Conclusion

Fluorouracil is stable in 5% dextrose injection for at least 16 weeks when stored at 5 °C in the two types of containers studied.

[a] Efudex, Roche Laboratories, Nutley, NJ 07110, lot 1911–01072.
[b] PL 146 MVP Ambulatory Pump, Travenol Laboratories, Inc., Deerfield, IL 60015.
[c] Travenol Laboratories, Inc.
[d] Aldrich Chemical Company, Inc., Milwaukee, WI 53233, lot 2213AE.
[e] Waters Associates, Inc., Milford, MA 01757.
[f] Waters Associates, Inc.
[g] Waters Associates, Inc.
[h] Alltech Associates, Deerfield, IL 60015.
[i] Aldrich Chemical Company, Inc., lot 72F–0477.
[j] Model 773, Kratos Analytical Instruments, Ramsey, NJ 07446.

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Stability of azacitidine in infusion fluids

YUEN-WAH CHEUNG, B. RAO VISHNUVAJJALA, NANCY L. MORRIS, AND KARL P. FLORA

Abstract: The stability of azacitidine in four infusion fluids was studied.

Azacitidine was reconstituted and diluted to final concentrations of 0.2 mg/ml and 2.0 mg/ml in glass bottles and plastic i.v. bags containing 0.9% sodium chloride injection, 5% dextrose injection, lactated Ringer's injection, or Normosol-R. All admixtures containing azacitidine 2.0 mg/ml and both drug concentrations in Normosol-R were prepared in glass bottles only. The pH of each solution was measured before mixing, immediately after mixing, and after six hours. Duplicate samples of each solution were removed for assay by high-performance liquid chromatography at zero time, then at hourly intervals for six hours, and again at 25 hours. Three experimental runs were performed for each combination of drug concentration, infusion fluid, and type of container. The percentage of initial (zero time) concentration remaining was determined and plotted versus time. This plot was used to calculate the t_{90} (time at which 90% of the initial concentration remained) for each solution.

The t_{90} values did not exceed three hours for any of the solutions studied. At the 0.2 mg/ml concentration, the t_{90} value in 5% dextrose injection (0.8 hours) was much smaller than that of the other solutions, which had t_{90} values of about two hours. In plastic bags, the percentage of initial azacitidine concentration remaining after six hours was less in 5% dextrose injection than in any other solution. The t_{90} values for all solutions containing azacitidine 2.0 mg/ml were similar, ranging from 2.4 to 3.0 hours.

The instability of azacitidine in the solutions studied suggests that the drug should be administered immediately after preparation; dilution in 5% dextrose solution at lower drug concentrations is not recommended. Infusion solutions should be changed at appropriate intervals to prevent unacceptable decomposition of azacitidine.

Index terms: Additives; Antineoplastic agents; Azacitidine; Concentration; Containers; Dextrose; Drugs; Glass; Incompatibilities; Injections; Normosol-R; Plastics; Ringer's injection, lactated; Sodium chloride; Stability; Vehicles

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Azacitidine is a cytidine analog that possesses antitumor activity. It has been used clinically in the treatment of human leukemias[1,2] but lacks noteworthy activity in human solid tumors.[3] The drug is often administered by continuous infusion to minimize its substantial gastrointestinal toxicity[4,5] or to increase its effectiveness.[6,7]

The stability and mechanism of decomposition of

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azacitidine have been studied in biological fluids and in various buffer solutions at several pH values.[5,8,9] Investigations have shown that azacitidine undergoes facile hydrolytic cleavage in aqueous solutions with maximum stability within a pH range of about 6.5–7.[8,9] Published information concerning the stability of azacitidine in infusion fluids is limited and in some cases confusing. Stability studies of the drug in infusion fluids have generally used 0.9% sodium chloride[9] and lactated Ringer's injections.[5,9,10] Lactated Ringer's injection has been recommended as a suitable diluent because of its optimal pH for azacitidine stability. However, conflicting stability data have been reported. The time required for 10% drug decomposition in lactated Ringer's injection at room temperature has been reported to vary from between two to three hours[10] to 14–15 hours.[5]

These conflicting reports have generated much confusion among practitioners involved with the administration of azacitidine. Drug stability is obviously important when the drug is to be administered by continuous infusion. Adequate arrangements must be made to ensure that unacceptable levels of decomposition do not occur during drug administration. In this study we hoped to eliminate the confusion surrounding azacitidine stability and to extend the available data to include other infusion fluids, drug concentrations, and types of containers.

Methods

The formulated product of azacitidine used in these studies[a] was supplied by the National Cancer Institute as a white lyophilized powder containing 100 mg of azacitidine and 100 mg of mannitol in a 30-ml vial. The following infusion fluids were used: 0.9% Sodium Chloride Injection, USP[b]; 5% Dextrose Injection, USP[c]; Lactated Ringer's Injection, USP[d]; and Normosol-R[e] (pH 7.4). The former three fluids are available in glass bottles and plastic bags; the latter is available only in glass bottles.

The high-pressure liquid chromatography (HPLC) apparatus used included a constant-flow pump[f] that delivered a mobile phase of 0.01*M* phosphate buffer (pH 6) at a flow rate of 2 ml/min to a radially compressed liquid-chromatography cartridge.[g] Injections were made with a rotary-valve injector[h] equipped with a 10-μl injection loop. The column eluant was monitored with an ultraviolet detector[i] at 254 nm with an attenuation of 0.08–0.16 absorbance units full scale. The detector output signal was recorded with a strip chart recorder.[j]

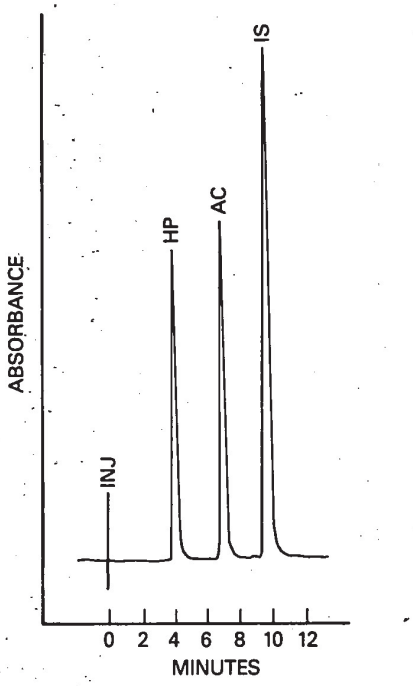
The stability-indicating capability of the HPLC method was demonstrated using partially and totally decomposed solutions of azacitidine. The azacitidine chromatographic peak was well resolved from all other peaks (e.g., decomposition products) in the chromatogram. Partially decomposed azacitidine solutions analyzed by HPLC using a range of mobile-phase compositions produced no peak asymmetries that would suggest a decomposition product eluting with the azacitidine peak.

Retention volumes of the hydrolysis product (HP), azacitidine (AC), and the internal standard (IS) uridine,[k] were 8.0 ml, 14 ml, and 19 ml, respectively. A representative chromatogram is shown in Figure 1. When the peak-height ratios of azacitidine to the internal standard were plotted against the concentration of azacitidine in the working range of 50–200 μg/ml, a linear relationship was observed ($r > 0.998$).

The precision of the HPLC method was determined by assaying seven azacitidine solutions (200 μg/ml) prepared individually by diluting a stock solution of the drug in dimethylsulfoxide. The relative standard deviation based on peak-height ratios for these seven solutions was 0.65%.

For stability studies, the formulated product was reconstituted with 20 ml of ice-cold water and immediately further diluted with the appropriate volume of thermally equilibrated (25 ± 0.1 °C) infusion fluid. Two concentrations of azacitidine in infusion fluids were studied: 0.2 mg/ml and 2.0 mg/ml. The pH values of the solutions were measured before mixing, immediately after mixing, and after six hours using a properly standardized pH meter.[l] At the lower concentration (0.2 mg/ml), duplicate 1-ml aliquots were removed at zero time,

Figure 1. A representative chromatogram for the assay of a solution containing azacitidine (AC), the internal standard (IS) uridine, and the hydrolysis product (HP); INJ represents the time of injection onto the column.



at hourly intervals for six hours, and at 25 hours. These aliquots were combined with an equal volume of ice-cold internal standard solution (uridine 0.15 mg/ml in the mobile-phase solution) and immediately subjected to HPLC analysis. The duplicate sample was kept in an ice bath for not more than 15 minutes before assay. No degradation was observed under such conditions.

For studies at 2.0 mg/ml, duplicate 100-μl aliquots were removed and combined with 900 μl of the internal standard solution (uridine 0.2 mg/ml in the mobile-phase solution) before HPLC analysis. Duplicate samples were treated as above. Three separate experimental runs were made for each drug concentration–infusion fluid–container variation. The percentage of drug present at each time point was calculated considering the time-zero concentration to be 100%. The t_{90} values (time at which drug concentration is 90% of the initial value) for azacitidine solutions were determined graphically from the plot of percentage of drug present versus time for each experimental run.

Results and Discussion

The stability of azacitidine in various infusion fluids is presented in Table 1. The drug was unstable in all infusion fluids studied. The t_{90} values did not exceed three hours in any case. This is in agreement with most t_{90} values reported in the literature[10,11] but greatly deviates from the findings of Israili et al.[5] (t_{90} = 14–15 hours in lactated Ringer's solution at room temperature). Chatterji[12] previously disputed the interpretation of stability data in Israili's article.

Previous studies suggested that the degradation of azacitidine occurs through hydration of the 5,6-imine double bond, followed by bond cleavage to yield the formyl derivative, *N*-(formyl-amidino)-*N*-β-D-ribofuranosylurea.[8,11] These steps are reversible. The formyl derivative further undergoes irreversible decomposition to other products. Because the formation of the formyl derivative is reversible, the kinetic profile observed is not usually pseudo first-order. Biphasic declines were observed when the log of the drug concentration was plotted against time in the stability studies of azacitidine in buffer solutions,[8,9] lactated Ringer's injection, and 0.9% sodium chloride injection.[9] Similarly, in our study, the observed kinetic profiles deviated from simple pseudo first-order plots, particularly when a low drug concentration was used. The disappearance of the drug was rapid initially, followed by a more gradual decrease. In contrast, Israili et al.[5] reported monoexponential degradation of azacitidine in aqueous buffers and human plasma under various conditions. The t_{90} values reported in their article were obtained based on simple pseudo first-order kinetics, which led to a substantial overestimation of these values.

In this study the stability of solutions containing azacitidine 0.2 mg/ml was essentially identical in 0.9% sodium chloride injection, lactated Ringer's injection, and Normosol-R (in glass bottles). The mean t_{90} values at 25 ± 0.1 °C were about two hours (Table 1). However, a faster decomposition rate was observed in 5% dextrose injection, especially during the first three hours. This mean t_{90} value in glass bottles (0.8 hr) was considerably smaller than those of the other infusion fluids; however, the percentage of drug remaining after six hours was only 2–5% less than that of the other solutions. The more rapid

Table 1.
Stability of Azacitidine in Various Infusion Fluids at 25 ± 0.1 °C

Infusion Fluid and Type of Container	Initial Concentration (mg/ml)	pH[a,b]	t_{90}[b,c] (hr)	% Remaining after Six Hours[b,d]
0.9% Sodium chloride injection				
Plastic	0.2	5.6 ± 0.18	1.6 ± 0.1	77.2 ± 1.3
Glass	0.2	5.9 ± 0.04	1.9 ± 0.1	78.6 ± 1.7
Glass	2	6.72 ± 0.1	2.4 ± 0	81.6 ± 0.6
5% Dextrose injection				
Plastic	0.2	4.72 ± 0.5	0.7 ± 0	63.0 ± 0.4
Glass	0.2	5.10 ± 0.05	0.8 ± 0	74.1 ± 0.35
Glass	2	6.4 ± 0.11	3.0 ± 0.2	83.9 ± 1.5
Lactated Ringer's injection				
Plastic	0.2	6.46 ± 0.03	2.03 ± 0.15	80.3 ± 0.4
Glass	0.2	6.5 ± 0	1.93 ± 0.06	79.2 ± 0.75
Glass	2	6.7 ± 0.03	2.87 ± 0.12	81.7 ± 0.9
Normosol-R (pH 7.4)				
Glass	0.2	7.1 ± 0.1	1.87 ± 0.46	75.9 ± 2.6
Glass	2	7.0 ± 0.05	3.03 ± 0.13	82.2 ± 0.2

[a] After addition of drug at time zero.
[b] Reported as mean ± S.D. of determinations on duplicate samples for each of three experimental runs for each drug concentration–infusion fluid–container combination.
[c] Time at which drug concentration is 90% of the initial value.
[d] Percentage of initial concentration remaining.

decomposition of azacitidine in 5% dextrose injection at this concentration may have been largely related to the substantially lower pH of the admixture. Investigations have shown that azacitidine is most stable at a pH of 6.5–7.[8,9] As shown in Table 1, the mean pH of the 5% dextrose injection admixture was the lowest while the other admixtures possessed mean pH values nearer this optimal pH.

In all the infusion fluids the drug was more stable at a concentration of 2 mg/ml than at the lower concentration. The mean t_{90} values ranged from 2.4 to 3 hours. The stability of azacitidine in 5% dextrose injection in this case was not vastly different from the stability of the drug in the other infusion fluids. However, at this concentration, the mean pH of the 5% dextrose injection admixtures was similar to the mean pH values determined for the other admixtures. The amount of drug added to each infusion fluid affected the pH of the admixtures. The range of mean pH values for the admixtures containing azacitidine 2.0 mg/ml was 6.4–7.0. This closely parallels the reported pH range for optimal stability of this drug. In contrast, the mean pH values for the admixtures containing azacitidine 0.2 mg/ml varied from 5.1 in 5% dextrose injection to 7.1 in Normosol-R.

Factors other than pH may have also influenced azacitidine stability. At a concentration of 0.2 mg/ml, the drug was equally stable in 0.9% sodium chloride injection, lactated Ringer's injection, and Normosol-R, which had mean admixture pH values of 5.9–7.1. Different rates of decomposition were seen for the two drug concentrations in Normosol-R at essentially the same pH. These observations cannot be explained on the basis of admixture pH alone. Perhaps infusion-fluid composition and other factors are responsible for the observed results. Previous studies have shown that the stability of azacitidine is influenced by differing compositions of buffers at the same pH.[8]

Infusion fluids supplied in polyvinyl chloride bags have found widespread acceptance. The stability of azacitidine at either of the concentrations studied was not substantially different in bags of 0.9% sodium chloride injection or lactated Ringer's injection (Table 1). However, in bags of 5% dextrose injection, azacitidine appeared less stable. The difference was most apparent at six hours (Table 1). The mean pH of the 5% dextrose injection admixture was consistently lower in bags than in glass bottles. (The USP ranges for infusion-fluid pH values are rather broad). This decrease in stability may have been related to the type of container or the pH of the admixture, or both.

Conclusion

The instability of azacitidine in the aqueous solutions studied suggests that the drug should be administered immediately after preparation; dilution in 5% dextrose injection at lower drug concentrations is not recommended. Infusion solutions should be changed at appropriate intervals to ensure that unacceptable decomposition of azacitidine does not occur.

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[a] NSC-102816, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD, lot BV-81-232.

[b] Abbott Laboratories, North Chicago, IL 60064, lots 48-072-DE-4 (glass bottles) and 47-929-FJ (plastic bags).

[c] Abbott, lots 49-079-DE-8 (glass bottles) and 45-584-FJ (plastic bags).

[d] Abbott, lots 42-518-DE-1 (glass bottles) and 50-407-FJ (plastic bags).

[e] Abbott, lot 45-501-DM-04.

[f] Model 3500 B, Spectra-Physics Inc., Santa Clara, CA 95051.

[g] C-18, 5μ Radial-Pak cartridge, 8 mm i.d. × 115 mm, Waters Associates, Inc., Milford, MA 01757.

[h] Model CV-6-UHPa-N6O, Valco Instrument Co., Houston, TX 77024.

[i] Model SP 8200, Spectra-Physics Inc.

[j] OmniScribe, Houston Instrument Division, Bausch & Lomb, Inc., Austin TX 78753.

[k] Aldrich Chemical Co., Milwaukee, WI 53201, lot 101177.

[l] Beckman Zeromatic, Beckman Instruments, Inc., Fullerton, CA 92634.

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