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Stability of amphotericin B and nystatin in antifungal mouthrinses containing sodium hydrogen carbonate

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Abstract

Amphotericin B and nystatin are two polyene antibiotics that are potent antifungal agents. These drugs are active against most pathogenic fungi like *Aspergillus* and *Candida*. Mouthrinses containing these drugs are used for preventive and curative treatment of fungal infections like oral candidiasis, which can cause multiple diseases in cancer patients. Because there were no marketed antifungal mouthrinses available, their preparations were performed at the hospital and town pharmacies. To date, there are no data available on the stability of both these drugs in the form of mouthrinses. Therefore, each mouthrinse had to be prepared extemporaneously. The aim of this study was to investigate the stability of amphotericin B (Fungizone®) and nystatin (Mycostatine®) in the form of mouthrinses containing 1.4% sodium hydrogen carbonate. The stability of these solutions was tested at different temperatures (4–37 °C) with or without electric- or sunlight exposure and in two types of containers (glass and polypropylene) over a 15-day period. The admixtures were also monitored for colour change and pH. Amphotericin B and nystatin were quantified by high-performance liquid chromatography. At 4 °C, amphotericin B and nystatin were stable for 15 days in polypropylene. When stored in polypropylene at room temperature, with or without light protection, amphotericin B and nystatin were stable for 3 and 4 days, respectively.

Keywords: Amphotericin B; Nystatin; Antifungal mouthrinse; Oral candidiasis; Stability

1. Introduction

Amphotericin B (AmB) and nystatin (Fig. 1) are both polyene macrolides produced by different strains of *Streptomyces*. Amphotericin B occurs as a yellow to orange, odourless or almost odourless powder containing 750 mg/g of anhydrous amphotericin B. Nystatin is a yellow to light brown hygroscopic powder with a characteristic odour suggestive of cereals containing 5000 unit/mg. Both drugs were stored in airtight containers at a temperature of between 2 and 8 °C and protected from light. Amphotericin B and nystatin were stored at 25 °C maximum after opening for 10 and 7 days, respectively [1]. Amphotericin B and nystatin are used as mouthrinses in oral suspension form to treat severe mucocutaneous candidal infections in the mouth. Amphotericin B and nystatin are potent fungistatic and fungicidal drugs which have been used for almost 30 years [2]. The wide use of amphotericin B and nystatin in recent years results from a dramatic increase in fungal infections in cancer patients [3,4].

The antifungal activity of polyene antibiotics is related to their ability to form micelles with ergosterol molecules present in the cell membranes of fungal cells. This bonding causes structural damage and membrane permeabilisation leading to the loss of electrolytes and other cytoplasmatic components like proteins. As a consequence of which the fungal cells die [5,6].

Mouthrinses are usually prepared in aqueous, saline or alkaline medium. The latter medium is effective against hyposalivation and acidity which contribute to the development of mucocutaneous candidal infections.

The stability of nystatin in various acid, aqueous and alkaline media, with and without colloidal silver has been studied by Vermerie et al. [7]. Vincent et al. [8] have studied the stability of mouthrinses containing nystatin or amphotericin B in sodium hydrogen carbonate (with or without 0.1% hexetidine) in relation to their activity against *Candida* strains.

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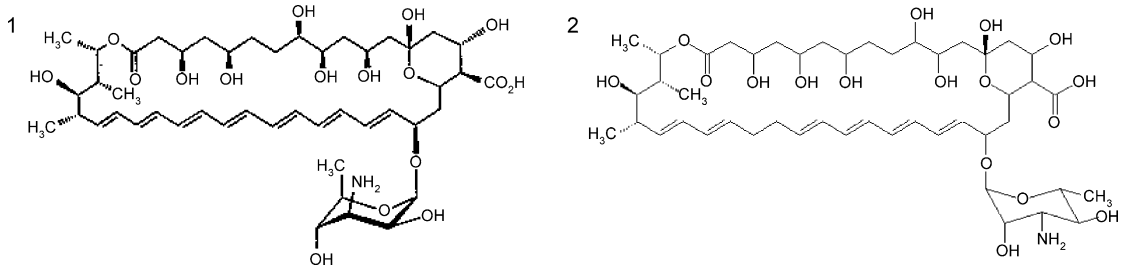

Fig. 1. Chemical structure of amphotericin B (**1**) and nystatin (**2**).

In view of the little data available in the literature concerning the stability of nystatin or amphotericin B in mouthrinses despite their wide use in clinical practice, we undertook to study the effects of temperature and light (electric- or sunlight) on the stability of amphotericin B and nystatin in 1.4% sodium hydrogen carbonate in two types of reservoirs (polypropylene and glass) over a period of 15 days. The aim of our study was to reproduce the different conditions of use and storage encountered both at the hospital and at home.

2. Experimental

2.1. Material

Flasks containing amphotericin B for oral suspension (Fungizone®, 100 mg/ml, flasks of 40 ml) and nystatin for oral suspension (Mycostatine®, 100,000 IU/ml, flasks of 24 ml) were obtained from Bristol-Myers Squibb (Rueil-Malmaison, France). The 1.4% sodium hydrogen carbonate was obtained from Fresenius Kabi (Sèvres, France).

2.2. Preparation of admixtures

Six sets of test solutions for amphotericin B (7.4 mg/ml) and for nystatin (4580.2 IU/ml) were prepared in 1.4% sodium hydrogen carbonate (500 ml). The drugs were assayed under various physical conditions encountered clinically. Thus, we studied the influence of temperature under five different conditions: (i) 4 °C (flask 3); (ii) 19–23 °C with electric light exposure (flask 1) and with light protection (flask 4); (iii) 37 °C with light protection (flask 5) and (iv) 25–28 °C with sunlight exposure (flask 6). The storage conditions are given in Table 1.

During a 15-day period, at 0, 1, 2, 3, 7, 10 and 15 days, a 5 ml sample was removed from each container and observed visually. Before sampling, each container was manually shaken to ensure a uniform suspension (10 min for amphotericin B and 2 min for nystatin). The samples were immediately subjected to pH measurements then frozen at −80 °C until analysis. The pH of the suspension was measured initially and at each test interval. Drug concentrations were determined in triplicate by high-performance liquid chromatography (HPLC).

Table 1
Storage conditions of the mouthrinses containing amphotericin B and nystatin

Container no.	Light	Temperature (°C)	Type of container
1	Electric light	19–23	Polypropylene
2	Electric light	19–23	Glass
3	Protected from light	4	Polypropylene
4	Protected from light	19–23	Polypropylene
5	Protected from light	37	Polypropylene
6	Sunlight[a]	>25	Polypropylene

[a] 13 h/day.

2.3. Analysis by high-performance liquid chromatography

Amphotericin B and nystatin drugs were quantified in each sample using a high-performance liquid chromatography (HPLC) method previously published [6]. This method was adapted for the purpose of our study; piroxicam (Feldene®) was used as internal standard.

All HPLC assays were performed isocratically at ambient temperature (20 °C). The LC system consisted of a high-pressure pump (LC10AT, Shimadzu, Croissy Beaubourg, France), an automatic sample injection system (SIL-10AD, Shimadzu) equipped with a 100 μl sample loop set at 4 °C and a UV–vis variable-wavelength detector (SPD-10AV, Shimadzu) operating at 405 nm for amphotericin B and 305 nm for nystatin. Integration of the peaks was performed with an integrator (C-R5A Chromatopac, Shimadzu). Liquid chromatographic analyses were performed on a Kromasil C18 column (5 μm, 250 mm × 4.6 mm i.d., Interchrom, Montluçon, France). The isocratic mobile phase consisted of a mixture of 1% acetic acid in acetonitrile–water (43:57, v/v). The flow rate was 1 ml/min. The injection volume was 100 μl.

Stock solution of amphotericin B (10 μg/ml), nystatin (45.8 IU/ml) and piroxicam (10 μg/ml) were prepared in water then stored at −20 °C. Working standard solutions were prepared daily from the stock solutions by suitable dilutions in mobile phase and used to prepare calibration standards and quality control samples. The calibration standards were of 30, 80, 100, 250, 500 and 1000 ng/ml for amphotericin B and 0.2, 0.8, 2, 3, 4, 5 IU/ml for nystatin. The linear relationship between the peak area ratios (analyte/internal standard) and concentrations was statistically confirmed. For each point of the calibration standards, the concentrations were back-calculated from the equation of the linear regression curves. The residuals (differences between nominal and back-calculated concentrations) showed random variations, the number of positive and negative values being approximately equal. Moreover, they were normally distributed and centred on 0. The quality control (QC) samples were of 60, 125, 750 ng/ml for amphotericin B and

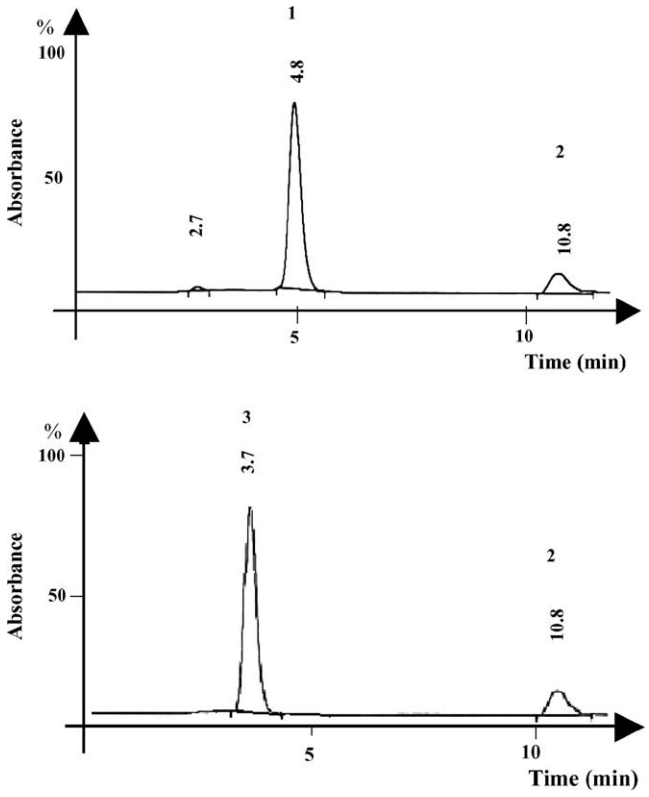

Fig. 2. Typical chromatograms of amphotericin B (740 ng/ml) and nystatin (4.58 IU/ml) in mouthrinses: 1, amphotericin B; 2, piroxicam; 3, nystatin.

0.5, 2.5, 4.58 IU/ml for nystatin. The within-day and between-day precisions expressed as relative standard deviation (R.S.D.) were below 2% and accuracy values ranged from 97 to 103%. Under the chromatographic conditions described above, retention times were 4.8 min for amphotericin B, 3.7 min for nystatin and 10.8 min for piroxicam. Representative chromatograms are given in Fig. 2.

QC samples were included in each analytical sequence to verify the accuracy and precision of analysis (three QC samples at low, medium and high concentrations, in duplicate). Before injection, the test samples were thawed and diluted in the mobile phase to achieve final concentrations of 740 ng/ml for amphotericin B and 4.58 IU/ml for nystatin. The internal standard was added to the diluted samples.

Triplicate HPLC determinations were performed on all samples of each test solution.

2.4. Analysis of data

The initial concentrations (time 0) of amphotericin B and nystatin were referenced as 100% and all subsequent concentrations were expressed as percentages of the initial concentration.

The stability of the two drugs in the admixtures was assessed by evaluating the percent change in amphotericin B or nystatin concentrations from time 0. A decrease of >10% of the initial concentration was considered to represent a significant loss of drug [2,5].

3. Results

3.1. Stability of amphotericin B

Stability of amphotericin B in borosilicate glass and polypropylene flasks is reported in Table 2. For both types of containers, at 19–23 °C, concentrations remained above 90% of the initial value for 3 days. However, we observed that degradation occurred faster in glass containers than in polypropylene flasks. Therefore, we only studied the influence of light exposure and temperature in the polypropylene flasks.

We found that the stability of amphotericin B is temperature dependent. At 4 °C, amphotericin B was stable throughout the 15-day survey; all concentrations remained above 90% of the initial value. At room temperature (19–28 °C with and without light exposure) losses of more than 10% of the initial concentration of amphotericin B occurred after 3 days. No differences occurred between electric- and sunlight exposures. At 37 °C, the duration of stability was 2 days.

Table 2
Stability of amphotericin B (7.4 mg/ml) in antifungal mouthrinse under different conditions of storage

Flask no.	Initial drug concentration[a] (%)	% of the initial concentration[a]					
	T0	Day 2	Day 3	Day 4	Day 7	Day 10	Day 15
19–23 °C in polypropylene (1) and glass (2) with electric light exposure							
1	100.0 ± 2.7	104.7 ± 1.4	97.1 ± 4.0	91.4 ± 1.6	89.7 ± 2.8	87.6 ± 4.2	82.1 ± 2.5
2	100.0 ± 3.1	92.2 ± 1.4	95.3 ± 3.7	87.8 ± 3.4	82.2 ± 0.9	79.4 ± 1.5	77.6 ± 2.9
4 °C in polypropylene							
3	100.0 ± 4.3	100.5 ± 2.9	95.9 ± 3.4	96.0 ± 3.0	95.6 ± 0.5	92.9 ± 2.7	96.4 ± 2.5
19–23 °C in polypropylene with light protection							
4	100.0 ± 1.2	93.3 ± 2.0	90.9 ± 0.6	88.4 ± 2.8	86.9 ± 4.0	85.3 ± 1.3	83.1 ± 3.0
37 °C in polypropylene with light protection							
5	100.0 ± 0.8	95.9 ± 1.8	94.1 ± 4.4	89.7 ± 0.5	87.4 ± 0.5	85.3 ± 4.5	88.1 ± 3.0
25–28 °C in polypropylene with sunlight exposure[b]							
6	100.0 ± 4.5	97.6 ± 4.8	92.4 ± 1.7	91.0 ± 1.4	87.4 ± 3.2	83.4 ± 7.3	80.2 ± 5.4

[a] Mean ± S.D. ($n = 3$).
[b] 13 h/day.

Table 3
Stability of nystatin (4580.2 IU/ml) in antifungal mouthrinse under different conditions of storage

Flask no.	Initial drug concentration[a] (%)	% of the initial concentration[a]					
	T0	Day 2	Day 3	Day 4	Day 7	Day 10	Day 15
19–23 °C in polypropylene (1) and glass (2) with electric light exposure							
1	100.0 ± 1.5	101.3 ± 2.1	96.6 ± 2.5	99.6 ± 1.5	92.1 ± 2.6	86.2 ± 2.5	86.9 ± 2.3
2	100.0 ± 2.2	100.0 ± 1.4	105.1 ± 1.0	103.4 ± 2.2	92.0 ± 2.3	89.9 ± 1.1	89.9 ± 1.4
4 °C in polypropylene							
3	100.0 ± 3.7	94.3 ± 2.0	95.5 ± 3.2	93.6 ± 2.1	92.8 ± 1.5	92.4 ± 1.6	91.9 ± 0.1
19–23 °C in polypropylene with light protection							
4	100.0 ± 0.6	103.1 ± 3.9	103.0 ± 4.5	98.4 ± 1.2	90.1 ± 3.2	89.5 ± 2.1	88.0 ± 3.6
37 °C in polypropylene with light protection							
5	100.0 ± 4.1	100.1 ± 3.8	92.3 ± 1.9	87.3 ± 0.6	78.3 ± 0.8	72.4 ± 6.1	64.8 ± 2.1
25–28 °C in polypropylene with sunlight exposure[b]							
6	100.0 ± 0.9	101.4 ± 0.6	93.6 ± 3.1	92.6 ± 2.0	91.3 ± 1.6	88.9 ± 1.8	87.6 ± 3.2

[a] Mean ± S.D. ($n = 3$).
[b] 13 h/day.

All admixtures showed a bi-exponential decay of the initial amphotericin B concentration with an initial half-life value of 1.6–2.9 days which could correspond to an adsorption of the drug on the container and/or the formation of degradation products. The half-lives of the terminal part of the curves ranged from 80 to 110 days.

3.2. Stability of nystatin

Results are given in Table 3. At room temperature with electric light exposure, nystatin was stable for 4 days, in both the polypropylene and glass containers, with most concentrations remaining close to 100%. At 4 °C in polypropylene, nystatin was stable throughout the 15-day study period. All concentrations remained above 90% of the initial value. At room temperature (19–23 °C with light protection) and at 25–28 °C with sunlight exposure, the concentrations of nystatin decreased below 90% of the initial value after 4 days. No significant differences in the stability occurred between electric- and sunlight exposures.

The mouthrinses stored at 37 °C were stable for only 3 days. The decrease in nystatin concentrations followed a bi-exponential decay with half-life values of 3.6 and 29 days.

3.3. Measure of pH and visual examination

The pH of all tested mouthrinses was initially 8 and did not differ by more than one decimal in any of the tested solutions over the period of time considered.

The amphotericin B suspension was initially slightly opaque and yellow to orange. At each sampling time, visual examination of admixtures showed no detectable change in colour or odour and no visible microbial growth when stored at 4 °C, 19–23 °C with or without electric light exposure or 37 °C with light protection. All samples were easily resuspended at each test interval.

A change in colour was observed for the mouthrinses exposed to sunlight. From day 7, the suspension turned brownish and a flocculation appeared in the solution.

Mouthrinses containing nystatin were slightly opaque and yellow at the beginning of the study. The colour of all tested admixtures did not change during the study period and there was no precipitation. Exposure to electric- or sunlight made no difference.

4. Discussion and conclusion

Mouthrinses are used in the prevention and treatment of candidiasis infections in cancer patients [9]. Nystatin and amphotericin B mouthrinses in 1.4% sodium hydrogen carbonate are widely used in clinical practice without any addition of stabilizers. Since they are prepared extemporaneously, it was important to determine the stability of these preparations in a clinical setting and for the patients at home. The problem we encountered was the little amount of available data in the literature concerning the stability of these two drugs under those conditions. Data were found but mainly concerned the influence of stabilizers on the stability of nystatin or amphotericin B [5,7,8]. Moreover, in the work published by Vermerie et al. [7], the stability of nystatin in mouthrinses was evaluated by using microbiological assays.

We observed that amphotericin B and nystatin in sodium hydrogen carbonate are stable in polypropylene bottles at 19–28 °C for 3 and 4 days, respectively. They were more stable at 4 °C (15 days) without any modification of colour. For nystatin the stability at 4 °C was higher than the 7 days reported by Vermerie et al. [7]. However, the concentration of nystatin used by these authors, was three times higher (14,400 IU/ml) than in our study. Moreover, light did not affect the stability of the solutions in a detectable way. Such results are of great importance for clinical practice and for the patients using mouthrinses to prevent or treat drug-induced stomatitis. The ranges of pH values recommended to optimize nystatin stability and antifun-

gal activity were 5–7 and 6–8, respectively [10]. A pH of 8 did not seem to alter the stability of nystatin when compared to the results published by Vermerie et al. [7].

In conclusion, since nystatin and amphotericin B in association with sodium bicarbonate have the same indication and antifungal activity when stored at room temperature with or without light protection, nystatin proves more advantageous than amphotericin B. Today, at our centre, nystatin is no longer discarded after 3 days, thus limiting waste and preparation time. It will also allow us to provide patients with 4 days worth of preparation to take home.

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