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Long-term stability of 5-fluorouracil stored in PVC bags and in ambulatory pump reservoirs

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Abstract

Prolonged infusions of 5-fluorouracil (5FU) have been used since the early 1960s, but recently there has been a major resurgence of interest, partly because of the advent of electronically controlled portable infusion pumps. Admixtures of new formulation 5FU were subjected to stability studies to establish the feasability of continuous infusions. In the first study, the stability of 5FU, 1 or 10 mg ml^{-1}, was determined in poly(vinyl chloride) (PVC) bags (0.9% sodium chloride injection or 5% dextrose injection) at 4 and 21°C after storage for 0, 1, 2, 3, 4, 7 and 14 days. In the second study, the stability of undiluted 5FU was tested at different temperatures (4 or 33°C) in ethylene–vinyl acetate (EVA) or PVC ambulatory pump reservoirs after storage for 0, 3, 5, 7 and 14 days. For each condition, samples from each admixture were tested for drug concentration by stability-indicating high-performance liquid chromatography. The admixtures were also monitored for precipitation, colour change and pH. Evaporative water loss from the containers was measured. The stability of 5FU in PVC bags was unaffected by 14 days of storage at either 4 or 21°C. When stored in EVA reservoirs, 5FU was stable for at least 2 weeks at 33°C and for 3 days at 4°C (a precipitate was observed after 3 days). In PVC reservoirs, 5FU was stable for over 14 days at 33°C, but at 4°C a precipitate appeared after 5 days. Loss of water through the reservoirs was substantial only at 33°C for 14 days, and gave falsely high readings.

Keywords: Ambulatory pump reservoir; Drug stability; Fluorouracil; High-performance liquid chromatography; Poly(vinyl chloride) bag

1. Introduction

More than two decades after its synthesis [1], 5-fluorouracil (5FU) continues to be one of the major agents used in the treatment of breast [2], gastrointestinal [3], head and neck [4] cancers. 5FU is a drug with a very short plasma half-life of approximately 11 min and has cytotoxic activity mainly against cells in S phase [5].

The antineoplasic effects of 5FU are caused by its metabolites. There are two main pathways for the incorporation of 5FU into nucleic acids [6]. It is metabolized initially to nucleotides including fluoridine 5′-triphosphate (FUTP) and 5-fluoro-2′-deoxyuridine 5′-monophosphate (FdUMP), al-

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though it is unclear which is the most important pathway clinically. FdUMP inhibits thymidilate synthetase activity and the synthesis of DNA, and FUTP forms fraudulent RNA. FdUMP forms a tightly binding covalent bond with thymidilate synthetase and 5,10-methylenetetrahydrofolate, and is a competitive inhibitor of deoxyuridine monophosphate binding. FUTP incorporates primarily into nuclear RNA and smaller quantities also incorporate into other RNA [7]. 5FU is inactivated initially by conversion into 5-fluorodihydrouracil by the enzyme dihydropyrimidine dehydrogenase. This occurs in all tissues, but its activity is most intense in the liver, which therefore plays a major role in the degradation of 5FU.

Its mode of action illustrates the arguments for using prolonged infusions in the treatment of cancers. It is well known that longer infusions produce less toxicity, increase efficacy and permit an increased total dose administration [6,8].

In France, a new formulation of 5FU is available in vials containing 50 mg ml^{-1} of drug with sodium hydroxide for pH adjustment. The old formulation contained 5FU with Tris buffer.

The cardiotoxicity of 5FU was attributed to degradation compounds present in the injected vials, fluoroacetaldehyde and fluoromalonaldehydic acid. These compounds are formed with time in the basic medium necessary to solubilize 5FU. In 5FU–Tris vials, fluoroacetaldehyde and fluoromalonaldehydic acid are stored in stable "depot" forms, which are adducts with Tris, whereas in 5FU–NaOH vials, they are extensively chemically transformed. Cardiotoxic fluoroacetate, arising from fluoroacetaldehyde metabolization, was found in the urine of patients, with a fluoroacetate to 5FU catabolites ratio 10–30 times lower in patients treated with 5FU–NaOH than in those treated with FU–Tris [9].

More information about the stability of 5FU in infusion fluids or in portable infusion-pump reservoirs is required because of the recently modified formulation and the now frequently encountered centralized preparation of anticancer drugs. Indeed, according to the package insert, the stability of diluted 5FU solutions was reported to be 8 h after preparation.

The purpose of this study was to determine the effects of temperature and drug concentration on the stability of 5FU in 0.9% sodium chloride injection and 5% dextrose injection and in reservoirs.

2. Materials and methods

2.1. Materials

Vials containing fluorouracil, 250 mg in 5 ml of aqueous solution (pH 9.4), were obtained from Roche Laboratories (Neuilly sur Seine, France; lot B0/7X).

The drug was assayed under different physical conditions corresponding to those encountered clinically: (i) poly(vinyl chloride) (PVC) plastic bags obtained from Baxter Laboratories (Maurepas, France): 0.9% sodium chloride injection (lot 93J04G50) and 5% dextrose injection (lot 93I30G50); and (ii) cassettes to simulate ambulatory treatment: the types of containers used were an 80 ml ethylene–vinyl acetate (EVA) reservoir (RES 80A, Celsa Laboratories, Chasseneuil, France; lot 93H13) and a 50 ml PVC Medication Cassette from Pharmacia Laboratories (St. Paul, MN, USA; lot 31180).

All other chemicals and reagents were of analytical grade or high-performance liquid chromatography (HPLC) grade.

2.2. Preparation of admixtures

All admixtures were prepared under aseptic conditions in vertical laminar-air-flow biological safety cabinet.

Duplicate test solutions of fluorouracil, 1 and 10 mg ml^{-1}, were prepared in 5% dextrose injection and in 0.9% sodium chloride injection, not protected from light, and stored at 4 or 21°C. At 0, 1, 2, 3, 4, 7 and 14 days, 3 ml samples were removed and observed visually.

Each drug reservoir was filled with 50 ml of undiluted solution (50 mg ml^{-1}). All air was removed from the reservoirs. The filled drug reservoirs were protected from light and stored at 4 or 33°C. Storage at 33°C was chosen to mimic the

conditions of prolonged infusion of a drug kept underneath the patient's clothing and administered via a pump. At 0, 3, 5, 7 and 14 days, samples were removed from each cassette.

The samples were immediately subjected to pH measurement and chromatographic analysis. Drug concentrations were determined in triplicate by HPLC for each combination of concentration, vehicle and temperature.

2.3. Measurement of evaporative water loss

Two EVA reservoirs and two PVC reservoirs were filled with 50 ml of undiluted 5FU and weighed. One of the two reservoirs was then stored at 4°C and the other at 33°C. The containers were reweighed after 3, 5, 7 and 14 days.

2.4. Analysis by high-performance liquid chromatography

5FU concentrations were determined by using the stability-indicating HPLC assay described by Pattyn et al. [10], modified to achieved satisfactory chromatography in this laboratory.

All HPLC assays were performed isocratically at ambient temperature. The LC system consisted of a high-pressure pump (LC-9A, Shimadzu, Kyoto, Japan), a manual injection valve equipped with a 20 μl sample loop, a 5 μm Kromasil C_{18} column (150 × 4.6 mm i.d.) (Touzart et Matignon, Vitry/Seine, France) and an ultraviolet absorbance detector operating at 254 nm (SPD-6A variable-wavelength UV detector, Shimadzu). Integration of the peaks was performed with an integrator (C-R5A Chromatopac, Shimadzu).

The mobile phase was 0.05 M phosphate buffer (dibasic potassium phosphate) at pH 3.0. The flow rate was 1.3 ml min^{-1}. The retention time for 5FU was 3 min. Samples of 1, 10 and 50 mg ml^{-1} 5FU solution were diluted 100-fold with sterile water before analysis. Triplicate HPLC determinations were performed on each sample of each test solution.

A six-point calibration curve of 5FU diluted in water showed good linearity from 10 to 500 μg ml^{-1}, as can be seen from the equation $y = 33722.2x + 14196$, $r = 0.99980$, where x and y are the concentration of the compounds (μg ml^{-1}) and the peak area, respectively, and r is the correlation coefficient.

For a nominal 0.050 mg ml^{-1} solution of 5FU, the mean ± SD of the assay, determined from six replicate injections, was 0.0509 ± 0.0008 mg ml^{-1}. The precision expressed as relative standard deviation (RSD) was 1.6%. The within-day and betwen-day RSDs were 1.3% and 2.4%, respectively.

2.5. Analysis of data

The initial concentration of 5FU was designated as 100% and all subsequent concentrations were expressed as percentages of the initial concentration.

Stability was defined as a concentration 90–105% of the initial concentration, in accordance with the Health Registration of France, the French regulatory agency for drug and drug-related products.

3. Results and discussion

3.1. Stability in PVC bags

As detailed in Table 1, 5FU at 1 or 10 mg ml^{-1} in 0.9% sodium chloride injection or 5% dextrose injection, at 4 or 21°C, was stable throughout the 14 day survey. All concentrations remained above 90% of the initial value, and most were near 100%. These findings are consistent with those of Milano et al. [11].

The effect of exposure to light was not evaluated because, as observed by Pinguet et al. [12], protection of PVC minibags and perfusor from light is not required.

At each sampling time, visual examination of all tested admixtures showed them to be clear and without any change in colour when stored at 4 and 21°C.

The pH of different samples did not vary over the period of time considered in any of the tested solutions.

Table 1
Stability of 5FU at 1 or 10 mg ml^{-1} in 0.9% sodium chloride injection or 5% dextrose injection in PVC bags

Temperature (°C)	Theoretical concentration (mg ml^{-1})	Actual initial concentration (mg ml^{-1})	Percentage of initial concentration remaining [a]					
			1 Day	2 Days	3 Days	4 Days	7 Days	14 Days
0.9% Sodium chloride injection								
4	1	0.94 ± 0.06	97.85 ± 0.26	97.90 ± 0.30	99.54 ± 0.60	99.58 ± 0.38	98.58 ± 1.00	97.80 ± 0.51
21	1	0.99 ± 0.05	98.03 ± 0.26	97.79 ± 1.77	97.69 ± 1.61	99.27 ± 0.58	99.65 ± 0.12	98.20 ± 0.61
4	10	9.89 ± 0.03	98.79 ± 0.32	98.25 ± 0.61	99.19 ± 0.67	99.87 ± 0.97	99.01 ± 1.05	99.57 ± 0.36
21	10	9.85 ± 0.19	98.24 ± 0.49	97.25 ± 1.04	98.57 ± 0.84	98.33 ± 2.78	98.06 ± 1.89	97.59 ± 0.54
5% Dextrose injection								
4	1	0.93 ± 0.02	99.51 ± 0.92	100.08 ± 1.00	97.16 ± 0.28	98.52 ± 2.23	98.65 ± 0.57	97.08 ± 0.90
21	1	0.93 ± 0.04	99.37 ± 1.68	98.62 ± 2.66	97.57 ± 1.05	98.50 ± 1.99	98.72 ± 2.56	98.69 ± 1.26
4	10	9.91 ± 0.03	99.14 ± 1.34	97.55 ± 0.91	97.86 ± 0.97	98.45 ± 1.04	97.28 ± 1.89	97.86 ± 1.87
21	10	9.99 ± 0.35	99.71 ± 0.43	99.24 ± 0.97	99.58 ± 0.42	97.47 ± 1.07	99.28 ± 0.54	98.49 ± 0.67

[a] Values are mean ± S.D. of triplicate determinations for two samples.

3.2. Stability in portable infusion-pump reservoirs

Concerning evaporative water loss from the containers, at 4°C no significant decrease in the weight of the containers occurred. Loss of water through the reservoirs was substantial only at 33°C. 5FU weight decreases of 3.8% and 4.5% were observed in the PVC and EVA reservoirs, respectively, after storage for 14 days.

Data on the stability of 5FU under each condition are listed in Table 2.

At 4°C, 5FU was stable throughout the study as long as the drug remained dispersed in the infusion solutions. The average loss of 5FU during 7 days was less than 10% of the initial concentration. The loss was more than 10% at 14 days in the reservoirs that showed heavy precipitation. A flocculent precipitate appeared in EVA containers after 3 days of storage and after 5 days in PVC reservoirs. The precipitation is a solubility effect dependent on temperature and concentration but no pH. In fact, the pH was stable throughout the storage period. These data do not corroborate those of investigators [13–15] who used the old formulation.

At 33°C, a 5% increase in the concentration of 5FU (107.13%) was observed in EVA reservoirs, because of water permeation through EVA. Rochard et al. [15] observed these similar results. No change in 5FU concentration was detected at

Table 2
Stability of 5FU (50 mg ml^{-1}) in portable infusion-pump reservoirs

Temperature (°C)	Theoretical concentration (mg ml^{-1})	Actual initial concentration (mg ml^{-1})	Percentage of initial concentration remaining [a]			
			3 Days	5 Days	7 Days	14 Days
Celsa						
4	50	50.10 ± 0.08	98.17 ± 0.67	99.31 ± 0.47	90.79 ± 0.78	86.72 ± 0.44
33	50	50.26 ± 0.02	98.26 ± 0.20	100.35 ± 1.27	101.63 ± 1.25	102.35 ± 0.08
Pharmacia						
4	50	50.06 ± 0.06	98.20 ± 0.24	97.80 ± 0.67	95.10 ± 0.48	85.18 ± 0.21
33	50	50.30 ± 0.03	95.88 ± 0.72	100.67 ± 0.34	97.72 ± 0.76	98.33 ± 0.43

[a] Corrected values for water loss. Values are mean ± S.D. of triplicate determinations for two samples.

any time interval throughout the 14 day period for PVC containers. These results support the findings of Stiles et al. [16]. However, after correction for the evaporative water loss, 5FU showed less than 5% variation of the initial concentration. The total dose of 5FU in the reservoirs was in the range 90–105% of the initial value. No change in pH or precipitation was observed in any of the containers at this temperature.

4. Conclusions

5FU at 1 or 10 mg ml^{-1} in 0.9% sodium chloride injection or 5% dextrose injection in its new formulation was found to be stable in PVC bags for at least 2 weeks at 4 or 21°C.

At 33°C, undiluted 5FU was stable for 14 days in EVA and PVC reservoirs. In contrast, at 4°C precipitation is the primary factor limiting the storage time and may occur after 3 days in EVA containers and after 5 days in PVC.

Loss of water through the reservoirs was substantial only at 33°C.

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