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Stability of intravenous admixtures of 5-fluorouracil and spirogermanium, a novel combination of cytotoxic agents

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Summary

When used in combination, spirogermanium (SG) and 5-fluorouracil (5-FU) have to be administered at separate intravenous-infusion sites. To ease the administration of this potentially useful combination of antineoplastic agents, the stability of SG and 5-FU admixtures was studied. Direct mixing of SG and 5-FU formulations resulted in the formation of a precipitate. This precipitate was the free base form of SG, which has a low solubility at the pH of the i.v. admixtures. The appropriate dilution in D5W needed to prevent the precipitation was calculated from the pH-solubility curves of SG and 5-FU and the stability of 250 mg SG and 1000 mg 5-FU in 250 ml infusion containers was then investigated. There was no loss of SG or 5-FU in glass bottles after storage for 7 days at 4°C followed by 2 days at room temperature. However, in polyvinylchloride (PVC) and polyolefin infusion bags at pH > 8.0, the SG concentration decreased by 68 and 28%, respectively, after 7 days at room temperature. This decrease was independent of the 5-FU concentration and was believed to be due to sorption or adsorption of SG by the plastic. Loss of SG was also found when the admixtures were passed through PVC or polyolefin tubing at 20 ml h^{-1}. By lowering the pH of the admixture to a value of less than 6.0, the loss of SG to PVC containers or tubing was prevented. Simultaneous administration of SG and 5-FU is possible if adequate dilution is maintained to prevent precipitation and the resulting pH of the admixture is kept below 6.0.

Introduction

Spirogermanium (SG, Fig. 1) is a novel cytotoxic agent that has shown activity against a number of tumors both in vitro (Hill et al., 1982, 1984, 1986; Slavik et al., 1982, 1983; Schwartz et al., 1983; Yang and Rafler, 1983) and in vivo (Slavik et al., 1982, 1983). Phase I and II investigations revealed the lack of bone marrow toxicity of SG (Schein et al., 1980; Budman et al., 1982; Slavik et al., 1982, 1983; Legha et al., 1983; Ajani et al., 1986; Goodwin et al., 1987; Saiers et al., 1987a, b). Unfortunately, these same clinical trials revealed substantial neurological side effects which have limited the dose of SG that can be administered safely. Although the dose-limiting side effects of SG are reversible and can be reduced by the use of continuous infusions (Saiers et al., 1987a, b), they prevent the achievement of the optimal plasma

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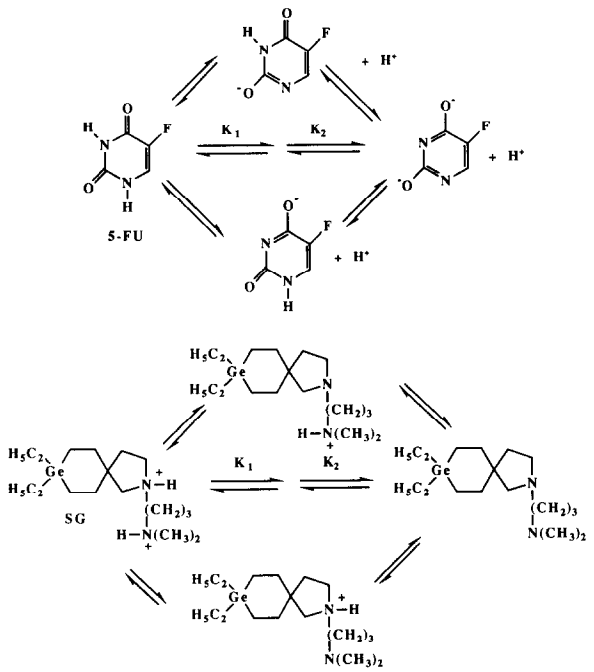

Fig. 1. The structures of spirogermanium (SG) and 5-fluorouracil (5-FU) and their respective acid dissociation species.

levels that were predicted from pre-clinical investigations (Hill et al., 1982, 1984, 1986; Slavik et al., 1982, 1983; Schwartz et al., 1983; Yang and Rafler, 1983).

Following Phase I and II studies in which SG was investigated as a single agent, it is now being evaluated in combination with other drugs including 5-fluorouracil (5-FU). Combining SG and 5-FU (Fig. 1) was prompted by the encouraging finding of Hill et al. (1984) who identified the synergism of these two agents using a range of human tumor cell lines in vitro. The lack of SG bone marrow toxicity is also an advantage since it allows 5-FU to be used without a reduction in its dosage levels. At present, researchers at this and other institutions are evaluating the effectiveness of continuous infusions of 5-FU (1000 mg m^{-2} day^{-1}) and SG (250 mg m^{-2} day^{-1}) against a variety of neoplastic diseases. The two drugs must be administered via separate intravenous catheters, since the compatibility of the two drugs is unknown. This is clearly an inconvenience to both patients and clinicians and the purpose of the present study was to devise a stable intravenous formulation containing both SG and 5-FU. SG is formulated as a 10 mg ml^{-1} solution of the dihydrochloride salt in saline (Spiro-32®) and the pH of the solution is approx. 6. In contrast, 5-FU is formulated as a 50 mg/ml^{-1} solution of the sodium salt (fluorouracil injection USP) at pH approx. 9. These facts alone suggest that the two drugs are likely to be physically incompatible and that the formulation of an aqueous solution containing both drugs represents a significant challenge. Various dosage regimens for the combination of SG and 5-FU have been suggested and the objective of the present study is to develop broad guidelines for the intravenous formulation of the two drugs.

Materials and Methods

Chemicals and reagents

5-FU was obtained from Sigma (St. Louis, MO) or as an injectable formulation (50 mg ml^{-1}, 5-FU injection USP, SoloPak Laboratories) from the University of Kansas Medical Center (Kansas City, KS). Both the SG · 2HCl and the consitituted vials of 10 mg ml^{-1} SG · 2HCl in 0.9% NaCl (Spiro-32®) were kindly provided by Unimed (Somerville, NJ). 9,10-Dimethoxyanthracene-2-sulfonic acid (DAS) was obtained as the sodium salt from Fluka (Ronkonkoma, NY) and the HPLC grade solvents from Fisher Scientific (St. Louis, MO). The chloroform was shaken with one half of its volume of water saturated with NaCl, dried over $CaCl_2$ and stored in the dark prior to use.

The 0.9% NaCl injections USP (NS), the 5% dextrose injection USP (D5W) and the various intravenous administration sets were obtained from either the Lawrence Memorial Hospital (Lawrence, KS) or the University of Kansas Medical Center (Kansas City, KS). The polyolefin intravenous administration sets were Tridilsets® from DuPont Critical Care (Wakegan, IL), flexible polyvinyl chloride (PVC) administration sets were 2C0006s from Travenol Labs (Deerfield, IL), polyolefin infusion bags were from Kendall McGaw Labs (Irvine, CA) and the glass bottles and

PVC (Viaflex) infusion bags were from Travenol. All the other chemicals and reagents were obtained from various sources and were used as received. Distilled, deionized water was used throughout.

Determinations of SG

SG was determined in aqueous solutions using a spectrophotometric method recently developed in these laboratories (Riley et al., 1989). This method involves the extraction of SG into chloroform as a fluorescent ion-pair with 9,10-dimethoxyanthracene-2-sulfonate. Daily, five-point calibration curves containing 5–20 μg ml^{-1} SG · 2HCl were prepared as follows: 500 μl of acetate buffer (0.5 M, pH 4.0), 300 μl of water, 1 ml of 0.6 mM aqueous DAS and 200 μl of an aqueous solution of SG · 2HCl (5–20 μg ml^{-1}) were combined in a 12 × 75 mm round-bottom polypropylene culture tube (5 ml, with polyethylene caps). Dried and alcohol-free chloroform (2 ml) was then added and the contents mixed with a vortex mixer for 60 s. The two phases were separated by centrifugation at 1000 × *g* for 5 min. The fluorescence of the chloroform layer was then determined with a Perkin-Elmer 650-40 spectrophotometer (Perkin-Elmer, Norwalk, CT). The excitation and emission wavelengths were 384 and 450 nm, respectively. Aqueous solutions were analyzed for SG in the same way after dilution with water so that their concentrations fell within the linear range of the calibration curve (5–20 μg ml^{-1}).

Determinations of 5-FU

5-FU was determined in aqueous solutions by high-performance liquid chromatography (HPLC) using a method based on that reported previously by Christophidis et al. (1979). The chromatograph was constructed from a Waters 6000A pump (Waters Associates, Milford, MA), a Kratos Spectroflow 783 UV detector (266 nm) (Kratos Analytical Instruments, Ramsey, NJ) and a Waters U6K manual injector (injection volume 20 μl). An ODS Hypersil column (5 μm, 15 × 4.6 cm, Keystone Scientific, State College, PA) was eluted with KH_2PO_4/H_3PO_4 (50 mM, pH 3.0) at a flow rate of 1 ml min^{-1}. Under these conditions, 5-FU had an elution volume of 3.8 ml and the void volume was 1.1 ml. An eight-point calibration curve was constructed on a daily basis over the concentration range of 5–75 μg ml^{-1}. Aqueous solutions were analyzed for 5-FU in a similar manner after dilution with mobile phase so that the concentrations of the solution injected were between 15 and 40 μg ml^{-1}. Each solution was injected in triplicate. The accuracy of the method for solutions containing 25, 30 or 35 mg ml^{-1} in NS and D5W was 100%. The precision of the assay was determined from the coefficient of variation obtained from the analysis of six aliquots of a mixture containing 30 mg ml^{-1} 5FU and 4 mg ml^{-1} SG · 2HCl. This coefficient of variation was 0.6% and there was no interference from SG.

Solubility determinations

The solubility of 5-FU in water was determined at room temperature (22 ± 1° C) at various pH values. Approx. 100 mg of 5-FU was weighed into glass screw-capped vials and mixed with 1 ml of various phosphate buffers (0.1 M; pH 2, 5, 7.5, 8 and 8.5). Two other solutions were prepared in water and 0.05 M NaOH. Each solution was prepared in duplicate. The solutions were shaken for 24 h at room temperature and then centrifuged at 1000 × *g* to separate the undissolved drug from the solution. The supernatant was then aspirated. After determining the pH, the supernatant was diluted (1 : 10) with water and stored in a refrigerator (4° C) prior to analysis by HPLC for 5-FU. The solubility of SG in water was determined in a similar manner using borate buffers (0.1 M, pH 8.5, 9 and 9.5) and NaOH (pH 10 and 12.5).

Titrations of SG / 5-FU admixtures

A solution containing 2.5 ml of SG · 2HCl injection (10 mg ml^{-1}), 2.0 ml of 5-FU injection USP (50 mg ml^{-1}), and 25 ml of D5W was titrated with either acetic acid (1 M), lactic acid (0.6 M), ascorbic acid (1 M) or hydrochloric acid (1 M). The acids were added in 5-μl aliquots and the pH was measured using an Orion Ross combination pH electrode and a Corning digital 112 research pH meter.

Compatibility studies

Preliminary compatibility studies involved mixing 50 mg ml^{-1} 5-FU injection USP, 10 mg ml^{-1}

SG · 2HCl injection and 5% dextrose injection (D5W) in various proportions. Aliquots of 1, 2, 3, 4, 5, or 6 ml of 5-FU injection USP, SG · 2HCl injection and D5W were mixed in glass test tubes, keeping the total volume constant at 6 ml (Table 1). The solutions were inspected for precipitation or any other changes in appearance. The degree of cloudiness was assessed on an arbitrary scale of + to + + + +, with + + + + being the most cloudy. Those solutions which had precipitated were centrifuged at $1000 \times g$ for 5 min, the clear supernatant removed and its pH measured. The supernatant was then diluted with water and assayed for 5-FU and SG concentrations.

Following these preliminary studies, the compatibility of 5-FU and SG was evaluated under conditions that might be encountered in the clinic. The effects of time, temperature (refrigeration and room temperature), container type (glass, polyolefin and polyvinylchloride) and tubing type (polyolefin and polyvinylchloride) on the stability of 5-FU and SG were determined. In these experiments, the concentrations of the two drugs were maintained constant at 0.8 mg ml^{-1} SG · 2HCl and 3.4 mg ml^{-1} 5-FU. Throughout these experiments, the diluent used was D5W and each admixture was prepared in duplicate in 250-ml prefilled containers. Diluent (25 ml) was removed from the reservoir, replaced by 25 ml of Spiro-32® (10 mg ml^{-1}, SG · 2HCl) and the solution mixed by inversion. 5-FU injection USP (20 ml, 50 mg ml^{-1}) was then added and again the solution was mixed by inversion. Samples (1 ml) were removed immediately after preparation and then periodically during storage. The pH of each aliquot was measured, followed by assaying for 5-FU and SG concentrations. For each experiment, an appropriate control admixture, containing only one of the two drugs, was prepared. The 5-FU control admixtures were prepared as previously described except that the 25 ml of Spiro-32® was replaced with 25 ml of D5W. The SG control admixture was prepared in a similar fashion.

The admixtures prepared in flexible PVC minibags were stored at room temperature for 7 days and those prepared in glass and rigid polyolefin bottles were stored for 7 days in the refrigerator followed by 48 h at room temperature (25 °C) under normal fluorescent lighting. After storage, the containers were inverted and the entire contents passed through either PVC or polyolefin tubing, at a flow rate of 20 ml h^{-1} to simulate an intravenous infusion. The concentrations of 5-FU and SG were determined in the 1-ml fractions collected after 0, 2.5, 5, 7.5, 10 and 12.5 h. Additionally, the cumulative amounts of 5-FU and SG were determined from the concentrations of 5-FU and SG in each 50 ml aliquot collected.

To determine the role of 5-FU and pH in the observed instability of spirogermanium, the following experiment was performed in polyvinylchloride minibags. The pH of an admixture containing both 5-FU and SG was adjusted to approximately that of the control (pH 5.5) using 1 N HCl and, conversely, the pH of an SG control was raised to that of the admixture (pH 9.0) with 1 N NaOH. The minibags were stored at room temperature under normal fluorescent lighting for 48 h. A 1 ml sample was removed immediately after preparation and then periodically during storage. The pH of each aliquot was measured, followed by the assays for 5-FU and SG concentrations.

The pH-adjusted experiments were performed only in PVC minibags. The intravenous admixtures were prepared and sampled as before except 50 ml of diluent (D5W) was removed from the reservoir, 15 ml of 1 M acetic acid was added prior to the addition of the drugs, and saline was used in place of SG in the controls. The minibags were stored for 7 days in the refrigerator followed by 48 h at room temperature under normal fluorescent lighting. After 48 h at room temperature, a simulated infusion was performed by inverting the containers and passing the entire contents through PVC tubing, at a flow rate of 20 ml h^{-1}.

Results and Discussion

Compatibility studies

SG injection (10 mg ml^{-1}), 5-FU injection USP (50 mg ml^{-1}) and D5W were mixed together in various proportions to determine their physical and chemical compatibilities. The results of these studies are presented in Table 1. Solutions that

contained SG in the presence of 5-FU (except no. 4) produced a precipitate almost immediately after preparation. As expected, the pH values of the solutions containing both SG and 5-FU were between 6 and 9, and the actual pH could be related to the ratio of the concentrations of SG and 5-FU in solution. However, the higher buffer capacity of the 5-FU injection resulted in a grouping of the pH values between 8.25 and 8.88. The fact that solution no. 4 (Table 1) was clear suggested that the formation of a precipitate could be prevented by dilution of the mixture with D5W. Subsequent investigations were aimed at characterizing this precipitate and developing strategies that would overcome the incompatibility of the two drugs.

Ionization states

SG is a diprotic base and can exist in three different ionization states, the free base (SG), the monocation (SGH^+) and the dication (SGH_2^{2+}) as shown in Fig. 1. The dissociation constants for SG have not been reported previously. However, the values for analogous compounds (Albert and Sergeant, 1971) suggest that the two pK_a values of SG are approx. 8.5 and 10.5. Clearly, both these pK_a values are relevant parameters to be consid-

TABLE 1

Analysis of solutions prepared by mixing commercial formulations of spirogermanium (di)hydrochloride (SG) (10 mg ml^{-1}), 5-fluorouracil (5-FU) (50 mg ml^{-1} as the sodium salt) and 5% dextrose injection

Solution No.	Volumes (ml)			pH [a]	Concentrations (mg ml^{-1}) [a]				Appearance [b]
	SG	5-FU	D5W		SG		5-FU		
					Calc. [c]	Found [a]	Calc. [c]	Found [a]	
1	5	1	0	8.15	8.33	8.17	8.33	8.26	−
2	5	0	1	6.25	8.33	8.78	0.00	0.00	−
3	4	2	0	8.40	6.66	3.93	16.7	16.0	+ + +
4	4	1	1	8.25	6.66	6.85	8.33	8.25	−
5	4	0	2	6.22	6.66	6.90	0.00	0.00	−
6	3	3	0	8.66	5.00	2.72	25.0	24.5	+ + + +
7	3	2	1	8.53	5.00	3.14	16.7	16.2	+ + +
8	3	1	2	8.38	5.00	3.84	8.33	8.26	+ +
9	3	0	3	6.16	5.00	5.15	0.00	0.00	−
10	2	4	0	8.76	3.33	2.00	33.3	33.0	+ + + +
11	2	3	1	8.70	3.33	2.10	25.0	24.5	+ + + +
12	2	2	2	8.61	3.33	2.03	16.7	16.1	+ + + +
13	2	1	3	8.48	3.33	2.50	8.33	8.26	+ + + +
14	2	0	4	6.01	3.33	3.44	0.00	0.00	−
15	1	5	0	8.84	1.67	1.53	41.7	40.5	+
16	1	4	1	8.83	1.67	1.49	33.3	32.4	+
17	1	3	2	8.77	1.67	1.39	25.0	24.7	+
18	1	2	3	8.71	1.67	1.34	16.7	16.6	+ +
19	1	1	4	8.59	1.67	1.53	8.33	8.47	+
20	1	0	5	5.65	1.67	1.60	0.00	0.00	−
21	0	5	1	8.88	0.00	0.00	41.7	42.3	−
22	0	4	2	8.88	0.00	0.00	33.3	33.1	−
23	0	3	3	8.85	0.00	0.00	25.0	24.7	−
24	0	2	4	8.83	0.00	0.00	16.7	17.1	−
25	0	1	5	8.80	0.00	0.00	8.33	8.37	−
26	6	0	0	6.27	10.0	10.3	0.00	0.00	−
27	0	6	0	8.91	0.00	0.00	50.0	49.6	−
28	0	0	6	4.80	0.00	0.00	0.00	0.00	−

[a] Mean of two determinations at ambient temperature (22 ± 1° C).

[b] The solutions were classified qualitatively as clear (−) and cloudy (+ to + + + +).

[c] Concentrations calculated from the nominal concentrations of the formulations.

TABLE 2

Solubilities of spirogermanium and 5-fluorouracil in various aqueous buffers

Spirogermanium			5-Fluorouracil		
pH	Buffer	Solubility [a,b] (mg ml^{-1})	pH	Buffer	Solubility [a] (mg ml^{-1})
8.50	borate (0.1 M)	1.18	4.97	phosphate (0.1 M)	11.9
9.01	borate (0.1 M)	0.183	6.52	none	12.6
9.51	borate (0.1 M)	0.0710	7.25	phosphate (0.1 M)	15.4
10.00	NaOH	0.0272	7.79	phosphate (0.1 M)	20.1
12.50	NaOH	0.00840	7.89	NaOH	23.0
			8.03	phosphate (0.1 M)	24.6
			8.25	phosphate (0.1 M)	36.6

[a] Mean of two determinations at ambient temperature (22 ± 1° C).
[b] As the dihydrochloride.

ered in the formulation of SG in aqueous solutions of pH 6–9. The commercial injection is a 10 mg ml^{-1} aqueous solution of the dihydrochloride salt which has a pH of about 6. In contrast with SG, 5-FU is a diprotic acid and can exist as the free acid (H_2FU), the monoanion (HFU^-) and the dianion (FU^{2-}) as shown in Fig. 1. The pK_a values of 5-FU have been reported to be 8.00 and 13.0 (Rudy and Senkowski, 1973) and 5-FU injection USP is a 50 mg ml^{-1} aqueous solution of 5-FU adjusted to a pH of about 9 with NaOH. The relationships between the fractions of the various ionic species and pH are given below:

$$f(SGH_2^{2+}) = (\text{or } f(H_2FU) =) \frac{[H^+]^2}{A} \quad (1)$$

$$f(SGH^+) = (\text{or } f(HFU^-) =) \frac{[H^+]K_1}{A} \quad (2)$$

$$f(SG) = (\text{or } f(FU^{2-}) =) \frac{K_1K_2}{A} \quad (3)$$

where

$$A = [H^+]^2 + K_1[H^+] + K_1K_2 \quad (4)$$

and K_1 and K_2 refer to the dissociation constants of either SG or 5-FU. Over the range pH 6–9, SG should exist predominantly as a mixture of its mono and dicationic forms. On the other hand, 5-FU will exist as a mixture of its free acid and monoanion. Consequently, the observed precipitation could be the result of the formation of an insoluble salt of SG and 5-FU. Alternatively, it is also possible that the intrinsic solubilities (S_0) of the free acid of 5-FU or the free base of SG could be exceeded, again leading to the formation of a precipitate.

Further chemical analysis of SG/5-FU mixtures revealed that the concentrations of SG in the solutions were generally much less than those expected (Table 1). In contrast, the concentrations of 5-FU found were all within 3% of the expected values. These results indicate that the incompatibility of 5-FU and SG arises from the precipitation of the free base form of SG due to the high pH values of the solutions (Fig. 1).

Solubility

To confirm the hypothesis that the incompatibility of SG and 5-FU is due to the precipitation of SG, their respective solubilities (S) in various aqueous buffers were determined. The results of these studies are listed in Table 2 and displayed graphically in Fig. 2. These data show that the solubility of 5-FU is independent of pH below 7 and then increases with increasing pH. In contrast, the solubility of SG increases gradually with decreasing pH between pH 12.5 and 9.5 and increases dramatically at lower pH values. These data are consistent with the ionization states of 5-FU and SG, such that the total solubility (S) of the two drugs is limited by their intrinsic solubilities (S_0) and is related to the H^+ concentrations

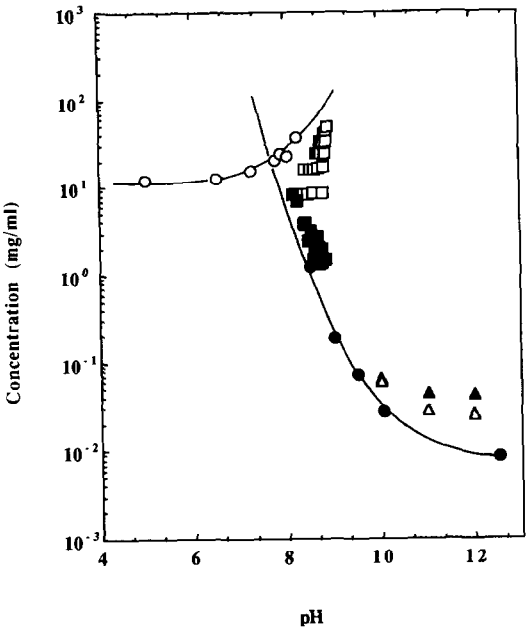

Fig. 2. Solubilities of spirogermanium (SG, closed circles) and 5-fluorouracil (5-FU, open circles) as a function of pH (Table 2). The solid lines have been drawn according to Eqns 7 and 5 for SG and 5-FU, respectively, using the following values: $pK_1(SG) = 8.51$, $pK_2(SG) = 10.31$, $S_0(SG) = 8.40$ $\mu g\ ml^{-1}$, $pK_1(5\text{-FU}) = 8.00$, $pK_1(5\text{-FU}) = 13.0$, $S_0(5\text{-FU}) = 12.2$ mg ml^{-1}. The closed and open squares correspond to the concentrations of SG and 5-FU, respectively, found in the various admixtures described previously in Tables 1 and 2. The open and closed triangles represent the solubilities of spirogermanium in the presence of 22.2 mg ml^{-1} 5-FU and 20.8 mg ml^{-1} dextrose, respectively.

by eqns 5–9 (Asuero, 1988). The relationship between the solubility of 5-FU and the H^+ concentration is given by:

$$S = S_0\left\{1 + \frac{K_a}{[H^+]}\right\} \tag{5}$$

where K_a is the first dissociation constant of 5-FU. The second pK_a of 5-FU was considered too high ($pK_2 = 13.0$) to influence the properties of aqueous solutions whose pH was less than 9. Rudy and Senkowski (1973) reported the first pK_a of 5-FU to be 8.00 and the intrinsic solubility to be 12.2 mg ml^{-1}. These parameters were used in conjunction with Eqn 5 to simulate the relationship between the solubility of 5-FU and pH. Fig. 3 shows that the agreement between theory and the values for the solubility of 5-FU found in this study to be excellent.

The relationship between the solubility of SG and the H^+ concentration is given by:

$$S = S_0\left\{1 + \frac{[H^+]}{K_2} + \frac{[H^+]^2}{K_1K_2}\right\} \tag{6}$$

where K_1 and K_2 denote the acid dissociation constants for SG. The intrinsic solubilities and the pK_a values of SG have not been reported. Consequently, the values of these parameters were determined from the data obtained in this study (Table 2). The value of S_0 was obtained by fitting the solubility data (Table 2) to Eqn 7 which is a rearranged, logarithmic form of Eqn 6.

$$\log\left(\frac{S}{S_0}\right) = \log\{1 + A'[H^+] + B'[H^+]^2\} \tag{7}$$

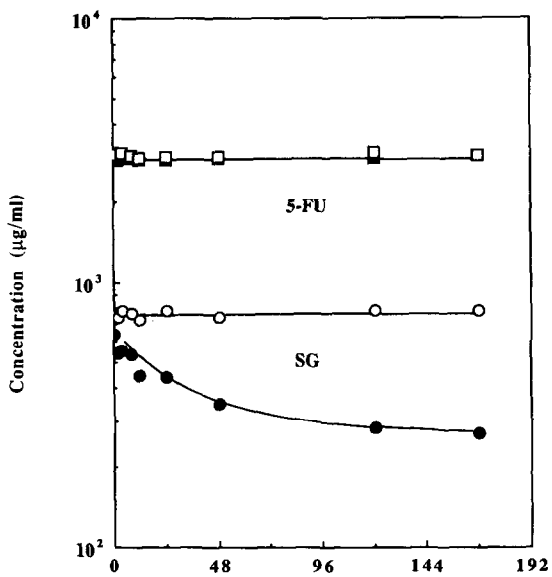

Fig. 3. Concentrations of spirogermanium (SG, circles) and 5-fluorouracil (5-FU, squares) in polyvinylchloride minibags (250 ml) after storage at room temperature. The closed symbols represent the concentrations of the drugs in the mixtures and the open symbols represent the concentrations of drugs in the controls.

where

$$A' = \frac{1}{K_2} \tag{8}$$

$$B' = \frac{1}{K_1 K_2} \tag{9}$$

Values of 8.40 μg ml^{-1} for S_0 and 3.06×10^{-9} and 4.86×10^{-11} for K_1 and K_2, respectively, were calculated. These dissociation constants correspond to values for pK_1 and pK_2 of 8.51 and 10.31, respectively, which agree very well with the values of analogous compounds (Albert and Sergeant, 1971). The very low intrinsic solubility of SG explains why a precipitate formed when the pH of admixtures was between 8.25 and 8.88, even though the fraction in the free base form is between 1 and 3%.

Fig. 2 (squares) also shows the data obtained from the compatibility studies described previously (Table 1) and as expected the 5-FU concentrations are below its solubility curve. In contrast with 5-FU, the data for SG lie slightly above its theoretical solubility curve. This suggests that the concentrations of SG found in the compatibility studies reflect supersaturated solutions. An alternative explanation for the slightly elevated solubilities of SG in the admixtures is that the dextrose or 5-FU present increases the intrinsic solubility of the free base form of SG. Accordingly, the solubility of SG was determined between pH 10 and 12 in the presence of (a) 22.2 mg ml^{-1} 5-FU and (b) 20.8 mg ml^{-1} dextrose. Fig. 2 (triangles) shows that the solubility of SG is enhanced by the presence of both 5-FU and dextrose. The mechanism of this enhancement is not known.

Fig. 2 was useful not only in explaining the precipitation of SG in admixtures with 5-FU, but also in predicting the maximum concentrations of SG that could be mixed with 5- FU without the production of a precipitate. Accordingly, the data in Fig. 2 were used to calculate the minimum volume of admixture that could be used to deliver daily doses of 250 mg m^{-2} SG and 1000 mg m^{-2} 5-FU which is one of the most commonly used combinations of these cytotoxic agents. It was found that these doses could be admixed in 295 ml of solution without the formation of a precipitate at room temperature (22° C).

Effects of containers and tubing on the stability of proposed formulation

Consistent with the solubility data (Table 2, Fig. 2), admixtures containing 1 g 5-FU and 250 mg SG could be prepared in approx. 295 ml of D5W without the formation of a precipitate. However, the stability of the SG/5-FU admixtures was not necessarily ensured. The hydrophobic nature of SG free-base (Fig. 1) coupled with its low intrinsic solubility made it an ideal candidate for plastic uptake. Alternatively, the protonated forms of SG (Fig. 1) can interact with the negatively charged sites on the surface of the container and/or tubing. Consequently, the nature of the container was expected to have an effect on the stability of the admixture. Subsequent studies were performed to test the hypothesis and develop a strategy that would produce a stable formulation of the two drugs, which is suitable for clinical use.

These admixtures were prepared in flexible PVC minibags, glass bottles and rigid polyolefin bottles and the concentrations of the two drugs monitored as a function of time and storage temperature. The admixtures were prepared by removing the 10% overage from a 250 ml container of D5W and adding 25 ml of 10 mg ml^{-1} SG injection and 20 ml of 50 mg ml^{-1} 5-FU injection USP. Each container was assayed initially and then at suitable intervals during storage. The 'initial concentrations' of SG and 5-FU found were all within 5% of the control values. The only exception to this was that the initial concentration of SG found in the PVC containers which was substantially lower than expected.

Fig. 3 shows the relationships between the concentrations of 5-FU and SG and time for admixtures prepared in PVC minibags and stored at room temperature (22 $\pm$ 1° C). It can be seen (Fig. 3) that there was a substantial decrease in the concentration of SG with time from the admixtures which also contained 5-FU. The mean initial concentration of SG in these admixtures was 0.630 mg ml^{-1} which represents a loss of 25.9%. After 7 days the mean concentration had decreased to

0.270 mg ml^{-1}, a total loss of 68.2%. In contrast, there was no loss of drug from control admixture containing SG alone (Fig. 3). In addition, there was no loss of 5-FU from either the control admixture or admixture containing both drugs (Fig. 3). Furthermore, the PVC containers containing both drugs turned distinctly yellow after 7 days.

In the subsequent studies, admixtures containing 1 g 5-FU and 250 mg SG · 2HCl in approx. 295 ml of diluent (D5W) were studied under simulated clinical conditions using both glass and polyolefin containers. These admixtures were stored for 7 days in the refrigerator (4 °C) followed by 48 h at room temperature (22 ± 1 °C), after which the solutions were passed through either PVC or polyolefin tubing at a flow rate of 20 ml h^{-1}. No loss of SG in the glass bottles was observed and the mean concentration of SG in the admixture after storage for 7 days in the refrigerator and 48 h at room temperature was 0.918 mg ml^{-1} compared with 0.913 mg ml^{-1} in the SG control. The 5-FU concentration remained constant throughout these experiments (Fig. 3).

Admixtures of SG and 5-FU in semi-rigid, polyolefin containers were also investigated, since several researchers have found substantially less uptake of lipophilic drugs by polyolefin- and polyethylene-based materials (Illum and Bundgard, 1982; Kowaluk et al., 1983; Hancock and Black, 1985; Lee, 1986; Paborji et al., 1988). The concentration of SG in the controls remained constant; however, in the admixtures containing 5-FU, the drug concentration decreased by 11.5% after 7 days in the refrigerator and by 6.7% (i.e. for a total of 18.2%) after 48 h at room temperature. The 5-FU concentration remained constant throughout the experiments. Thus, even in polyolefin containers, a significant amount of SG was lost from the admixtures containing both drugs.

Following storage of the formulations in glass or polyolefin (PO) containers, the solutions were passed through PVC or polyolefin tubing at a flow rate of 20 ml h^{-1} and the concentration profiles for SG are shown in Fig. 4. Some of the initial concentrations of SG shown in Fig. 4 were less than those in the control because of prior losses of drug in previous experiments investigating the effects of containers. Upon passage through PVC

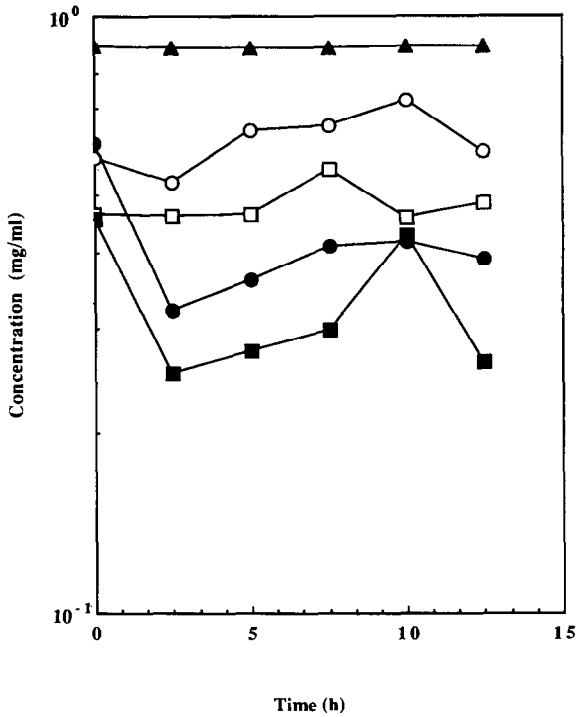

Fig. 4. A comparison of delivery systems for SG and 5-FU admixture. Glass (GL) and polyolefin (PO) containers were investigated with both types of tubing, polyolefin or polyvinylchloride (PVC). (▲) Control solution of SG was passed through PVC tubing; (○) glass container and polyolefin tubing; (□) polyolefin container and polyolefin tubing; (●) glass containers and PVC tubing; (■) polyolefin container and PVC tubing.

tubing, the SG concentration decreased substantially (Fig. 4) and only 46.5 and 52.4% of the dose were recovered from the glass/PVC and PO/PVC intravenous infusion sets, respectively. On the other hand, 88.7 and 84.7% of the SG were recovered from the glass/PO and PO/PO intravenous infusion sets, respectively.

Decreases in the concentration of SG were observed only in those containers in which 5-FU was also present. No loss of SG was seen in any of the SG controls. The major difference between the control admixture containing SG alone and the admixture which also contained 5-FU was the pH of the solutions. The pH of the control was 5.5 whereas the pH of the admixture containing both drugs was much higher (8.7). The most likely explanation for the loss of SG from the admix-

tures was sorption of the free base form of SG by the different plastic containers. Illum and Bundgard (1982) have studied the interaction of several drugs with PVC, PO and various other plastics. They concluded that the sorption of weak acids and bases is strongly dependent on the pH of the admixture and is greatest when the drug is in its neutral, unionized, form. The higher pH of the SG/5-FU admixture promotes the production of the SG free base (Fig. 1), which in turn increases the tendency of SG to partition into the matrix of the plastic. The observed concentration/time profile for SG during the simulated infusion studies (Fig. 4) is consistent with sorption of SG by the layer of PVC or PO tubing in immediate contact with the infusate (Illum and Bundgard, 1982; Paborji et al., 1988).

Illum and Bundgard (1982) have noted reduced sorption of drugs by plastics that contain no plasticizer, such as polyolefin. Even in polyolefin containers and tubings, where the observed loss of SG was less than that in PVC, the results were still unacceptable. Glass bottles proved to be the only suitable container for intravenous admixtures of SG and 5-FU; however, no suitable i.v. administration set in which there was no uptake of drug was identified. The loss of SG can be attributed to the sorption or adsorption of the drug by the plastic at higher pH values. This observation is further supported by the fact that no sorption occurred in the SG control whose pH value was approx. 5.5. Consequently, in order to obtain an acceptable formulation of the two drugs, the pH of the admixture had to be lowered to a value where the concentration of the free base form of SG was insignificant.

The pH dependence of SG sorption

In the case of SG (Fig. 1), higher pH values promote the production of the free base, thus sorption should increase with increasing pH. Furthermore, if the pH of the SG control was raised to approx. 7, loss of drug to the plastic container would be anticipated. As expected, in a solution at pH 7, a 23% loss of SG was seen after 48 h at room temperature. When the pH of the solution was then increased to 9, an additional 27% loss of SG was observed (i.e. the total loss of SG at pH 9 was 50%). Conversely, lowering the pH of the admixture containing both SG and 5-FU to approx. 6 prevented the sorption of the SG (Fig. 6). The 5-FU concentration was found to be within 5% of the predicted value (3.39 mg ml^{-1}) throughout this phase of the study and no loss of drug to any of the plastic surfaces was detected.

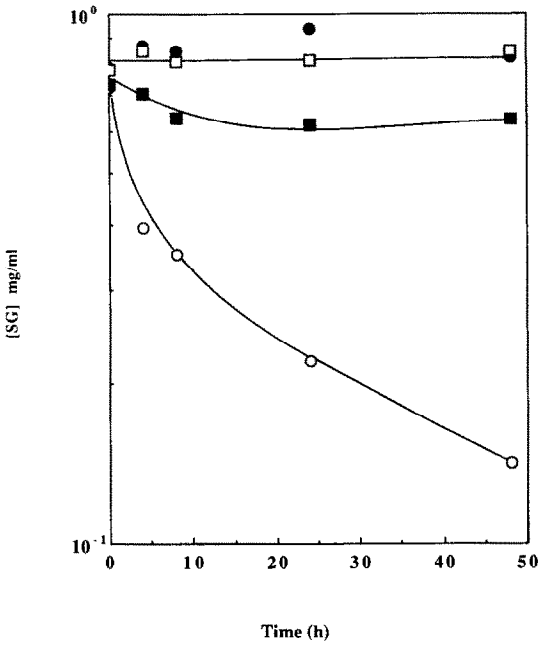

Fig. 5. Spirogermanium concentrations in admixtures of various pH values after storage in PVC minibags for 48 h at room temperature ($22 \pm 1°$ C). The circles represent the admixtures containing SG and 5-FU before (open) and after adjustment to pH 6 (closed). The squares represent the admixtures containing SG alone before (open) and after adjustment to pH 7 (closed).

The sorption of SG was found to be independent of the 5-FU concentration in the admixture and strongly dependent on the solution pH (Fig. 5). These results suggest that intravenous admixtures of SG and 5-FU would be physically stable if formulated at an acidic pH below a value of 6.

An acceptable formulation

The successful approach to the formulation of an intravenous admixture containing 250 mg SG and 1 g 5-FU involved reduction of the pH of the previously developed admixture of SG · 2HCl injection (10 mg ml^{-1}), 5-FU injection USP (50 mg ml^{-1}) and D5W mixed in the volumetric ratios

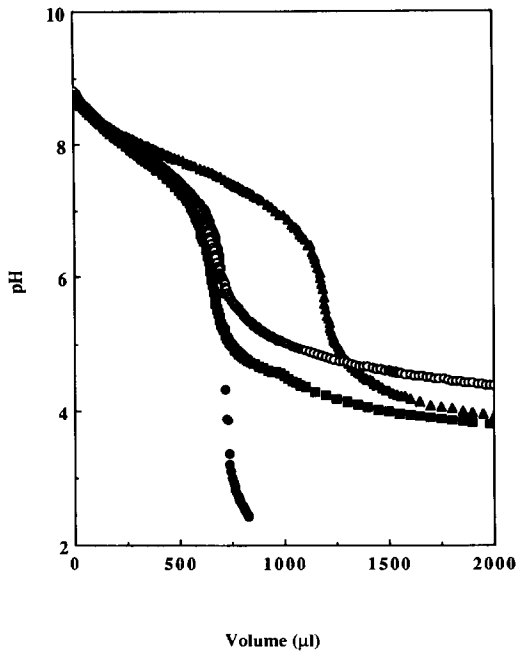

Fig. 6. Titration of a 29.5 ml aliquot of the admixture (2 : 2.5 : 25 solution of 5-FU, SG and D5W) with HCl (1 M, ○), lactic acid (0.6 M, ▲), acetic acid (1 M, ◆) and ascorbic acid (1 M, ■).

2 : 2.5 : 25. Several acids were investigated as being potentially suitable for reducing the pH of the solution. A 29.5 ml aliquot of the admixture was titrated with either 1 M HCl, 1 M ascorbic acid, 1 M acetic acid or 0.6 M lactic acid and the titration curves are shown in Fig. 6. Since neither 5-FU nor SG possesses any buffer capacity below a pH value of 6, the main purpose of this experiment was to identify an acid which could maintain the pH of the admixture between 4 and 6. Figure 6 shows that reduction of the pH with hydrochloric acid is unacceptable since the final pH of the solution rapidly approaches a value of 2 after neutralization of the admixture's components. On the other hand, titration with lactic acid, acetic acid or ascorbic acid produced pharmaceutically acceptable, plateau pH values of greater than 4, after neutralization of the admixtures. In the final formulation, acetic acid was preferred to ascorbic or lactic acid because of concerns over the stability. The degradation products of ascorbic acid are dehydroascorbic acid which is yellow and oxalic acid (Connors et al., 1986) which is toxic. Lactic acid was not chosen since it took a greater volume of acid to neutralize the admixture and reach the buffer plateau. Furthermore, in more concentrated solutions of lactic acid, one of its degradation products is highly insoluble polylactic acid.

Admixtures containing 250 mg SG and 1 g 5-FU were formulated by removing 50 ml of diluent (D5W) from the reservoir and adding 15 ml of 1 M acetic acid prior to the addition of 25 ml of Spiro-32® (10 mg ml^{-1} SG · 2HCl). After thorough mixing, 20 ml of 5-FU injection USP (50 mg ml^{-1}) was added and the solution again mixed by inversion and stored in PVC minibags for 7 days in the refrigerator followed by 48 h at room temperature (22 ± 1° C), after which the solutions were passed through PVC tubing at a flow rate of 20 ml h^{-1}. No sorption of SG into the PVC was observed and the mean concentration of SG in the admixture was 0.805 mg ml^{-1} which was identical to the value found for the SG control; the theoretical concentration was 0.808 mg ml^{-1}. Furthermore, the 5-FU concentration was found to be within 5% of the predicted values.

Conclusions

Two major problems have inhibited the formulation of admixtures containing both SG and 5-FU. Firstly, the free base form of SG has an intrinsic solubility of 8 μg ml^{-1} which, without substantial dilution, resulted in the formation of a precipitate when the two formulations were mixed. Secondly, without pH adjustment, sorption or adsorption by PVC containers, polyolefin containers, PVC tubing and polyolefin tubing caused substantial losses of SG from the admixtures. This sorption was found to be independent of the 5-FU concentration in the admixture but strongly dependent on the solution pH. However, simultaneous administration of SG and 5-FU is possible if adequate dilution is made, so that (a) the concentrations of the two drugs are below their respective solubilities and (b) the resulting pH of the admixture is kept below 6.0 to prevent sorption or adsorption of the SG by plastic materials.

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