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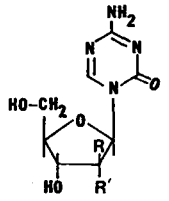
Development of an intravenous formulation for the unstable investigational cytotoxic nucleosides 5-azacytosine arabinoside (NSC 281272) and 5-azacytidine (NSC 102816)

P. MOJAVERIAN† AND A. J. REPTA*

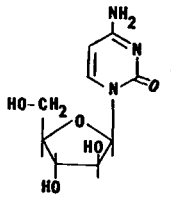
School of Pharmacy, Pharmaceutical Chemistry Department, The University of Kansas, Lawrence, Kansas 66045 USA

In aqueous solutions 5-azacytosine arabinoside (aza-A) (NSC 281272) exhibits complex and rapid degradation of a type analogous to 5-azacytidine (aza-C) (NSC 102816). Consequently, it is not amenable for use as slow i.v. infusions. This study has determined that both compounds are relatively stable in dry dimethylsulfoxide (DMSO) or dimethylacetamide (DMA). In mixed aqueous-organic solvents, as the water content is reduced the rate of degradation is descreased. Based on these findings, aza-A may be dissolved in DMSO at 100 mg ml^{-1}, sterile filtered, and sealed in ampoules. The contents appear to be adequately stable at 4 °C, and may at the time of use be diluted with water to yield a 70% DMSO solution which retains >90% potency for 24 h at 25 °C and is compatible with commercially available i.v. infusion tubing. The diluted solution may be added in-line to a flowing i.v. vehicle, resulting in a physiologically acceptable solution in which the drug is unstable (t_{90} 2 h). Its short residence time before reaching the bloodstream precludes any significant loss.

5-Azacytosine arabinoside (I, aza-A, NSC 281272) is a nucleoside analogue with antitumour activity related to both 5-azacytidine (II, aza-C, NSC 102816) and cytosine arabinoside (III, ara-C) (Beisler et al 1979).



aza-A, I, R = OH, R′ = H
aza-C, II, R = H, R′ = OH



III, ara-C

Initial data suggest that aza-A exhibits less host toxicity than either aza-C or ara-C (Beisler et al 1979). In man, the use of these antitumour nucleosides is often limited by severe and sometimes dose-limiting nausea and vomiting (Chatterji & Gallelli 1979). Although such toxicity can be controlled effectively by administering the drug as a slow infusion, instability of a drug such as aza-C poses a serious problem (Chatterji & Gallelli 1979). Even when aza-C is infused in lactated Ringer injection, at pH 6·4, which represents conditions at maximum stability in aqueous media, ~10% of aza-C is lost in 2–3 h at room temperature (Chatterji & Gallelli 1979).

Considering that the instability of aza-C is associated with the 5-azacytosine moiety (Notari & DeYoung 1975; Beisler 1978; Benjamin 1979; Benjamin et al 1981), it was expected that similar instability problems would be encountered with aza-A.

We have assessed the relative stability of aza-A and aza-C and designed a formulation allowing slow i.v. infusion.

MATERIALS AND METHODS

Materials

Aza-A and aza-C were used as obtained from the National Cancer Institute (NCI). DMSO was also supplied by NCI in sterile, vacuum sealed vials and was used as received. Spectrophotometric grade DMA was stored over CaO overnight and fractionally distilled under reduced pressure. The first 10 ml was discarded then a constant boiling fraction was collected. Di-(2-ethylhexyl)phthalate was purchased from Aldrich Chemical Co. (Milwaukee, WI) and tri-(2-ethylhexyl)trimellitate was supplied by Cutter Laboratories (Berkeley, CA). Double distilled water

* Correspondence.
† Present address: Division of Clinical Pharmacology, Jefferson Medical College, Philadelphia, PA 19107.

was used in all experiments. All other chemicals were of reagent grade. The intravenous tubings were from sets i.v. made available by: Travenol Laboratories (Deerfield, IL), #2C006 solution administration set (Lot #6J063R3) and Cutter Laboratories (Berkeley, CA) Saftiset i.v. fat emulsion administration set #808-40 (Lot #NJ1625).

Hplc procedure for determination of aza-A and aza-C

Degradation kinetics of aza-A and aza-C in water and aqueous-organic media were studied at drug concentrations of 5 mg ml^{-1} and 25 °C using a reverse-phase hplc system consisting of a C_{18} μ-Bondapak (~10 μm particle size, 30 × 0·46 cm) column. Aqueous phosphate buffer (0·01 M, pH 6·8) was the mobile phase. Flow rates of 2·0 ml min^{-1} (for aza-A) or 1·5 ml min^{-1} (for aza-C) were employed. Detection was at 254 nm at a sensitivity of 0·1 AUFS. Each drug concentration was diluted 1 : 20 along with the internal standard (0·6 mg ml^{-1} uracil for aza-A and 1·2 mg ml^{-1} cytosine for aza-C) and chromatographed. Samples of 5 μl were injected. For aza-A, the internal standard (uracil), degradation product (IV) and aza-A were eluted with k′ values of 0·5, 1·2 and 1·8, respectively. Similarly for aza-C, the capacity factor for internal standard (cytosine), first degradation compound and the aza-C itself were 0·3, 0·73 and 1·6, respectively. Degradation of aza-A and aza-C were followed qualitatively and quantitatively (via standard curve) constructed from 62·5, 125, 250 and 500 μg ml^{-1} concentrations in distilled water. The plot of ratio of peak areas (aza-A/internal standard) vs concentration was linear (r = 0·9999) regression line being described by: y = 0·01x + 0·001. The inter-assay coefficient of variation (cv) was 1·6%. Under similar conditions, the regression line for aza-C was best described by y = 0·01x + 0·02, r = 0·9997 and cv = 1·5%. The pH of solutions of the drug in distilled water and in mixed solvent systems was measured several times throughout the experiment. In all solutions, the pH appeared to remain constant with the solution in distilled water exhibiting a pH ~5.

Plasticizer assay

The plasticizer di-(2-ethylhexyl)phthalate (DEHP) and tri-(2-ethylhexyl)trimellitate (TEHT) were assayed with a hplc system consisting of a 5 μm Hypersil ODS column (15 × 0·46 cm, 10% carbon load) and using a mobile phase compound of 97 volumes methanol and 3 volumes aqueous 1% acetic acid at a flow rate of 1·0 ml min^{-1}. Sample volumes injected were 20 μl and detection was at 254 nm.

Quantitation employed standard curves and a sensitivity setting of 0·05 AUFs. Standards were prepared in chloroform in the range of 0·1 to 50 μg ml^{-1}. A typical plot of peak height vs concentration for TEHT is given by the regression line: y = 1·6x + 0·7 (r = 0·9997). The inter-assay cv was 3%. For DEHP, the regression line was y = 0·8x + 0·1, r = 0·9998 and cv = 4%. The detection limit of the assay was 0·1 μg ml^{-1}. The k′ values for DEHP and TEHT were 0·5 min and 2·0 min, respectively.

Mixing compatibility

Compatibility and uniform mixing/dilution of the concentrated preparation of aza-A in 70% DMSO (70 mg ml^{-1}) and 70% DMA (60 mg ml^{-1}) in water was assessed in the following way. A sterile commercial i.v. solution of (5% dextrose : 0·45% sodium chloride injection, USP) or sodium chloride was delivered at a rate of 250 ml h^{-1} through a commercial i.v. administration set. The concentrated drug solution was introduced through a side arm tube at the rate of 6 ml h^{-1} using a calibrated infusion pump. The diluted drug solution was subsequently passed through a Whatman filter. Visual observation of the flowing solution at the point of mixing, and examination of the filter, indicated no precipitate formation had occurred.

Equilibrium solubility and long-term stability

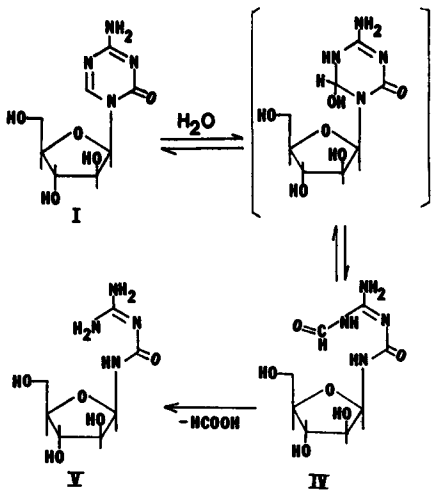
The apparent equilibrium solubility of aza-A was studied in 60, 70 and 100% (v/v) DMA and DMSO in water. Because of drug instability, apparent equilibrium solubilities in the aqueous-organic solution were approximated by hplc analysis of solutions prepared both (a) by agitation of excess solid aza-A with the solvent and (b) by precipitation from supersaturated solutions. In the latter method, the initial concentrations of aza-A in the aqueous DMA and DMSO solutions (before precipitation) were 80 and 160 mg ml^{-1}, respectively. Supersaturated solutions were prepared by dissolution of drug in the organic phase followed by addition of aqueous phase. Each drug solution was first shaken and then centrifuged just before the sampling time and 25 μl of the supernatant was diluted along with the internal standard to 10 ml. A 5 μl aliquot was analysed by hplc. The apparent equilibrium solubility of aza-A in 70% DMSO : 30% H_2O and 70% DMA : 30% H_2O was estimated to be 70 and 60 mg ml^{-1}, respectively (see Fig. 3). Aza-C exhibited a 40 mg ml^{-1} solubility in the 70% DMSO : water system.

Long-term stability studies of aza-A in pure organic solvents were carried out at concentrations

of 100 mg ml^{-1} in DMSO and 85 mg ml^{-1} in DMA. Sealed ampoules were set up at 6, 25, 37 and 50 °C (±1 °C).

RESULTS AND DISCUSSION

Initial studies on the stability of aza-A in aqueous media using hplc demonstrated that it degraded at a comparable rate and by a route analogous to that of aza-C as proposed by Notari & DeYoung (1975) and Benjamin (1979) and substantiated by Beisler (1978). In addition, chromatographic results indicated biphasic kinetics. The data we obtained together with those of the previous workers on aza-C, appear to support a degradation mechanism of the type proposed in Scheme 1, which involves



Scheme 1

Proposed degradation pathway of I in aqueous media.

nucleophilic addition of water to the 5–6 double-bond of the azacytosine moiety accompanied by a proton transfer followed by hydrolysis of IV. It thus appears that the instability could be avoided by use of a solvent that could not participate in one or both of these key steps. Accordingly, the stability of aza-A in DMSO and DMA was investigated since neither solvent should participate in the reactions shown in Scheme 1. Over three weeks, solutions of aza-A in both solvents yielded no evidence of degradation.

The stability at 25 °C of aza-A in solvent systems containing water and either DSMO or DMA in various concentrations was assessed by hplc. Since the degradation kinetics of aza-A are biphasic, the time required for 10% loss (t90) of drug was used as a stability index that usefully reflects drug potency.

The stability of both aza-A (Table 1) and aza-C (Table 2) can be improved by increasing the fraction of organic solvent in the mixed solvent system. The effects of buffer concentration and pH of the mixed aqueous-organic system on the t90 value of aza-A was also examined (Table 1), and at organic solvent

Table 1. Stability characteristics of 5-azacytosine arabinoside (I) at 25 °C in various solvent systems.

Medium	[I] mg ml^{-1}	Measured pH of the solution[a]	t90 (h)
Distilled H_2O	0·25	6·8	1·8
Buffer[b]	0·25	7·0	1·7
5% DMSO in Buffer[c]	5·0	7·0	2·0
10% DMSO in Buffer	5·0	7·1	2·5
25% DMSO in Buffer	5·0	7·4	2·8
5% DMA in Buffer[c]	5·0	7·0	1·9
10% DMA in Buffer[c]	5·0	7·1	2·2
25% DMA in Buffer[c]	5·0	7·4	2·7
50% DMA in Buffer[c]	5·0	8·1	4·5
55% DMA in Buffer[c]	5·0	8·3	6·0
60% DMA in Buffer[d]	5·0	7·4	15·0
60% DMSO in Buffer[d]	5·0	7·8	16·0
70% DMSO in H_2O	5·0	8·0	28·0

[a] In mixed aqueous-organic system, the value represents apparent pH of the final mixture.
[b] Phosphate buffer, 0·01 M, pH 7·0, μ = 0·1 ml^{-1} with NaCl.
[c] Phosphate buffer, 0·01 M, pH 6·8, μ = 0·1 ml^{-1} with NaCl.
[d] Phosphate buffer, 0·03 M, pH 6·0, μ = 0·3 ml^{-1} with NaCl.

Table 2. Stability characteristics of 5-azacytidine (II) at 25 °C in various solvent systems.

Medium	[II] mg ml^{-1}	t90 (h)
Distilled H_2O	5·0	2·0
50% DMSO in H_2O	5·0	7·8
70% DMSO in H_2O	5·0	22·0
70% DMA in H_2O	5·0	19·0

concentrations of >60%, distilled water, rather than buffers, resulted in a slight improvement of the stability index of aza-A. In addition, buffer precipitation, which tended to occur in the organic-aqueous (70:30%, v/v) solution, was avoided. Chromatograms of aza-A in a purely aqueous system and in a aqueous-DMSO (30:70%, v/v) system clearly demonstrated that in the aqueous-organic system, as well as a decreased rate of loss of aza-A, there was

only an insignificant accumulation of IV when compared with the pure aqueous system. A plot of log aza-A remaining as a function of time for both these systems is shown in Fig. 1.

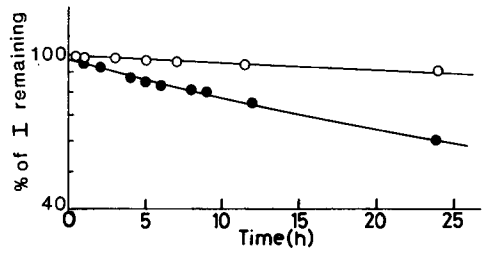


FIG. 1. Semilog plot of degradation of I (aza-A) as a function of time when dissolved in water (●) or 70% (v/v) DMSO-30% water (○) at 25 °C.

While kinetic studies in aqueous solutions at neutral pH exhibited a biphasic degradation profile, in the 70% DMSO : 30% H_2O system the degradation of aza-A appeared to be a simple first-order process in accord with observations that there was no accumulation of IV in such systems. The plot of t90 values as a function of percent (v/v) organic cosolvent is shown in Fig. 2. It is clear that in mixtures with greater than 50% DMA or DMSO the stability of aza-A increased greatly.

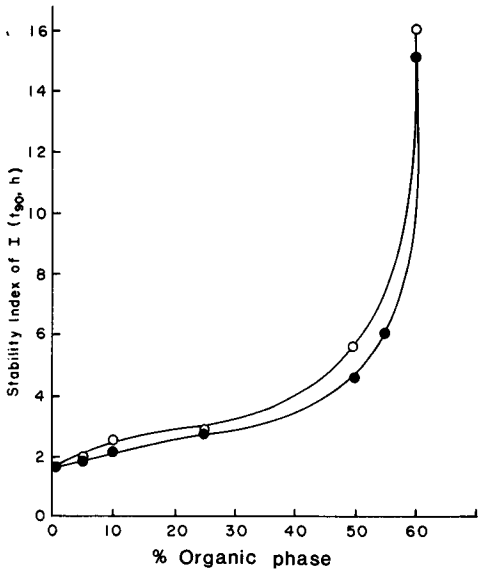


FIG. 2. Plot of stability index of I (aza-A) vs per cent (v/v) organic-aqueous solvent (●, DMA; ○, DMSO) at 25 °C. Phosphate buffer pH 6·8, 0·01 M, ionic strength 0·1 was used as the aqueous phase.

Although the t90 in the cosolvent systems was substantially increased, it was clear that the stability of aza-A in such mixed aqueous-organic solvent systems would not be satisfactory for purposes of manufacturing and distribution as a drug solution. Alternatively, while the stability of aza-A in DMA or DMSO might be suitable for such purposes, the i.v. administration of such solutions would not be pharmaceutically acceptable. In light of the above problems and findings, an alternative strategy was developed.

The approach involved the use of an anhydrous solution of aza-A (in a water miscible organic solvent such as DMSO or DMA) which may be sterilized by filtration, is chemically stable and subsequently diluted at the time of administration. In order to assess the utility of the above approach, the solubility of aza-A as a function of DMSO and DMA concentration in water was evaluated at 25 °C by estimates based on the use of solubility data obtained by hplc monitoring, as a function of time, of the intact drug concentration obtained by agitation of excess drug with the particular solvent system. Values so obtained were compared with values obtained by preparing supersaturated solutions of the drug in the solvent system and monitoring the intact drug concentration as precipitation occurred. Similar asymptotic values of drug solubility were obtained in (Fig. 3) and the solubility data shown in Fig. 4 were obtained. While the solubility of aza-A in

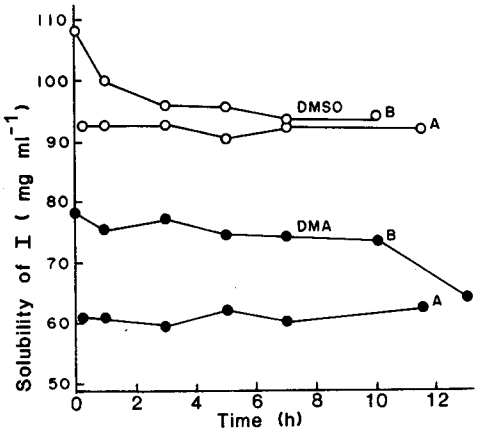


FIG. 3. Determination of apparent equilibrium solubility of I (aza-A) in 70% DMSO–30% water (○) and 70% DMA–30% water (●) as a function of time when the solution was prepared by agitation of excess drug with the solvent (A) or by precipitation from supersaturated solution (B).

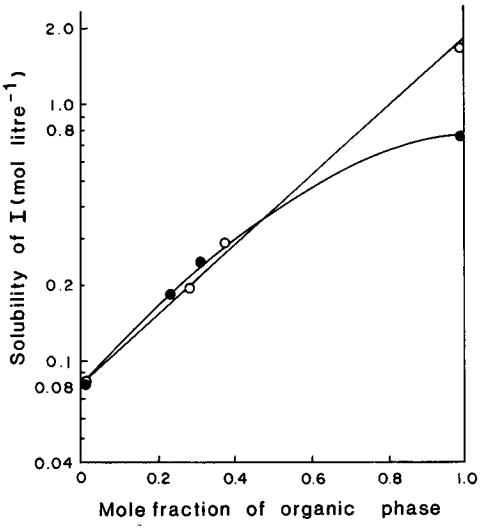


FIG. 4. Plot of apparent saturation solubility of I (aza-A) at 25 °C in solvents composed of various proportions of DMA (●) or DMSO (○) with aqueous phosphate buffer (pH 6, 0·03 M, μ = 0·1).

The compatibility of two samples of commercial tubing with the neat organic solvents and with aqueous-organic solvents was examined. While both systems containing <0·4 mole fraction DMA or DMSO in water were similar, the solubility in DMSO was much greater than in DMA (75 mg ml^{-1} in 70% (v/v) DMA in water and 100 mg ml^{-1} in 70% (v/v) DMSO in water at 25 °C).

were primarily composed of a polyvinyl chloride polymer, they differed in the plasticizers used. In one tubing (Cutter) was tri-(2-ethylhexyl)trimellitate (TEHT) while in the other, (Travenol) it was di-(2-ethylhexyl)phthalate (DEHP). In neat DMA or DMSO, both tubings dissolved in minutes while in 70% organic : 30% water solvents, neither tubing appeared to be physically altered, with both remaining clear and flexible after soaking in the solvent for >24 h.

To ascertain if plasticizer is leached from the tubing, the plasticizer levels in the solvent were determined after various types of exposure of the tubing to the 70% organic : 30% aqueous solvents. The 70% DMSO in water was found to extract less of either plasticizer than 70% DMA in water (Table 3). Also less TEHT is extracted than DEHP by either solvent system. The data suggest that the tubing and solvent of choice in minimizing plasticizer extraction are the TEHT plasticized tubing and the 70% DMSO : 30% water solvent system. Such a combination resulted in <1·5 µg plasticizer extracted from 50 cm lengths of tubing under the most severe treatment investigated.

Table 3. Extraction of plasticizers from intravenous tubing under various treatment conditions at 25 °C.

		Plasticizer extracted[b]			
		30% water–70% DMA		30% water–70% DMSO	
Tubing used (50 cm length)	Treatment[a]	Total (µg)	(µg h^{-1})	Total (µg)	(µg h^{-1})
Travenol	A	1380	345	56·0	14·0
	B	1460	365	64·0	16·0
	C	1800	450	270·0	68·0
Cutter	A	44·0	11·0	0·2	0·05
	B	44·0	11·0	0·6	0·15
	C	112·0	28·0	1·4	0·35

[a] Treatment A = 3·2 ml of liquid flowing through tubing for 4 h. Treatment B = tube filled with 3·2 ml of liquid and allowed to stand for 4 h. Treatment C = tube filled and allowed to stand for 1 h, then removed and tubing refilled with fresh liquid at 1 h intervals for 4 h.
[b] Travenol tubing is plasticized with DEHP, while Cutter uses TEHT.

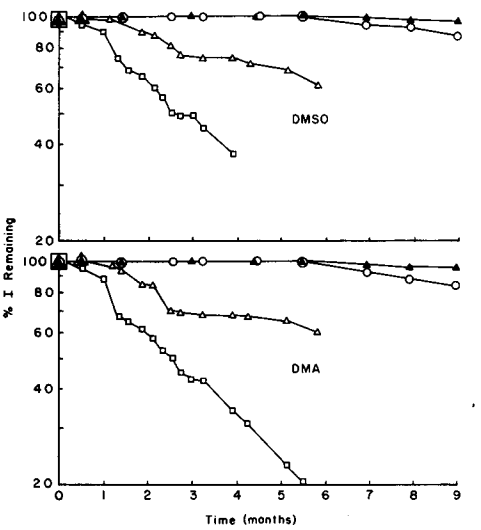


FIG. 5. Log % I (aza-A) remaining as a function of time at various temperatures in DMSO ([aza-A]$_{init}$ = 100 mg ml^{-1}) and in DMA ([aza-A]$_{init}$ = 85 mg ml^{-1}). ▲, 6 °C; ○, 25 °C; △, 37 °C; □, 50 °C.

The ease of mixing and dilution of a 70% DMSO : 30% water solution of aza-A (at 70 mg ml^{-1}) with both saline and 5% dextrose solution was assessed. When the drug solution was infused slowly (6 ml h^{-1}) through Cutter tubing connected to a 20-gauge needle inserted into either of the commercial i.v. vehicles (flowing at a rate of 250 ml min^{-1}), there was no evidence of precipitation nor of any other perceptible incompatibility, and the two solutions appeared to mix readily.

Long-term studies on the stability of aza-A in neat DMA and DMSO have been undertaken at various temperatures for solutions containing aza-A at concentrations of 85 mg ml^{-1} in DMA and at 100 mg ml^{-1} in DMSO. The results, in Fig. 5, show that the stability of aza-A at 37 and 50 °C is inadequate in either DMSO or DMA with DMSO giving a slightly more stable solution. However, the results at 6 °C indicate a high probability that ≥90% potency of aza-A will be retained for a year or more.

Based on the data thus far obtained relative to stability, solubility, and extent of extraction of plasticizer for i.v. tubing, it appears that DMSO is preferable to DMA as a solvent for aza-A. Less extensive studies similar to those described above for aza-A have also been carried out for aza-C. Results obtained again indicate that the general formulation approach described above for aza-A is similarly applicable for amelioration of the stability/formulation problems presented by aza-C.

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