients with primary tumours originating from a large variety of

sites, the majority of which were from the gastrointestinal or gynaecological tracts. Cisplatin and epirubicin were given at fixed doses of 50 and 60 mg/m^2, respectively. The irinotecan dose was escalated in 10-mg/m^2 increments from a starting dose level of 70 mg/m^2. The treatment was carried out in two courses; all three drugs were administered sequentially (epirubicin followed by cisplatin, followed by irinotecan) on day 1 every 3 weeks in course 1, and irinotecan was given on day 1 and epirubicin and cisplatin on day 3 in course 2, in order to evaluate possible pharmacokinetic interactions between irinotecan and epirubicin. Epirubicin, irinotecan and their metabolites were measured by HPLC. Pharmacokinetic parameters of epirubicin were not affected by the sequence of drug administration. However, the AUCs of irinotecan and its metabolites were increased significantly when irinotecan and epirubicin were administered concurrently. The dose-limiting toxicity was neutropenic fever. Of 34 patients with evaluable disease, 1 had a complete response and 9 had a partial response, yielding an overall response rate of 29.4%.

As in the two phase I studies described above, the two drugs, irinotecan as an infusion and epirubicin as a bolus, were given sequentially. But if the two drugs could be injected into the same infusion solution, the risk of extravasation and irritation of epirubicin may be decreased, as the number of procedures applied to the patient would be reduced.

There is a lack of information regarding the stability of multidrug admixtures over prolonged periods, i.e. beyond that which is necessary for traditional bolus or short-term delivery of chemotherapy [18]. Although the chemical compatibility studies of admixed drugs in infusion solutions are inadequate, they include important results for clinical applications. For example, Cohen et al. studied the visual compatibility of some anticancer drugs after Y-site injection of the infusion solution [3]. Radford et al. [12] investigated the stability of mesna and ifosfamide, each one alone and in admixtures, at room temperature and daylight, at 27°C in the dark and at 37°C in the dark. Trissel et al. [14] investigated the chemical compatibility of fludarabine phosphate with other anticancer and anti-infective drugs by simulating the Y-site injection of the infusion solution. McRae and King [9] studied the chemical compatibility of anticancer drugs in the same infusion solution using visual and spectrophotometric methods.

Materials and methods

Materials and equipment

Epirubicin-HCl and Irinotecan-HCl·3H_2O USP and reference standards were provided by Pharmacia-Italia and Aventis-France. Campto (Rhoné Poulenc Rorer-France) and Farmorubicin (Carlo Erba-Italy) were provided by Marmara University Hospital Department of Oncology. These two drugs are cytotoxic and require the use of gloves and maximum caution. All reagents were of analytical grade. Other solutions and equipment used included NaCl (Carlo Erba), pH 4.01 and pH 7.00 WTW STP 10 technical buffer solutions, a Shimadzu UV-1601 spectrophotometer, an Epson LX-300 printer, a WTW Inolab terminal level pH meter, a combination HI 1131 pH electrode, a Shimadzu A $\times$ 200 balance, and GFL-2004 distilled water apparatus. All solutions were prepared using distilled water.

Procedure

Visual compatibility was assessed by means of effervescence, colour change, precipitation and pH change [3, 14] after epirubicin-HCl and irinotecan-HCl had been injected into same infusion solution. Chemical interaction was further investigated quantitatively using a spectrophotometric method. High drug concentrations are usually more likely to exhibit visual incompatibilities than lower concentrations [14]. It was considered that chemical incompatibility might be observed more easily with higher drug concentrations, thus high concentrations of each drug which could be used in combination therapy were used.

In the spectrophotometric and visual assessment of chemical compatibility, in all experiments the drug concentrations used were within the ranges used in combination chemotherapy in 0.9% NaCl infusion solution assuming a patient surface area of 1.7 mg/m^2: irinotecan-HCl concentration ranges 0.400–0.815 mg/ml when injected to 250 ml infusion solution and 0.184–0.379 mg/ml when injected to 500 ml infusion solution; epirubicin-HCl concentration ranges 0.107–0.580 mg/ml when injected to 250 ml infusion solution and 0.060–0.339 mg/ml when injected to 500 ml infusion solution.

The intravenous preparations used clinically were simulated on a smaller scale using analytical procedures with reference standards of epirubicin-HCl and irinotecan-HCl. In the second part of the study, simulation was performed with the commercially available pharmaceutical forms of the drugs. Spectrophotometric measurements were made after dilution to appropriate concentrations.

Visual assessment of chemical compatibility

Visual compatibility was assessed by means of effervescence, colour change, precipitation and pH change [14] with irinotecan at concentrations in the range 7.20–1.17 $\times$ 10^{-4} M and epirubicin at concentrations in the range 7.32–7.53 $\times$ 10^{-4} M after the drugs were mixed.

Spectrophotometric assessment of chemical compatibility

Experiments using USP standards Epirubicin HCl (56.0 mg) was dissolved in 28.0 ml distilled water and

mixed with 103.2 ml NaCl infusion solution (of which 100.0 ml was 0.9% NaCl solution and 3.2 ml was distilled water) containing 64.0 mg irinotecan-HCl. The final concentrations of epirubicin-HCl and irinotecan-HCl were 7.36×10^{-4} *M* and 7.20×10^{-4} *M* respectively. The infusion solution was stirred and diluted at a ratio of 1:10 for spectrophotometric measurements.

In order to obtain UV absorption spectra of individual components, circumventing the acquisition of composite spectra and additive effects [9], all spectral changes of each drug in the mixture were observed by placing the other drug solution as the reference.

The spectra of the mixture were obtained against reference solutions containing irinotecan-HCl and epirubicin-HCl at the same concentrations prepared by the same procedures as for the mixture and blank-mixed 0.9% NaCl solution (Figs. 3, 5, and 6). The spectrum of each epirubicin and irinotecan reference solution were obtained by placing the blank mixed 0.9% NaCl solution as the reference (Figs. 2 and 4).

Experiments using commercially available pharmaceutical dosage forms A volume of 3.2 ml Campto, containing 64.0 mg irinotecan-HCl, was mixed with 100.0 ml 0.9% NaCl infusion solution. Farmorubicin, containing 56.0 mg epirubicin-HCl, was dissolved in 28.0 ml distilled water and was mixed with the same 100.0 ml 0.9% NaCl infusion solution. The final concentrations of epirubicin-HCl and irinotecan-HCl were 7.36×10^{-4} *M* and 7.20×10^{-4} *M*, respectively. The infusion solution was stirred and diluted at a ratio of 1:10. The same method as described above for the experiments using USP standards was used to circumvent the acquisition of composite spectra and additive effects. The spectra of the mixture were obtained against irinotecan-HCl and epirubicin-HCl reference solutions, which were prepared at the same concentrations using the same procedures as for the mixture. The spectrum of each epirubicin and irinotecan reference solution were obtained by placing the blank mixed 0.9% NaCl solution as the reference.

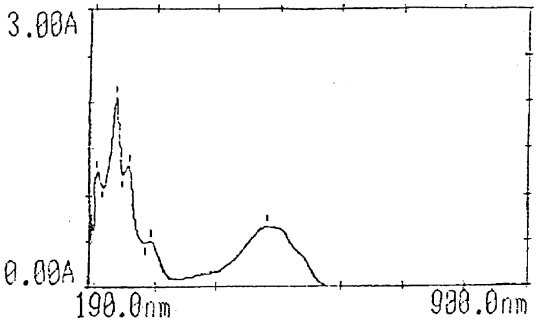


Fig. 2 The spectrum of 7.36×10^{-5} *M* epirubicin-HCl USP standard in 0.9% NaCl infusion solution (ref: 0.9% NaCl infusion solution)

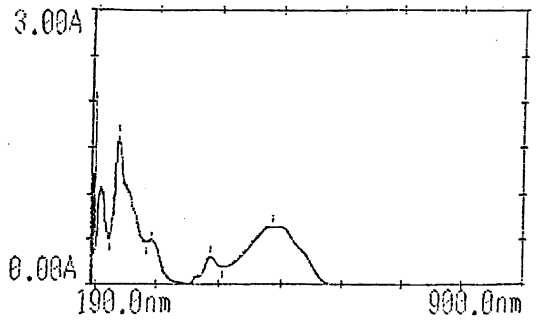


Fig. 3 The spectrum of 7.20×10^{-5} *M* irinotecan-HCl and 7.36×10^{-5} *M* epirubicin-HCl USP standards in 0.9% NaCl infusion solution (ref: 7.20×10^{-5} *M* irinotecan-HCl USP standard in 0.9% NaCl infusion solution)

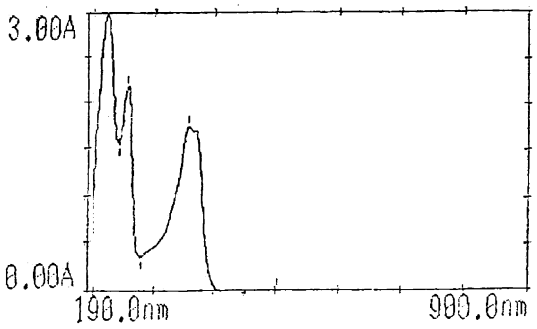


Fig. 4 The spectrum of 7.20×10^{-5} *M* irinotecan-HCl USP standard in 0.9% NaCl infusion solution (ref: 0.9% NaCl infusion solution)

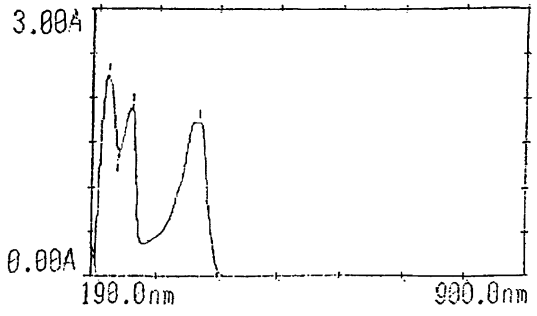


Fig. 5 The spectrum of 7.20×10^{-5} *M* irinotecan and 7.36×10^{-5} *M* epirubicin USP standards in 0.9% NaCl infusion solution (ref: 7.36×10^{-5} *M* epirubicin-HCl USP standard in 0.9% NaCl infusion solution)

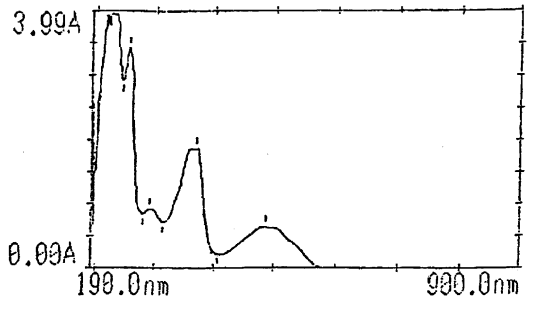


Fig. 6 The spectrum of 7.20×10^{-5} *M* irinotecan and 7.36×10^{-5} *M* epirubicin USP standards in 0.9% NaCl infusion solution (ref: 0.9% NaCl infusion solution)

Table 1 Absorbance values of epirubicin-HCl/irinotecan-HCl solutions at different molar ratios

	Epirubicin-HCl/irinotecan-HCl molar ratio							
	0.25	0.50	1.00	2.00	3.00	4.00	5.00	6.00
Absorbance (A)	0.26	0.47	0.81	1.22	1.41	1.51	1.50	1.51

Molar ratio

To determine the molar ratio for the reaction to proceed quantitatively, each 1.0 ml of 1.60×10^{-3} *M* irinotecan-HCl solution was mixed with 0.5–6.0 ml epirubicin-HCl solutions, with the same concentration as the irinotecan-HCl solutions, so that the final volume of each mixture was 7.0 ml. The absorbance values obtained using irinotecan-HCl solution as the reference are shown in Table 1. Extrapolating the curve, absorbance was found to reach maximum when the epirubicin-HCl/irinotecan-HCl molar ratio was 2:1.

Results and discussion

No sign of chemical incompatibility of these two drugs has been found in qualitative studies related to visual incompatibility from the point of view of gas production and precipitation. The infusion solution took the red–orange colour of epirubicin-HCl and the pH of the infusion solution took the pH of the drug which was used at the higher concentration. It is possible to predict drug–infusion fluid or drug–drug incompatibilities due to changes in pH, provided that the pH range for optimal stability of the chemotherapeutic drug is known [18]. In our study, the pH of the irinotecan was dominant in the mixture solutions (the pH of irinotecan infusion solutions and mixture solutions were almost the same, about 4.5 in the 7.20×10^{-4} *M* solutions and about 3.2 in the more concentrated solutions as 7.30×10^{-3} *M*) and since epirubicin is stable at acid pH, no pH-related stability problem was seen in our study. There were no changes in the spectra and absorbances when each irinotecan-HCl and epirubicin-HCl solution was investigated alone at various pH values between 3.0 and 7.0. These results show that the interaction peak was not a peak of ionization of either of the two drugs. Also no changes in the spectra of the mixtures was seen at pH values between 3.0 and 7.0 after mixing the two drugs.

The pH of infusion solution containing 7.20×10^{-4} *M* irinotecan-HCl was 4.5. It is known that at pH values of 4.0 and higher, hydrolysis of the lactone ring occurs. Because of this, care should be taken when working with solutions containing concentrations lower than 1.0×10^{-3} *M* (0.7 mg/ml). In combination therapy, injection using 100-ml infusion bags for lower doses and 250-ml bags for higher doses might be considered in order to maintain the pH for maximum lactone configuration.

Serious changes in the spectrum of epirubicin-HCl, especially a formation at 385 nm, were observed after the two drugs were mixed (Figs. 2, and 3). This suggests a structural change in epirubicin-HCl in infusion solution in the presence of irinotecan-HCl. This peak showed a maximum absorbance with an epirubicin-HCl/irinotecan-HCl molar ratio of 2:1 (Table 1). In the presence of epirubicin-HCl in the infusion solution, small decreases were observed in the absorbances of the peaks of irinotecan-HCl (Figs. 4, and 5). The spectrum of the cumulative peaks of the mixture with blank-mixed 0.9% NaCl infusion solution as reference is shown in Fig. 6.

The order of mixing did not affect the interaction spectrum. Chemical interaction occurred immediately after mixing and no visual or spectral change were noted for 24 h after the interaction had occurred. Also, since there were no differences between the spectra of mixtures at different pH values between 3.0 and 7.0, it can be concluded that this chemical interaction is not reversible under these conditions.

It has been considered that these two drugs are chemically incompatible. It is thought that, with administration of irinotecan and epirubicin in combination, the risk of cardiotoxicity can be reduced by decreasing the epirubicin doses. Each agent works through the inhibition of topoisomerases and inhibition of topoisomerases I and II may possibly result in significant clinical synergy. There are only two studies in the literature on the administration of these two drugs in the same combination, both of which are only phase I trials. No phase II trials have been published yet, and for further pharmacological studies on concurrent administration of irinotecan and epirubicin, the chemical compatibility of these two antineoplastic drugs in the same infusion solution, especially through the infusion period, has gained importance.

In further pharmacological studies of these two drugs in combination, their chemical interaction should also be considered. The positive or negative contibution of the chemical interaction through the infusion period found in this study to the pharmacological effect of the combination might be of clinical importance and should be investigated in further clinical trials.

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