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A study on the stability of three antineoplastic drugs and on their sorption by i.v. delivery systems and end-line filters

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Summary

Sorption by i.v. delivery systems and stability in different infusion fluid containers were investigated for bleomycin, epidoxorubicin and nimustine. Two infusion fluids were used: 0.9% NaCl and 5% glucose. A solution of 15 $\mu g\ ml^{-1}$ bleomycin can be stored at room temperature, unprotected from daylight, for up to 24 h after reconstitution. Solutions of 50 $\mu g\ ml^{-1}$ epidoxorubicin or 2 mg ml^{-1} nimustine kept at 4°C, protected from daylight, are stable for at least 25 and 6 days, respectively. During the stability testing no influence of the infusion fluid or packaging materials was observed. Dynamic experiments showed no sorption of any of the tested drugs by the infusion bags or administration sets. The use of end-line filters caused an initial, clinically unimportant loss of bleomycin and epidoxorubicin.

Introduction

Next to radiation therapy and surgery, chemotherapy has been shown to be effective in the treatment of a wide range of tumours. In a hospital, antitumour drug admixtures should be prepared by the pharmacy department. Including these drugs in a reconstitution program reduces costs and optimizes the use of the pharmacist's time.

The chemical stability, light sensitivity and sorption by delivery systems of antineoplastic agents have been studied by several authors (Hoffman et al., 1979; Tavoloni et al., 1980; Benvenuto et al., 1981; Poochikian et al., 1981; Beynen et al., 1985, 1986; Bosanquet, 1985, 1986; Lafollette et al., 1985; Bouma et al., 1986; Sewell et al., 1987; McElnay et al., 1988), but the results are often contradictory especially in the case of doxorubicin and nimustine. Since many factors such as pH, differences in infusion fluids, temperature, drug concentration and flow rates are important in stability and sorption testing, it is not unexpected that some studies report different results.

The aim of the present study was to investigate the stability of bleomycin, nimustine and epidoxorubicin in situations simulating clinical practice relating to storage before use and during continuous infusion. In addition, sorption of these

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antineoplastic agents by the different parts of an administration system was investigated.

Materials and Methods

Materials

Bleomycin sulphate (Bleomycin®, Laboratoire Roger Bellon, Paris, France) was supplied by Wellcome (Aalst, Belgium) in vials containing 15 mg (activity) bleomycin sulphate. Epidoxorubicin (Farmorubicin®, Farmitalia Carlo Erba, Italy) was supplied by Farmitalia (Brussels, Belgium) in vials containing 10 mg 4′-epidoxorubicin HCl lyophilised powder and 50 mg lactose. The vials of nimustine (ACNU®) contained 50 mg nimustine HCl lyophilisate and 150 mg sodium chloride and were supplied by ASTA-Pharma (Bielefeld, F.R.G.).

The drug concentrations used in this study were chosen to simulate clinical practice. The concentrations, flow rates and administration times are listed in Table 1.

The main specifications of the infusion bags, infusion sets and end-line filters are set out in Table 2. The infusion tubings and end-line filters that were tested during dynamic experiments are detailed in Table 3.

Two infusion solutions were used to deliver the drugs: 0.9% NaCl and 5% glucose.

Analysis

Drug analyses were performed by HPLC, except for bleomycin, where a UV-spectrophotometric method was used. The HPLC system consisted of a solvent pump (Merck-Hitachi L-6000 pump, E. Merck, Darmstadt, F.R.G.), a syringe loaded injector (Valco Instrum. Corp., Houston, U.S.A.), a reversed-phase column (Lichrospher RP-18, 5 μm particles, 125 × 4 mm, E. Merck, Darmstadt, F.R.G.), a variable-wavelength detector (Merck-Hitachi, L-4000 UV-Vis detector, E. Merck, Darmstadt, F.R.G.) and a chromatointegrator (Merck-Hitachi D-2000, E. Merck, Darmstadt, F.R.G.).

The flow rate was 1 ml min^{-1}. Solvent composition, detector wavelength and calibration curves for the two methods are listed in Table 4.

All HPLC methods were validated using an additional diode array detector with complete UV spectral capability. In order to complete the response specificity of the drugs, the purity of the analyte response was evaluated using the numerical spectroscopic approach of absorbance indexing (Poile and Conlon, 1981). The bleomycin concentration was measured with a UV spectrophotometer at 210 nm (double-beam spectrophotometer UV-140-02, Shimadzu, Seisakusho Ltd., Kyoto, Japan). The HPLC method described by Benvenuto et al. (1981) for the determination of bleomycin was used for the validation of the spectrophotometric analysis of the drug. Experiments without bleomycin were run simultaneously to avoid interference from plasticizers, sodium chloride or glucose. Calibration curves for a bleomycin concentration between 5 and 40 μg ml^{-1} showed excellent correlation ($y = 1.548x - 0.053$; $r^2 = 0.9999$). The S.D. (%) on the slope was 1.61 ($n = 5$).

Methods

The drugs were reconstituted according to the manufacturer's specifications. Preliminary tests in-

TABLE 1

Concentrations, flow rates and administration times of bleomycin, epidoxorubicin and nimustine

Drug	Concentration used in the study (μg ml^{-1})	Volume of infusion fluid container (ml)	Flow rate (ml h^{-1})	Administration time (h)
Bleomycin sulphate	15	500	20	24
Epidoxorubicin HCl	50	1000	42	24
Nimustine HCl	2000	50	100	0.5

TABLE 2

Specifications of infusion fluid containers, administration sets and end-line filters.

Code	Device	Material	Manufacturer
A	Infusion fluid container	Glass	NPBI bv Emmer Compascuum, The Netherlands
B	Infusion fluid container	High density polyethylene (HDPE)	Braun Melsungen, F.R.G. (Plasco®)
C	Infusion fluid container	PVC	Baxter Lab. Lessen, Belgium (Viaflex®)
D	Administration set	PVC	Abbott N.V., Ottignies, Belgium (Venisystems® Vented pump set)
E	Administration set	PVC	Baxter Laboratories, Lessen, Belgium
F	Administration set	Double polymer (PVC/PE)	Abbott N.V., Ottignies, Belgium (Venisystems®, Nitroglycerin pump set)
G	Administration set	Polybutadiene (PBD)	Avon Medicals, U.K. (Sureset A 261®)
H	Administration set	PVC with UV filter	Avon Medicals, U.K. (Amberset A 260®)
I	Tubing	HDPE	Rehau, F.R.G.
J	End-line filter	Cellulose ester	Braun Melsungen, F.R.G. (Sterifix®)
K	End-line filter	Cellulose ester	Millipore 67 Molstein, France (Ivex HP®)
L	End-line filter	Polyamide (Nylon 66)	Pall Fajardo, U.S.A. (FAE-020 LL)
M	End-line filter	Polyamide (Nylon 66) positively charged	Pall, Fajardo, U.S.A. (ELD-96LL)

dicated that samples could be taken with polypropylene syringes without sorption problems. Samples were stored in glass tubes for bleomycin (Benvenuto et al., 1981) and in polypropylene tubes for epidoxorubicin (Bosanquet, 1986; Bouma et al., 1986) and nimustine. Samples were diluted just before analysis. Analysis was always performed during the day of sampling.

TABLE 3

Administration sets and end-line filters tested during dynamic experiments

Drug	Infusion tubing	End-line filters
Bleomycin	D,E,F,G,I	J,K,L,M
Epidoxorubicin	E,G,H,I	J,K,L,M
Nimustine	E,H	J,K,L,M

Reconstituted drugs were added to the infusion fluid containers, to obtain the concentrations listed in Table 1. For epidoxorubicin and nimustine the pH of the infusion fluid, without drug added, was measured before each experiment.

During static experiments the drug solutions were stored in glass, HDPE or PVC containers. The glass and HDPE containers were stored upright in order to avoid sorption of the drug by the rubber stoppers. Samples were taken after 5, 10, 20, 30, 60, 120 and 180 min and then every 2 h over a period of 8 h and after 23 and 24 h. For

TABLE 4

HPLC conditions for epidoxorubicin and nimustine determinations

	Epidoxorubicin	Nimustine
Internal standard	4-chloropyridine	methyl-4-hydroxybenzoate
Solvent	CH_3CN/phosphate buffer (27.5 : 72.5) (pH 4.6)	MeOH/phosphate buffer (40 : 60) (pH 5.5)
Detector wavelength	254 nm	230 nm
Reference	Poochikian et al. (1981)	Product information ASTA Pharma
Calibration curves		
Concentration range	2–60 $\mu g\ ml^{-1}$	0–100 $\mu g\ ml^{-1}$
Parameters	$y = 3.6032x - 0.0050$	$y = 0.0218x - 0.0006$
Correlation coefficient	0.9998	0.9999
S.D.(%) on slope ($n = 5$):		
Within runs	0.22%	1.33%
Between runs	0.34%	1.35%

nimustine and epidoxorubicin solutions, further samples were taken once a day up to 6 (nimustine) or 30 days (epidoxorubicin) after drug addition.

The infusion bags supplemented with bleomycin were stored at room temperature, unprotected from light. For epidoxorubicin the infusion bags were protected from light and stored at $4 \pm 1°$ C. For nimustine the experiments were performed with and without protection from light, at room temperature and at $4 \pm 1°$ C.

During dynamic experiments, samples were taken in the infusion fluid containers and at the end of the infusion tubing with or without end-line filter. The first effluent from the administration

TABLE 5

Percent drug recovery during static experiments (bleomycin and epidoxorubucin; ± S.D.; n = 3)

Drug	Infusion fluid	Temperature	Period	Daylight protection	% Recovery infusion fluid container		
					A	B	C
Bleomycin	0.9% NaCl	RT	24 h	−	100.0 ± 4.2	96.9 ± 1.6	97.9 ± 0.8
		RT	48 h	−	98.3 ± 2.2	100.6 ± 3.8	98.8 ± 3.1
	5% glucose	RT	24 h	−	101.3 ± 2.2	98.9 ± 2.5	102.6 + 3.8
		RT	48 h	−	101.1 ± 2.2		
Epidoxo-rubicin	0.9% NaCl	4 ± 1° C	24 h	+	98.7 ± 1.0	96.0 ± 0.7	99.8 ± 4.7
			20 days	+	95.6 ± 1.0	94.0 ± 1.4	95.0 ± 0.7
			25 days	+	97.9 ± 3.7	95.6 ± 0.6	93.7 ± 1.9
			30 days	+	90.7 ± 1.0	87.8 ± 0.5	89.6 ± 0.7
	5% glucose	4 ± 1° C	24 h	+	99.5 ± 1.0	99.0 ± 1.9	99.0 ± 2.5
			20 days	+	96.1 ± 3.5	96.7 ± 1.2	99.0 ± 1.0
			25 days	+	90.6 ± 0.0	97.8 ± 1.9	96.5 ± 2.0
			30 days	+	91.0 ± 4.6	95.5 ± 1.5	92.5 ± 0.9

RT, room temperature; +, protected from light; −, not protected from light.

sets was sampled and then after 10, 20, 30, 60, 120 and 180 min. Further samples were taken every 2 h during a period of 8 h and after 23 and 24 h. When end-line filters were used samples were taken before and after the filter.

Besides the filter effluent was sampled after 5, 10, 20, 30, 60, 120 and 180 min. Further sampling was carried out at the same time intervals as mentioned for the infusion tubing. In the case of nimustine, the infusion period was only 30 min and samples were taken for all experiments after 0, 2, 5, 10, 15, 20 and 30 min.

In all cases the drug recovery was calculated as a function of the initial drug concentration.

Results and Discussion

The results of the static experiments are given in Tables 5 and 6. Bleomycin recovery in 0.9% NaCl and in 5% glucose using glass, PVC or HDPE containers remained above the 90% value during a 24 h period of storage at room temperature (Table 5). Bosanquet (1986) reported that a bleomycin solution (concentration unknown) was stable for 14 days when kept at room temperature in artificial daylight. Benvenuto et al. (1981) suggested that PVC containers should be avoided when storing a 0.3 mg/ml bleomycin solution. In our experiments no difference in drug levels was found between storage in glass or PVC containers.

Epidoxorubicin was stable in PVC, glass and HDPE infusion containers when stored at $4 \pm 1°C$, protected from light, for a period of 25 days (Table 5). The solutions were protected from light, since several authors have reported that photodecomposition of anthracyclines took place (Tavoloni, 1980; Beynen et al., 1986; Bouma et al., 1986). Storage at $4 \pm 1°C$ was chosen, since Bosanquet (1986) reported that most antitumour antibiotics, including doxorubicin and epidoxorubicin, were more stable at 5 °C.

The stability of epidoxorubicin was also studied by Beynen et al. (1985). They found that a 100 μg

TABLE 6

Percent recovery during static experiments (nimustine ± S.D., n = 3)

Infusion fluid	Temperature	Period	Daylight protection	% Recovery PVC infusion fluid container	
				A	C
0.9% NaCl	RT	5 h	−	92.4 ± 0.9	
	RT	7 h	−	87.3 ± 0.8	
[a]	RT	24 h	−	65.4 ± 2.5	
	RT	5 h	+	95.7 ± 1.4	
	RT	7 h	+	95.8 ± 0.5	
[a]	RT	24 h	+	81.6 ± 3.4	
	4 ± 1 °C	5 h	+	97.0 ± 1.5	
	4 ± 1 °C	3 days	+	93.7 ± 1.4	
[a]	4 ± 1 °C	6 days	+	90.9 ± 1.9	
5% glucose	RT	5 h	−	91.8 ± 5.8	
	RT	7 h	−	93.0 ± 4.4	
	RT	24 h	−	68.3 ± 4.4	
	RT	5 h	+	97.6 ± 1.0	
	RT	7 h	+	98.7 ± 1.0	
	RT	24 h	+	89.0 ± 1.5	
	4 ± 1 °C	5 h	+	100.2 ± 0.8	
	4 ± 1 °C	3 days	+	96.8 ± 0.6	
	4 ± 1 °C	6 days	+	92.4 ± 2.2	

+, protected from daylight; −, not protected from daylight. [a] No significant influence of the composition of the infusion fluid ($p = 0.05$ Mann-Whitney U test).

ml^{-1} solution, kept at room temperature in the dark, could be stored for 6 days in 0.9% NaCl and for 28 days in 5% glucose. The difference in stability of the drug was explained as being the result of a difference in pH of the infusion fluid. The pH values of the infusion fluids used in the study of Beynen et al. were 4.7 and 7 for the glucose and 0.9% NaCl solution, respectively. Thus, anthracyclines appear to be more stable in the lower pH range (Bosanquet, 1986). In this study the difference in observed pH between normal saline (5.5 ± 0.3) and 5% glucose (4.26 ± 0.1) did not result in a significant difference in degradation rate ($p = 0.05$; Mann-Whitney U test).

The only information on stability data for nimustine was made available by the supplier. The data indicated that the stability of the drug is much greater at 5°C than at 25°C and that problems due to photodegradation are to be expected. We observed that nimustine added to 5% glucose or 0.9% NaCl solutions and stored at room temperature was not stable over a 24 h period, even when protected from light. When these solutions were kept at 4 ± 1°C, and were protected from light, they could be used until the sixth day after addition. The use of 0.9% NaCl (pH 4.8) or 5% glucose (pH 4) solutions did not significantly influence the results ($p = 0.05$; Mann-Whitney U test). The stability in glass bottles was compared to the results obtained with PVC bags (kept at room temperature with and without light protection in 0.9% NaCl and 5% glucose solutions and stored in 5% glucose solution at 4°C with light protection). In none of these cases was there a significant difference with the results for the PVC infusion bags shown in Table 6 ($p = 0.05$; Mann-Whitney U test). It was obvious that the solutions should be protected from light (Table 6).

Dynamic experiments were performed in order to detect sorption of the drugs to administration sets. Sorption problems have been reported previously by McElnay et al. (1988) for the antitumour drugs methotrexate and vinblastine.

TABLE 7

Cumulative amount of drug loss during infusion through an endline filter for epidoxorubicin and bleomycin expressed as a function of the theoretical amount of drug that should be delivered at that moment

Drug	Infusion fluid	Filter	Time period (min)	Cumulative amount of drug lost ± S.D. (%)
Epidoxorubicin	0.9% NaCl	J	60	17.9 ± 3.56
		K	20	7.5 ± 1.85
		L	30	47.3 ± 2.51
		M	30	46.2 ± 3.40
	5% glucose	J	20	28.8 ± 1.22
		K	10	4.7 ± 2.85
		L	20	5.2 ± 1.18
		M	10	4.3 ± 1.58
Bleomycin	0.9% NaCl	J	18	2.8 ± 0.034
		K	20	5.7 ± 0.4
		L	8	23.3 ± 0.30
		M	4	0.8 ± 0.06
	5% glucose	J	18	42.5 ± 0.12
		K	20	49.6 ± 0.75
		L	8	15.2 ± 1.05
		M	17	24.5 ± 1.02

The data indicate the amount of drug as a percentage of the sorbed drug and the time at which saturation of the filter membrane occurs.

For the dynamic experiments, the continuous administration mode was chosen, since several studies demonstrated that drug toxicity can be lowered and the efficacy improved by this method of administration rather than by intermittent small volume infusion or i.v.-push (Garnick et al., 1983; Greidanus et al., 1988). For the three antineoplastic drugs no decrease in concentration due to sorption was observed.

Because many patients receiving antineoplastic drugs are immunosuppressed, the use of end-line filters is indicated to prevent septicemia and the passage of particulate matter generated by dissolving the lyophilised drug powder. Adsorption to end-line filters has been described for several drugs such as vincristin sulphate, mithramycin, dactinomycin and insulin (Rusmin et al., 1977; Butler et al., 1980).

We found that the connection of an end-line filter caused a reduction in drug recovery for epidoxorubicin and bleomycin solutions. The cumulative amount of drug that is lost is listed in Table 7. As an example, the loss of epidoxorubicin to the Pall ELD-96 LL filter in 0.9% NaCl is shown in Fig. 1. The drug loss is only temporary and is dependent on the filter type, the drug and the infusion fluid. Nevertheless, the total amount of drug that is lost during the whole period of infusion is small ($< 2\%$ for every filter-drug-infusion fluid combination tested). For nimustine no drug loss was detected when administered through the four tested end-line filters. This could be due to the high concentration of the drug in the infusion fluid through which an immediate saturation of the binding sites on the hydrophilic filter occurs. This is confirmed by the literature that indicates that only drugs administered in very low concentrations could present problems with regard to binding of drugs to the filter materials (Rusmin et al., 1977).

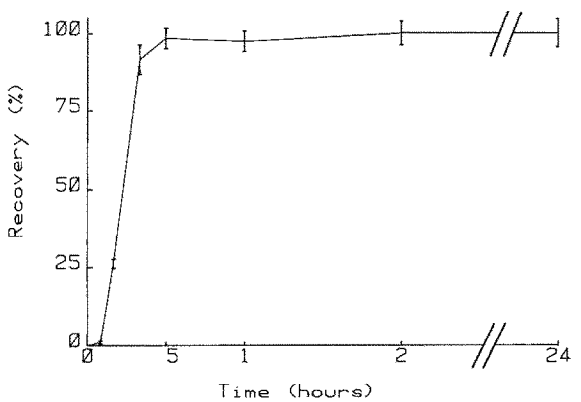

Fig. 1. Adsorption pattern of epidoxorubicin (when diluted in 0.9% NaCl) to the Pall ELD-96 LL filter.

Conclusion

From the static experiments, it can be concluded that a 15 μg ml^{-1} solution of bleomycin stored at room temperature, unprotected from light, and a 50 μg ml^{-1} solution of epidoxorubicin stored at $4 \pm 1°$ C, protected from light, are stable in 0.9% NaCl and 5% glucose in PVC, HDPE and glass containers for periods of 24 h and 25 days, respectively. A nimustine solution of 2 mg ml^{-1} in 0.9% NaCl and 5% glucose stored at $4 \pm 1°$ C, protected from light, can be used until the sixth day after addition of the drug to the infusion fluid in glass or PVC containers.

From the dynamic experiments, it can be concluded that no drug was sorbed by the infusion bag nor in the administration set when infused under the present experimental conditions. Infusion through an end-line filter could cause a temporary decrease in the amount of epidoxorubicin and bleomycin delivered. The total amount of drug loss seemed negligible.

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