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Development of parenteral formulations of experimental cytotoxic agents. I. Rhizoxin (NSC-332598)

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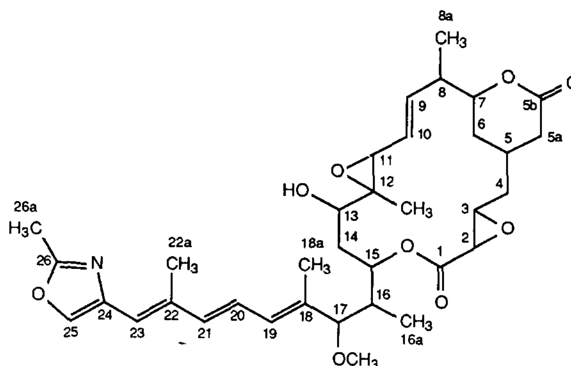
Summary

Rhizoxin (NSC-332598), is a macrocyclic lactone with antifungal and cytotoxic activity. To better evaluate the clinical potential of this poorly water-soluble (12 μg/ml), chemically unstable agent, a parenteral dosage form containing 1 mg/ml rhizoxin was desired. Therefore, the purpose of the present study was to evaluate the solubility and stability characteristics of rhizoxin and explore various strategies for the development of a dosage form with the desired stability and concentration goal. In aqueous solution rhizoxin appeared to degrade primarily via hydrolytic reactions resulting from cleavage of the lactone rings and the epoxide groups. In the solid state, there were some indications that rhizoxin cleavage might be oxidative in nature. Rhizoxin can be formulated for parenteral use at 1 mg/ml by preparing freeze-dried vials containing rhizoxin and mannitol (0.5%) from a 40% *t*-butanol/water solvent. The vials can be reconstituted with a solvent of 40% propylene glycol/10% ethanol/water. Optimization of the freeze-dried formulation is needed if a 2 year shelf-life at ambient temperatures is desired. The $t_{90\%}$ of the reconstituted solution was 18 h. Dilution (< 1 : 100) of the reconstituted solution with D5W and NS for intravenous infusion resulted in precipitation of rhizoxin. Dilution of the reconstituted solution with a commercial 10% soybean oil parenteral emulsion, Intralipid, appeared to be an alternative method of administering rhizoxin if parenteral infusion is desired. The $t_{90\%}$ of rhizoxin diluted in the emulsion was similar to that in water.

Introduction

Rhizoxin (**1**, NSC-332598), is a macrocyclic lactone with antifungal (Iwasaki et al., 1984) and cytotoxic activity (J. Cradock, personal communications, National Cancer Institute). Further evaluation of the clinical potential of this poorly



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water-soluble (12 μg/ml), chemically unstable agent, required the development of a parenteral dosage form of rhizoxin containing 1 mg/ml. Therefore, the purpose of the present study was to evaluate the solubility and stability characteristics of rhizoxin and explore various strategies for the development of a dosage form with the desired stability and concentration goal.

Experimental

Materials

Rhizoxin (**1**) was obtained from the National Cancer Institute, Bethesda, MD, U.S.A. (lot number, 090147L) through the supplier, Fujisawa Pharmaceutical Co., Osaka, Japan, and was used without any further purification. All chemicals including buffer components were ACS reagent grade and were used without further purification. Organic solvents except *t*-butanol were HPLC grade. Water used was deionized and glass-distilled (Mega-Pure system model MP-1, Corning). All pH measurements were made at 25°C with a digital pH meter, Corning model 155 (Medfield, MA).

HPLC analysis

Analysis of rhizoxin from aqueous and hydroalcoholic solutions used a Waters M-6000A pump, a Waters Model 440 UV detector set at 280 nm, a Rheodyne 7125 injector fitted with a 20 μl loop and a 150 mm × 5 mm column packed with 5 μm ODS-Hypersil (Shandon). The mobile phase consisted of 12 parts 0.025 M aqueous NaH_2PO_4, 7 parts tetrahydrofuran, and 1 part acetonitrile and the flow rate was 2 ml/min. Chart speed (Omni Scribe, Industrial Scientific) was 0.5 cm/min and the retention volume was 8.5 ml for rhizoxin and 7.4 ml for the principal alkaline degradation product. Samples, if necessary, were diluted with mobile phase prior to injection. Calibration curves, 0.002–0.05 mg/ml, were constructed from linear plots of peak height or peak area (Varian CDS 111 integrator) versus concentration.

Analysis of rhizoxin after addition to Intralipid involved extraction of rhizoxin from 3 ml of Intralipid with 6 ml of methylene chloride followed by centrifugation and direct injection of the methylene chloride solution on a normal phase HPLC system. The analysis used a Waters M-6000A pump, a Waters 440 UV detector set at 280 nm, a Rheodyne 7125 injector fitted with a 20 μl loop and a 30 cm column packed with Waters μPorasil. The mobile phase consisted of 6 parts chloroform to 4 parts acetonitrile and the flow rate was 2 ml/min. Chart speed (Omni Scribe, Industrial Scientific) was 0.2 inch/min and the retention volume was 6.4 ml for rhizoxin. Calibration curves, 0.004–0.05 mg/ml, were constructed from linear plots of peak heights or peak area (Varian CDS 111 integrator) versus concentration.

Solubility studies

The accurate determination of rhizoxin solubility in various solvents was complicated by its instability. Approximate solubilities in some solvents were determined by weighing a 5 mg rhizoxin sample into a test-tube, adding a known small volume of a solvent, briefly sonicating the solution and adding additional aliquots of solvent until solution was complete. In the case of safflower and soybean oil, rhizoxin at 40 mg/ml was first dissolved in dimethylacetamide and 50 μl aliquots were added with stirring to 2 ml of the corresponding oil. The solubilities recorded were those where the drug precipitated on addition of the concentrate to the vigorous stirring oil, and did not redissolve in 10–15 min. More accurate solubilities in selected solvents were determined by placing an excess of rhizoxin in screw-capped vials along with various solvents. The vials were protected from light by wrapping with aluminium foil and rotated in a 25°C constant temperature water bath for 5 h. The suspensions were then filtered through a 0.45 μm Metricel filter and assayed for dissolved rhizoxin by reversed-phase HPLC.

Chemical stability and pH-rate profile

Apparent first-order rate constants for the degradation of rhizoxin at 25 ± 0.1°C were determined in aqueous buffer solutions at various pH values and in various hydroalcoholic solvents in the presence and absence of laboratory lighting. The ionic strength in the buffer solutions was

adjusted to 0.15 M by the addition of sodium chloride. The following buffer systems were employed: HCl (pH 2.0 and 3.0), acetate (pH 4.6 and 5.6; 0.025–0.075 M), phosphate (pH 7.0 and 8.0; 0.01–0.03 M) and borate (pH 9.0; 0.01–0.02 M). Rhizoxin was added to the buffer, 1 : 25, dissolved in methanol, 0.125 mg/ml, giving a final rhizoxin concentration of 0.005 mg/ml and a methanol content of 4%. In all aqueous or hydroalcoholic solvents, the degradation rates of rhizoxin were followed using the reversed phase HPLC procedure reported earlier. Aliquots were withdrawn periodically and either injected directly, or diluted with mobile phase prior to injection, onto the HPLC. Quantification was performed by peak height or area analysis in relation to standard calibration curves. Observed first-order rate constants were calculated from the slopes of linear plots of log C versus time, where C is the concentration of the remaining intact rhizoxin at time t. Rhizoxin was subject to significant catalysis by some buffers; thus, rate constants extrapolated to zero buffer concentration were used to construct the pH–rate profile.

Freeze-drying cycle

Various lots of freeze-dried rhizoxin were prepared both for initial animal testing at NCI as well as for long-term stability testing in the laboratory. Generally, the required amounts of mannitol (5 mg/ml), rhizoxin (1 mg/ml) and other excipients were dissolved in 40% freshly distilled *t*-butanol in water by sonicating for 5 min, filtered through a 0.2 μm Metricel membrane and 5 ml aliquots were placed in 10 or 20 ml amber glass freeze-drying vials. The samples, partially stoppered, were either immediately frozen in a dry-ice–acetone mixture, or frozen in the freeze-dryer, Virtis Model Benchtop 1.5, with the shelf temperature initially set at $-45\,^{\circ}$C. On initiation of the freeze-drying cycle, the shelf temperature was allowed to rise over 40 h or until vials containing only the solvent were empty, after which the shelf temperature was increased in 8 °C increments every 30 min until 25 °C was reached. This temperature was then maintained at 25 °C for 1–7 h. Secondary drying times were varied to assess the effect of drying time on final moisture content. After the chamber was filled with air, samples were fully stoppered, removed from the freeze-dryer, crimped, and stored at either 37 °C in a temperature-controlled oven, ambient temperature ($\approx$ 23 °C), in a refrigerator at 4 °C, or in a freezer maintained at $-20\,^{\circ}$C. No studies were performed with nitrogen backfilling or vial sealing under vacuum.

GLC analysis of t-butanol

The procedure for detecting residual *t*-butanol in freeze-dried rhizoxin vials involved two methods, A and B. In method A, 3 ml of chloroform was injected into freeze-dried rhizoxin vials, also containing mannitol, shaken for 5 min, the suspension filtered through Whatman no. 40 filter paper, and 1 μl of the chloroform injected into a gas chromatograph. The calibration curves of *t*-butanol in chloroform were prepared using concentrations between 3.95 and 39.5×10^{-2} mg/ml. Gas chromatographic conditions for quantitation of *t*-butanol used a Varian Model 3700 gas chromatograph equipped with a flame ionization detector, a 10% carbowax 20 M on 80/100 Chromosorb WAW column maintained at 60 °C, injector temperature was 150 °C, detector temperature was 220 °C, carrier gas was nitrogen at 50 psi, detector gases were hydrogen at 40 psi, and air at 60 psi. Calibration curves were prepared from linear peak height versus concentration plots.

For method B, 3 ml of 40% propylene glycol/water was injected into bottles of freeze-dried rhizoxin, shaken gently for 5 min (or until clear), and 1 μl of solution was injected into the gas chromatograph. Calibration curves were prepared as for method A except 40% propylene glycol/water was used as the solvent for *t*-butanol. Gas chromatographic conditions and equipment were the same as in method A except that a temperature program was utilized. The program used was as follows; initial temperature and temperature hold were 60 °C and 3 min respectively, followed by a temperature increase of 20 °C/min to a final temperature of 180 °C and a final hold of 3 min.

The results indicated that the residual amount of *t*-butanol using method A was slightly higher than using method B. The reason may be that a peak resolution of less than 1 for *t*-butanol and

the preservative in chloroform was obtained using method A resulting in an overestimated residual *t*-butanol content. However, in method B, *t*-butanol was clearly separated from the propylene glycol peak. Method B was the desired assay procedure.

Results and Discussion

Solubility studies

Table 1 gives some approximate visual solubilities of rhizoxin in various solvents while Table 2 gives some solubilities determined at 25 °C after rotation in a constant temperature water bath for 5 h. Minimal decomposition was observed over this time period. Since rhizoxin has no ionizable groups, is relatively lipo-soluble, and is poorly water-soluble, the only major means of achieving the desired solubility goal of 1 mg/ml, other than chemical modification, is the use of complexation, micellar solubilization, or co-solvents. The use of complexation was ruled out because of the 100-fold increase in solubility that was needed, and while micellar solubilization with 5% Cremophor appeared to achieve the solubility goal, the use of Cremophor was ruled out due to the reported incidences of anaphylaxis resulting from its use in parenteral products (Attwood and Florence, 1983 and references therein; Watkins, 1981; Doenicke et al., 1981; Howrie et al., 1985; Davignon and Cradock, 1987). The desired goal was achieved by the co-solvent system, 40% propylene glycol/10% ethanol/water. There is ample literature precedent for the use of this and similar solvents in parenteral products (Yalkowsky and Roseman, 1981 and references therein). As can be seen from Table 2, partial dilution of this vehicle with water will result in the precipitation of rhizoxin. This point will be addressed later.

TABLE 1

Approximate room temperature visual solubilities of rhizoxin

Solvent	Approximate solubilities (mg/ml)
safflower oil	1–2
soybean oil	2
t-butanol	100
40% *t*-butanol/water	7–10
25% ethanol/water	0.1
50% ethanol/water	6

TABLE 2

Rhizoxin solubilities at 25 °C, in various solvents

Solvent	Solubilities (mg/ml)
water	0.012
1% Tween 80/water	0.42
5% Cremophor	1.84
20% ethanol/water	0.12
40% propylene glycol/10% ethanol/water	1.85
20% propylene glycol/5% ethanol/water	0.10
15% propylene glycol/3.75% ethanol/water	0.056
10% propylene glycol/2.5% ethanol/water	0.030

Chemical stability and pH–rate profile

The kinetics of hydrolytic degradation of rhizoxin was investigated at 25 ± 0.1°C ($\mu = 0.15$ M with sodium chloride) in aqueous solution at various pH values. Rhizoxin degraded via apparent first-order kinetics at all pH values. Table 3 contains the apparent first-order rate constants, k_{obs}, as a function of pH and buffer concentration. Plots of k_{obs} versus buffer concentration were used to determine k_{obs} at zero buffer concentration, k'_{obs}, which were then used to construct the pH–rate profile shown in Fig. 1. Fig. 1 can be adequately defined by Eqn. 1

$$k'_{obs} = k_H[H^+] + k_0 + k_{OH}[OH^-] \quad (1)$$

where k_H is the second order, acid catalyzed rate constant, k_0 is the spontaneous, or apparent first-order, water-catalyzed rate constant, and k_{OH} is the second-order, base-catalyzed rate constant for the degradation of rhizoxin. Values of 7.5 $M^{-1} \cdot min^{-1}$ for k_H, 8.7×10^{-6} min^{-1} for k_0, and 3.8×10^3 $M^{-1} \cdot min^{-1}$ for k_{OH} were used to draw the solid line in Fig. 1. The value of k_0 of 8.7×10^{-6} min^{-1} represents only an $\approx 20\%$ contribution to k_{obs} at pH 5.6. Because of the V-shaped nature of the pH–rate profile and the

TABLE 3

pH and buffer dependency of rhizoxin degradation ($\mu = 0.15$ with NaCl) at 25 ± 0.1 °C in the absence of light

pH	Buffer	Conc. (*M*)	k_{obs} (min^{-1})	$t_{1/2}$
2.0	HCl		6.72×10^{-2}	10.3 min
3.0	HCl		7.82×10^{-3}	88.6 min
4.6	Acetate	0.025	2.03×10^{-4}	57 h
	Acetate	0.05	2.22×10^{-4}	52 h
	Acetate	0.075	2.25×10^{-4}	51.3 h
5.6	Acetate	0.025	4.51×10^{-5} (8.58×10^{-5}) *	256.2 h
	Acetate	0.05	4.77×10^{-5} (7.93×10^{-5}) *	241.4 h
	Acetate	0.075	5.01×10^{-5} (7.84×10^{-5}) *	230.5 h
7.0	Phosphate	0.01	4.47×10^{-4}	25.8 h
	Phosphate	0.03	4.97×10^{-4}	23.3 h
	Phosphate	0.05	6.04×10^{-4}	19.1 h
8.0	Phosphate	0.01	3.41×10^{-3}	203 min
	Phosphate	0.03	3.51×10^{-3}	197 min
	Phosphate	0.05	3.58×10^{-3}	193.5 min
9.0	Borate	0.01	3.93×10^{-2}	17.6 min
	Borate	0.02	4.06×10^{-2}	17.1 min

* In the presence of laboratory lighting.

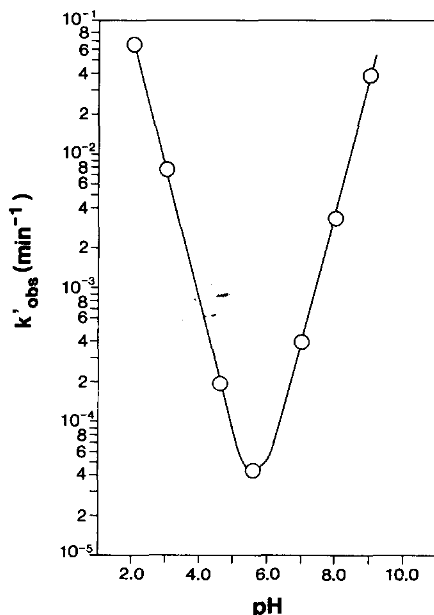

Fig. 1. pH–rate profile for the degradation of rhizoxin in aqueous solution at 25 °C, $\mu = 0.15$ (sodium chloride) in the absence of light.

normal uncertainty in the data, the contribution of k_0 to k_{obs} may not be statistically significant. The maximum stability of rhizoxin occurs at pH 5.6 ± 0.1. The $t_{90\%}$ at this pH is ≈ 38 h. Studies in the presence and absence of light were undertaken since preliminary information from the supplier (J. Cradock, personal communications, National Cancer Institute) indicated that rhizoxin, in the solid state, was subject to photochemical degradation. In aqueous and hydroalcoholic solutions, especially at acidic and alkali pH values, the kinetics of rhizoxin degradation did not appear to be particularly sensitive to light. At the pH of maximal stability, however, the kinetics in the presence of laboratory lighting showed an approximate two-fold increase in rate compared to solutions protected from light (see pH 5.6 data in Table 3). The lack of effect of light at more acidic and alkaline pH values probably is due to the greater hydrolytic sensitivity of rhizoxin at these pH values.

The apparent first-order kinetics of rhizoxin degradation in various hydroalcoholic solvents was

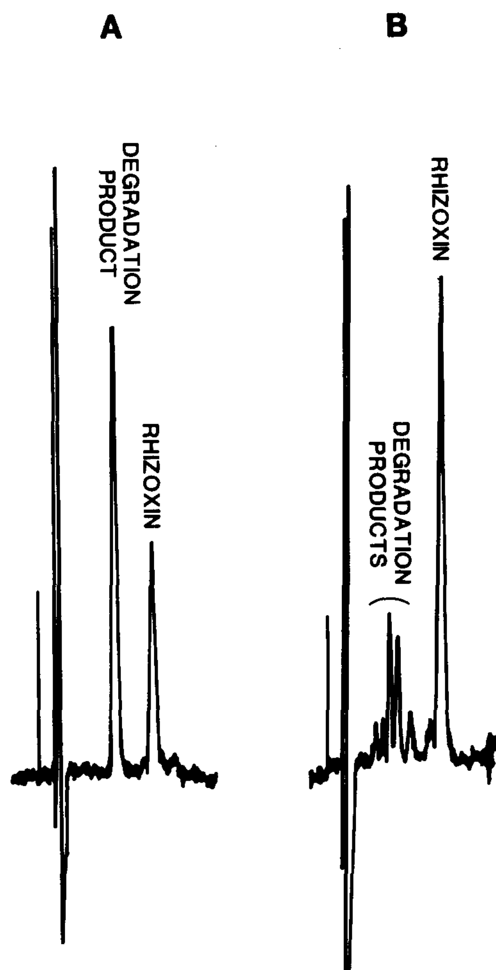

Fig. 2. HPLC chromatograms of partially degraded rhizoxin after 19 min exposure to a pH 9.0 borate buffer (A), and 62 min exposure to a pH 3.0 hydrochloric acid buffer (B).

also determined. In 40% propylene glycol/10% ethanol/water solvent, the $t_{90\%}$ was found to be 72 h when protected from light, and 69 h when exposed to room light. In 40% *t*-butanol/water, the $t_{90\%}$ was > 100 h. In both these solvent systems, rhizoxin was significantly more stable than in purely aqueous systems.

At pH values of 8 and 9, rhizoxin appeared to initially degrade to a single product since only one peak was initially observed in the HPLC chromatogram (Fig. 2A), while under acidic pH values multiple products were observed. Iwasaki et al. (1984) found that alkaline treatment of rhizoxin followed by acidification and extraction into ether and subsequent methylation and acetylation resulted in the isolation and characterization of *seco*-rhizoxin triacetate. This suggested that the major degradation product under aqueous alkali conditions was cleavage of the larger lactone ring by the attack of hydroxide ion at the 1-position of rhizoxin. Whether the neighboring 2,3-epoxide bond is also simultaneously cleaved is unclear. Using 0.025 M phosphate buffer of pH 4.6 as the aqueous portion of the HPLC mobile phase, rhizoxin eluted at 4.8 min and the alkali degradation product at 4 min. When the aqueous portion of the mobile phase was changed to a 0.025 M phosphate buffer of pH 5.5, rhizoxin eluted at 4.2 min while the degradation product eluted at 1.6 min. It appeared from these retention properties that the alkaline degradation product was probably a carboxylic acid resulting from cleavage of the larger lactone ring of rhizoxin (Iwasaki et al., 1984). At acidic pH values, multiple products were observed eluting from the HPLC column (Fig. 2B). Although not identified, this complex mixture of products was probably the result of acid-catalyzed cleavage of the lactone ring(s) and one or both of the epoxide rings of rhizoxin.

Parenteral formulation of rhizoxin

It is obvious from the stability data above that rhizoxin is not sufficiently stable to be formulated as ready-made aqueous or hydroalcoholic solutions. Its poor water-solubility also precludes freeze-drying from water. However, rhizoxin's excellent solubility in *t*-butanol and *t*-butanol/water mixtures and the high melting temperatures and sublimation properties of these solvents suggested that they could be used to prepare sterile freeze-dried samples of rhizoxin. There are few well-documented examples of the use of *t*-butanol and *t*-butanol/water mixtures for the preparation of parenteral pharmaceutical products (Seager et al., 1985). Excellent freeze-dried cakes in 10 or 20 ml vials containing 25 mg of mannitol and 5 mg of rhizoxin were prepared from a 40% *t*-butanol/water mixture. Mannitol was necessary since rhizoxin alone resulted in unsatisfactory cakes. Pure *t*-butanol was not tried as the freeze-drying solvent because of the poor solubility of mannitol

in *t*-butanol. The freeze-dried samples could be reconstituted with 5 ml of the sterile solvent, 40% propylene glycol/10% ethanol/water, to give a rhizoxin concentration of 1 mg/ml. On reconstitution, rhizoxin degradation was faster than that observed in the same co-solvent system in the absence of mannitol. A $t_{90\%}$ of 18 h was observed. The difference was probably the result of the pH lowering effect of mannitol.

Analysis of the freeze-dried vials showed no apparent degradation during processing. Residual water content of the vials analyzed using DMSO and a Metrohm Karl Fischer titrator gave values of 0.42% and 0.45% for lots having a secondary drying time of 4 h with 1 h at 25 °C and 0.36% and 0.39% for lots having a drying time of 7 h with 4.5 h at 25 °C. The content of *t*-butanol in the vials with the shorter secondary drying cycle was 0.33 (1.1%) and 0.34 (1.1%) mg/vial when analyzed using method A and 0.26 (0.87%) and 0.23 (0.77%) mg/vial when analyzed via method B.

Personal communications from the National Cancer Institute and reports from the primary supplier of rhizoxin indicated that rhizoxin was inherently unstable in the solid state, especially in the presence of light. Therefore, studies were initiated to study the stability of rhizoxin in the amber glass freeze-drying vials (vials that had been exposed to 1 h of secondary drying at 25 °C) after storage at 37 °C, ambient room temperature (≈ 23 °C), 4 °C and −20 °C as a function of storage time. The results, mean of two vials at each time point unless otherwise indicated, are given in Table 4. Note, that as expected, rhizoxin was quite unstable in the freeze-dried state even at room temperature, but appeared to have reasonable stability under refrigeration and freezer conditions. Light did not appear to have a significant effect on the relative stability at room temperature. This was not unexpected since amber glass vials were used. No difference in rhizoxin stability was seen between lots that had been dried at 25 °C for 1 h versus 4.5 h or longer in the secondary drying cycle.

Decomposition product analysis from freeze-dried samples did not give the same pattern as noted in the hydrolytic studies. HPLC chromatograms clearly showed the loss of rhizoxin but only minor product peaks. This suggested that the degradation of rhizoxin in the vials was different from that in aqueous and hydroalcoholic solutions. Because of the extended conjugated double-bond system in rhizoxin, oxidative breakdown was suspected. Oxidative breakdown would probably result in cross-polymerized products

TABLE 4

Stability of lyophilized rhizoxin after storage at 37 °C, ambient temperature, 4 °C and −20 °C

Temperature	Time (days)	Rhizoxin concentration after reconstitution (mg/ml)
37 °C, dark	0	1.01
	16	0.73
	29	0.64
	58	0.43
	107	0.30 *
	142	0.32 *
	183	0.24 *
	366	0.21 *
Ambient temp., light	0	0.98
	16	0.90
	28	0.88
	58	0.74 *
	107	0.64 *
	183	0.52
	366	0.39
Ambient temp., dark	0	0.98
	16	0.89
	28	0.88
	58	0.74 *
	107	0.65 *
	183	0.57
	366	0.45
4 °C	0	1.01
	28	1.00
	107	0.99
	183	0.98
	366	0.91
−20 °C	0	0.98
	28	1.02
	107	1.00
	183	1.06
	265	1.03
	366	1.12 **

* Only one sample reconstituted.
** Reason for the high values unknown.

TABLE 5

Stability of lyophilized rhizoxin after storage at 37 °C and at ambient temperature in the absence and presence of added neutralized ascorbic acid

Conditions of storage	Storage time (days)	Conc. of rhizoxin (mg/ml)	
		No ascorbic acid	With ascorbic acid
37 °C, dark	0	1.00	0.99
	26	–	1.00
	29	0.56	–
	54	–	0.93
	63	0.48	–
	112	0.30	–
	123	–	0.78
	194	0.17	0.92
Ambient temp.	0	1.00	0.99
	26	–	1.08
	29	0.75	–
	54	–	0.90
	63	0.76	–
	112	0.59	–
	194	0.45	0.98

which would probably elute poorly from the column.

To test for the possibility that oxidation might be important, 10 lyophilized samples were prepared with and without neutralized ascorbic acid added to the formulation. The mannitol and ascorbic acid were first dissolved in water and the pH adjusted to 5.6–5.7 (pH of maximum stability of rhizoxin) with sodium hydroxide, the required amounts of *t*-butanol and rhizoxin were then added and sonicated until solution was complete. The solutions were then sterile filtered and freeze-dried. Each ascorbic acid-containing vial contained 5 mg rhizoxin, 25 mg mannitol and 25 mg ascorbic acid. The secondary drying cycle was for 5 h with 1.25 h at 25 °C. Two vials were periodically removed, reconstituted with 5 ml each of 40% propylene glycol/10% ethanol/water, and analyzed for rhizoxin content. The fresh samples readily dissolved and contained 1.00 ± 0.1 mg/ml of rhizoxin. Stability data over 194 days is given in Table 5. Although the formulation was not optimized, the ascorbic acid appeared to significantly enhance the solid state stability of rhizoxin. No logical explanation for the erratic behaviour of the solid state stability data in the presence of ascorbic acid is available. Despite the erratic results, the data do suggest that the solid state stability of rhizoxin may be oxygen-sensitive. More extensive studies, such as sealing the vials under vacuum, head space displacement with an inert gas, and a more controlled and extensive antioxidant study are needed to demonstrate more conclusively the possible role played by moisture and oxygen in the solid state stability of rhizoxin.

Dilution of reconstituted rhizoxin

Because of the limited aqueous solubility of rhizoxin, dilution of the reconstituted rhizoxin with dextrose 5% in water (D5W) or normal saline (NS) was expected to present problems. The concentrated rhizoxin solution (1 mg/ml) was slowly added dropwise with vigorous stirring to NS and D5W. In NS, the solution was clear up to an added rhizoxin concentration of 0.0075 mg/ml but became slightly hazy at a concentration of 0.01 mg/ml. In D5W, a clear solution was observed up to a concentration of 0.01 mg/ml; at a concentration of 0.012 mg/ml the solution became very slightly hazy. The poor solubilities of rhizoxin in purely aqueous solutions limits the use of this drug in normal intravenous infusion sets.

The solubility of rhizoxin in soybean oil is significant (see Table 1). Therefore, the possibility of diluting rhizoxin in a commercial 10% soybean oil parenteral emulsion was explored. An aliquot of the reconstituted drug solution was added dropwise to Intralipid (Cutter Labs.) to a final rhizoxin concentration of 0.01 and 0.1 mg/ml. Samples of the resulting emulsions were centrifuged at 20,000 rpm for 30 min. No crystals of drug were evident in the aqueous layer. Microscopic evaluation of the emulsion showed no apparent changes in the emulsion or the presence of drug particles. The chemical stability of rhizoxin in the Intralipid solutions was studied by extraction of rhizoxin from the emulsions with methylene chloride and analysis by normal phase HPLC. Recovery of rhizoxin from fresh additions to the emulsions was quantitative. Loss of rhizoxin from the emulsions, followed over 48 h, gave apparent $t_{90\%}$ values of 16.7 and 18.5 h for duplicate runs

for the 1:10 dilution, and 19.6 and 27.7 h for duplicate runs for the 1:100 dilution. If rhizoxin must be given by slow intravenous infusion, the use of a commercial 10% parenteral lipid emulsion might be considered.

Conclusion

Rhizoxin is a large complex molecule with poor aqueous solubility and stability characteristics. In aqueous and hydroalcoholic solutions, rhizoxin appears to degrade primarily via hydrolytic reactions resulting from cleavage of the lactone rings and the epoxide groups. In the solid state, there were some indications that rhizoxin cleavage might be oxidative in nature. Rhizoxin can be formulated for parenteral use at 1 mg/ml by preparing freeze-dried vials (amber glass) containing rhizoxin and mannitol (0.5%) from a 40% *t*-butanol/water solvent. The vials can be reconstituted with a solvent of 40% propylene glycol/10% ethanol/water. Optimization of the freeze-dried formulation and freeze-drying cycle is needed if a 2 year shelf-life at ambient temperatures is desired. The $t_{90\%}$ of the reconstituted solution was 18 h. Dilution (< 1:100) of the reconstituted solution with D5W and NS results in precipitation of rhizoxin. Dilution of the reconstituted solution with a 10% soybean oil parenteral emulsion, Intralipid, appeared to be possible. The $t_{90\%}$ of rhizoxin in the emulsion was similar to that in water.

Acknowledgements

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References

Attwood, D. and Florence, A.T., *Surfactant Systems: Their Chemistry, Pharmacy and Biology*, Chapman and Hall, New York, 1983, pp. 614–697 and references therein.

Davignon, J.P. and Cradock, J.C., The formulation of anticancer drugs. In Hellmann, K. and Carter, S.K. (Ed.) *Fundamentals of Cancer Chemotherapy*, 1987, Ch.. 23, pp. 218–219.

Doenicke, A., Ennis, M. and Lorenz, W., Histamine release in anaesthesia and surgery: a systematic approach to risk in the perioperative period. *Int. Anesthesiol. Clin.*, 23 (1985) 41–66.

Howrie, D.L., Ptachcinski, R.J., Griffith, B.P., Hardesty, R.J., Rosenthal, J.T., Burckart, G.J. and Venataramanan, R., Anaphylactoid reactions associated with parenteral cyclosporine use: possible role of cremophor el. *Drug. Intel. Clin. Pharm.*, 19 (1985) 425–427.

Iwasaki, S., Kobayashi, H., Furakawa, J., Namikoshi, M., Okuda, S., Sata, Z., Matsuda, I. and Noda, T., Studies on macrocyclic lactone antibiotics, VII. Structure of phytotoxin "Rhizoxin" produced by *Rhizopus chinensis*. *J. Antibiotics*, 37 (1984) 354–362.

Seager, H., Taskis, C.B., Syrop, M. and Lee, T.J., Structure of products prepared by freeze-drying solutions containing organic solvents. *J. Parenteral Sci. Technol.*, 39 (1985) 161–179.

Watkins, J., Mechanisms and factors predisposing towards adverse response to intravenous anaesthetic substances. In Thornton, J.A. (Ed.), *Adverse Reactions of Anaesthetic Drugs, Vol. 8,* Elsevier, New York, 1981, Ch. 5.

Yalkowsky, S.H. and Roseman, T.J., Solubilization of drugs by co-solvents. In Yalkowsky, S. (Ed.) *Techniques of Solubilization of Drugs*, Marcel Dekker, New York, 1981, pp. 91–135.