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Stability of three aminoglycoside solutions in PVC and multilayer infusion bags

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The compatibility of drugs has been examined from solutions infused via PVC bags (Macopharma® Lab.) and multilayer bags, composed of polyethylene, polyamide and polypropylene (Bieffe Medital) connected with plastic infusion sets. HPLC was performed to analyse the following aminoglycosides: tobramycin, gentamicin and amikacin. After a derivatization reaction and organic extraction, the drugs were separated by HPLC and detected by fluorimetry. The data show stability of drug concentration in PVC and multilayer bags during 1 h simulated infusion or in reconstituted solutions 2 h before infusion. No difference was noticed between the two infusion solutions 5% dextrose or 0.9% sodium chloride.

1. Introduction

For many years infusion solutions have been packaged into collapsible plastic bags, in order to infuse them under optimal aseptic conditions. Many drug solutions such as antibiotics and anti-cancer agents, are perfused through these devices. Polyvinal chloride (PVC) is a frequently used material. Some studies have shown no interaction between PVC and many drugs [1–3], but some others have proved an incompatibility between PVC and dissolved drugs such as isosorbide dinitrate and diazepam [4–7]. Multilayer bags are an alternative to PVC packaging [8]. This multilayer material is a combination of polyethylene, polyamide and polypropylene. Numerous studies have shown no interactions for a number of drugs with polypropylene and polyethylene [5], especially with isosorbide dinitrate solutions [9]. Moreover, polyurethane glue is submitted to a high degree of reticulation which results in a very high stability. Antibiotics are frequently infused with injectable solutions of 5% dextrose or 0.9% sodium chloride. Most reconstituted antibiotic solutions are compatible with PVC bags and sets and with multilayer bags [10–12]. Yet, no compatibility of aminoglycosids with these two packaging devices has been proved.

The aim of our study was to verify the stability and the compatibility of three frequently infused aminoglycosids from PVC and multilayer bags, under usual hospital conditions. Preparation and infusion conditions were performed to detect possible interactions of drugs with plastic material. Tobramycin, gentamicin and amikacin were dissolved in 0.9% sodium chloride and in 5% dextrose, packaged in PVC by Macopharma® laboratories and packaged in multilayer material by Bieffé Médital® Laboratories. HPLC was choosen as analytic reference method to assess the stability of the aminoglycosids and to detect possible degradation products. This method allowed simple and quick determination of the three aminoglycosids in infusion solutions.

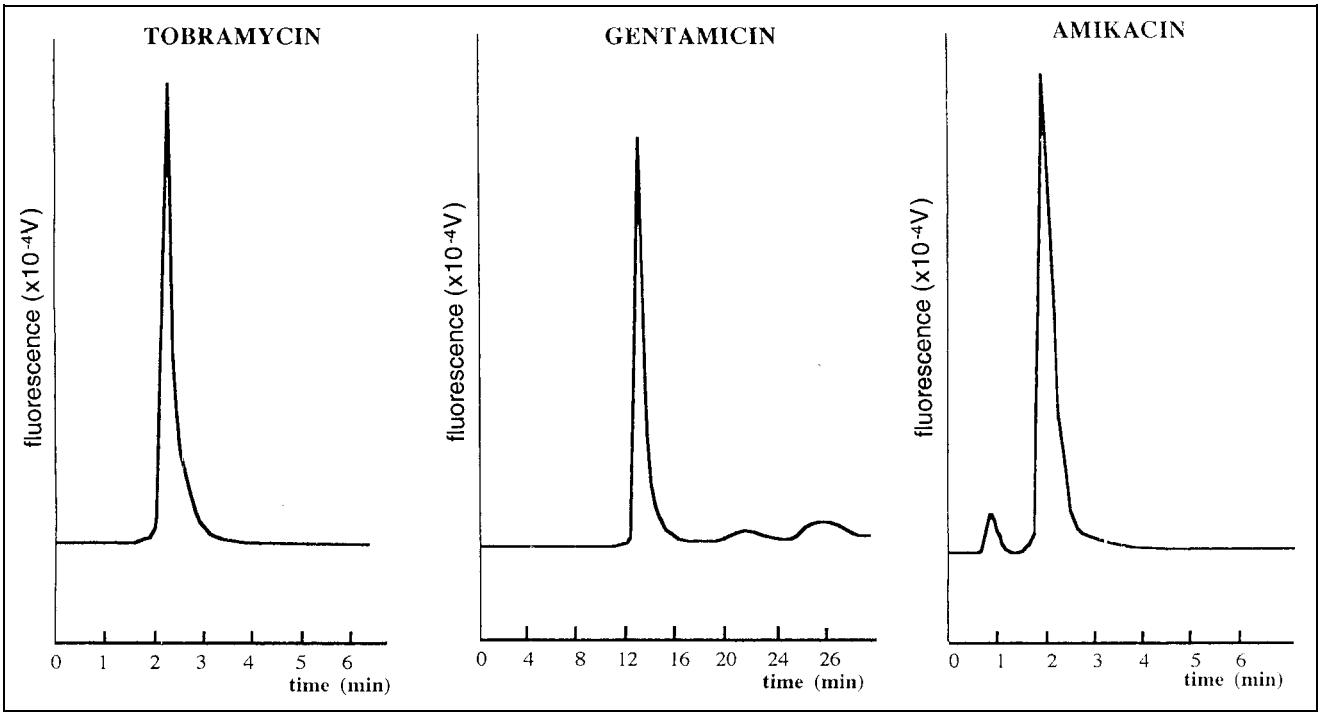

Fig.: Chromatograms of aminoglycosides (see 3.4.3.)

2. Investigations, results and discussion

Chromatograms of tobramycin, gentamycin and amikacin are shown in the Fig. Means (± SD) of retention times (RT) are 2.2 min (± 0.15) for tobramycin, 12.3 min (± 0.27) for gentamycin and 2.1 min (± 0.12) for amikacin. HPLC determination of aminoglycosids in blood samples usually needs a complex process to obtain separation of aminoglycosids from their fractions [13]. Because of the simple mediums in which we operated the different drugs and compounds are easy to separate under rapid and inexpensive conditions. The quantification has been performed from the main peak, neglecting the minor peaks for gentamycin, for both standards and samples. We did not use internal standards to avoid sophisticated equipment and the complex chromatographic processes required to separate drugs from the internal standards. These methods are adaptable for a rapid screening of various aminoglycosids.

Table 1 presents data for validation of the HPLC method for the different drugs as reported in the four trials of each test. The accuracy of the methods was assessed by the recovery of known standard amounts added to 0.9% sodium chloride and 5% dextrose solutions. The mean recovery was 92.1–106.2%. The precision of HPLC methods was estimated by a repetition test ($n = 8$), with variation rates $< 4\%$ and by a reproduction test ($n = 8$), with a variation rate $< 8\%$. The linearity was considered as conform with a correlation coefficient of the linear regression of 0.999 when the drug concentrations are between 2.5 to 10 µg/ml for trobramycin, between 2.5 to 25 µg/ml for gentamicin and between 0.625 to 5 µg/ml for amikacin. Finally, the detection limit of chromatographic methods was estimated as 1 µg/ml for tobramycin and gentamicin, and 0.3 µg/ml for amikacin. Consequently, the different methods proposed for the control of the aminoglycosid stability are satisfactory, especially when measuring high concentrations.

The observed concentrations of tobramycin, gentamycin and amikacin are reported for simulated and pre-prepared infusions in Table 2 for PVC and multilayer bags. These results are the mean of four determinations for each type of text and for each type of bag of specified aminoglycosid concentration in the different samples. 89.1–112.8% of active agent was recovered in bags and infusion sets.

Variations are first due to lack of precision in solution and dilution operations and secondly to variation in active agents mass and solution volume. These variations are below 5% according to pharmacopoeal standards and below 1% according to the manufacturer's controls. Finally, these variations are also due to the multiple steps of the analytical process.

No interactions have been reported in previous studies between amikacin and gentamycin and PVC bags [11, 14, 15]. No gentamycin retention on cellulose ester filters has been observed [16]. However, a strong fixation of gentamycin on polyamid filters has been shown [17]. Other studies have concluded a stability of tobramycin in polypropylene syringes [7].

These results are also confirmed by our own studies in which we found compatibility between aminoglycosids and plastic materials such as PVC, polypropylene, polyamide and high density polyethylene.

Table 1: Validation data of HPLC assay procedure (n = 5)

Sample substance	Concentration (mg/50 ml)	Average concentration found ± SD (mg/50 ml)		Precision		Accuracy	Linear regression equation	Correlation coefficient
		5% Glucose	0.9% NaCl	CV IntraAssay	CV InterAssay		y = a(x) + b	(r)
Tobramycin	75.0	79.6 ± 2.9	79.6 ± 3.1	3.7	7.2	106	$y = 0.347(x) - 37.66$	0.999
Gentamicin	80.0	77.6 ± 2.8	81.6 ± 1.9	2.2	5.1	98	$y = 0.15(x) + 2.02$	0.999
Amikacin	500.0	493.1 ± 16.7	460.5 ± 15.7	1.5	2.5	96	$y = 0.263(x) - 2.352$	0.998

Table 2: Aminoglycosid concentrations in 5% dextrose or NaCl 0.9% solutions during a simulated infusion using PVC or multilayer infusion bags

PVC

	Tobramycin 75 mg/50 ml				Gentamicin 80 mg/50 ml				Amikacin 500 mg/50 ml			
	0.9% NaCl		5% Dextrose		0.9% NaCl		5% Dextrose		0.9% NaCl		5% Dextrose	
	Bag	Pipe	Bag	Pipe	Bag	Pipe	Bag	Pipe	Bag	Pipe	Bag	Pipe
T 0 min	77.6 ± 8.5	73.0 ± 6.8	77.7 ± 7.2	80.5 ± 5.8	81.3 ± 2.5	79.8 ± 3.8	81.1 ± 7.3	85.4 ± 2.5	442.9 ± 23.1	453.0 ± 43.8	474.5 ± 52.5	517.1 ± 89.6
T 5 min	80.5 ± 5.8	82.2 ± 9.0	75.2 ± 8.0	71.7 ± 10.3	81.1 ± 3.8	79.0 ± 3.5	81.6 ± 4.5	78.5 ± 3.3	459.1 ± 37.9	511.4 ± 47.3	504.4 ± 36.8	487.6 ± 49.4
T 15 min	72.5 ± 12.0	71.8 ± 8.3	82.2 ± 6.8	79.9 ± 8.5	83.2 ± 5.3	84.5 ± 4.8	81.5 ± 2.9	80.7 ± 2.1	450.8 ± 45.2	469.4 ± 38.5	499.7 ± 38.6	469.5 ± 3.7
T 30 min	80.5 ± 9.0	81.1 ± 7.0	75.2 ± 3.5	77.6 ± 5.5	79.5 ± 2.4	83.5 ± 2.2	79.7 ± 6.3	79.3 ± 4.9	490.1 ± 50.3	465 ± 25.5	462.2 ± 50.4	480.5 ± 70.3
T 60 min		74.7 ± 10.5		81.6 ± 7.8		85.1 ± 4.6		81.4 ± 3.1		449.5 ± 15.5		471.5 ± 18.6

Multilayer

	Tobramycin 75 mg/100 ml				Gentamicin 80 mg/100 ml				Amikacin 500 mg/100 ml			
	0.9% NaCl		5% Dextrose		0.9% NaCl		5% Dextrose		0.9% NaCl		5% Dextrose	
	Bag	Pipe	Bag	Pipe	Bag	Pipe	Bag	Pipe	Bag	Pipe	Bag	Pipe
T 0 min	68.8 ± 2.1	69.7 ± 7.1	73.4 ± 1.7	76.0 ± 7.9	83.9 ± 4.4	84.4 ± 5.2	77.2 ± 2.6	75.1 ± 2.8	443.9 ± 37.4	456.7 ± 45.7	513.3 ± 19.6	460.3 ± 66.3
T 5 min	70.0 ± 6.1	68.5 ± 3.2	75.3 ± 4.1	75.7 ± 8.9	84.1 ± 4.9	83.3 ± 3.9	75.6 ± 3.4	76.9 ± 2.9	470.4 ± 4.2	505.6 ± 12.8	458.8 ± 28.6	466.3 ± 57.7
T 15 min	67.1 ± 5.1	73.8 ± 2.9	76.4 ± 8.8	71.9 ± 3.8	85.1 ± 4.8	82.3 ± 1.0	76.3 ± 3.6	76.3 ± 2.8	493.0 ± 5.1	487.4 ± 31.1	477.7 ± 41.4	430.5 ± 59.2
T 30 min	68.7 ± 5.6	72.8 ± 3.8	73.9 ± 7.0	71.3 ± 0.8	85.2 ± 6.4	83.9 ± 4.1	76.2 ± 4.3	77.7 ± 1.5	450.9 ± 38.3	486.9 ± 24.5	491.7 ± 8.25	440.6 ± 61.6
T 60 min		68.1 ± 3.5		74.4 ± 2.6		83.0 ± 4.6		77.3 ± 3.5		472.1 ± 53.3		446.5 ± 59.1

Under the experimental conditions used, no variation greater than 15% was observed (active agents dissolved in 0.9% sodium chloride and 5% dextrose).
Finally, no aminoglycoside degradation was observed during the different trials, considering first the concentration stability and secondly the lack of any degradation product peaks. These tests were performed without any special protection against temperature and light.
This study shows a good stability of various aminoglycosides with the two main types of bags used for human infusion. These plastic bags constitute an interesting alternative to glass vials, especially regarding their lightness, bacteriological security and handiness.

3. Experimental

3.1. Materials

Aminoglycoside concentrations were measured by HPLC comprising a pump (Waters® 6000), an automated injector (Waters® 712 Wisp), and a fluorimetric detector (Kratos®) connected with an integrator-recorder (Waters® M 730). Analysis of tobramycin, gentamicin and amikacin was performed with a Lichrosorb RP8 column (Merck®) packed with C8 10 μm grafted silica. PVC bags (50 ml) were supplied by Macropharma® Lab. and multilayer bags (100 ml) by Bieffé Médital® Lab. To simulate infusion, a PVC set (Abbott® Laboratory) and a flowmeter (Cair® Laboratory) were connected to the bag.

3.2. Chemicals

All chemicals were of analytic or chromatographic grades: methanol, orthophthalaldehyde, ethyl acetate, mercaptoethanol (Prolabo, Paris, France); acetonitrile (Carlo Erba – Farmitalia, France).
Standards of the studied drugs, (vacuum packaged) for calibration were supplied by the following laboratories:
Qualimed® Laboratory for tobramycin (Paris La Défense, France), Dakota® Laboratory for gentamicin (Créteil, France), Sigma® Laboratory for amikacin (Saint-Quentin-Fallavier, France).
Corresponding drugs were supplied by the central pharmacy of the University Hospital of Amiens and were selected according to the frequency of their prescription: Tobramycin® 75 mg (Qualimed® Laboratory, France), Gentamicin® 80 mg (Dakota® Laboratory, France), Amiklin® 500 mg (Bristol-Myers Squibb® Laboratory, France).
Antibiotics were dissolved in 0.9% sodium chloride and 5% dextrose solutions packaged in PVC and multilayer bags.

3.3. Study protocol

After dissolution of drugs in their vials and introduction into bags, two different tests were performed. Test "A" simulates a 1 h infusion, with a 0.8 ml/min flow for PVC bags and 1.6 ml/min for multilayer bags. After connecting the administration set and adjusting the flowmeter, samples (1 ml) were collected through a polypropylene syringe: directly from the bags, at to (corresponding to the initial time after homogenization and opening the flowmeter), then at $t_0 + 5$ min, $t_0 + 15$ min, and $t_0 + 30$ min or from the end of the set, at the same times, and also at $t_0 + 60$ min.
Test "B" consisted of dissolving the drug in a bag and waiting 2 h for simulating a pre-prepared infusion.
Samples were collected from the site of injection at t_0 (initial time after homogenization), then at $t_0 + 30$ min, $t_0 + 60$ min, $t_0 + 120$ min. Tests "A" and "B" were carried out in quadruplicate with 5% dextrose and 0.9% sodium chloride solutions. Studies were performed at room temperature and without special light protection.

3.4. Analytical methods

3.4.1. Preparation of standards

Tobramycin, gentamicin and amikacin were quantified by external standardization, using standard scales (range 2.5 to 10 μg/ml for tobramycin, range 2.5 to 25 μg/ml for gentamicin, range 0.625 to 5 μg/ml for amikacin).

3.4.2. Preparation of samples

After dilution of samples, theoretical concentrations of the different solutions were 33 μg/ml for tobramycin, 20 μg/ml for gentamicin and 25 μg/ml for amikacin in PVC bags, and 7.5 μg/ml, 20 μg/ml and 2.5 μg/ml respectively, in multilayer bags. The derivatization was performed in a borate buffer adjusted to pH 10.5 with a 40% sodium hydroxide solution. The derivatization reagent was prepared from orthophthalaldehyde (OPA) solutions in methanol 10% (w/v) for tobramycin, and 50% w/v for gentamycin and amikacin. After homogenization, mercaptoethanol (200 μl) and borate buffer (10 ml) were added. Aminoglycoside solutions (200 μl) were then mixed with water (800 μl) and the OPA solution (200 μl), and vortexed. The derivatives were then extracted in ethyl acetate (1 ml) and 800 μl of extracted solution immediately placed in sealed glass microvials.

3.4.3. HPLC conditions

Standards were injected before and after each serial determination. Aminoglycosides were detected by fluorimetry (λ_{ex} 340 nm, λ_{em} 418 nm) after injection of 30 μl tobramycin sample, 20 μl gentamicin sample and 15 μl amikacin sample. The tobramycin assay was performed using a mobile phase of methanol/acetonitrile (70 : 30 v/v), under isocratic conditions (1.5 ml/min). The gentamycin assay was performed using a mobile phase of TRIS buffer pH 2.1/acetonitrile (55 : 45 v/v), under isocratic conditions (1.5 ml/min). The amikacin assay was performed using a mobile phase of methanol/acetonitrile/water (70 : 10 : 20 v/v/v) under isocratic conditions (1.2 ml/min).

Acknowledgement: We thank Bieffe Medital and Macopharma laboratories for their financial suport.

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Received June 15, 1998
Accepted March 1, 1999

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