Stability of Preservative-Free Tobramycin in Half-Normal Saline

Tobramycin is the aminoglycoside antibiotic most frequently prescribed for the treatment of pulmonary infections in patients with cystic fibrosis. Given the continued susceptibility of *Pseudomonas aeruginosa* isolates to tobramycin, it remains the most effective drug available for treating such infections. Tobramycin is frequently administered by aerosol. This approach allows delivery of the antibiotic directly to the lungs, while minimizing systemic exposure and the associated risk of ototoxicity and nephrotoxicity.

Formulations of the drug for inhalation should be within certain limits of pH, osmolality, chloride concentration, and antibiotic concentration, to ensure both airway tolerance and efficient nebulization.[1]

To ensure tolerability, a solution for inhalation should have osmolality between 150 and 550 mOsm/kg and a chloride concentration between 31 and 300 mmol/L.[1] High osmolality can produce cough or bronchoconstriction or both.[2] Conversely, a tobramycin solution in sterile water would be hypo-osmolar and could cause airway hyperreactivity, which would be manifested by either cough or bronchospasm. Similarly, an aerosol with a pH outside the physiologically acceptable range of 4.5 to 8.7 might produce cough or bronchoconstriction.[3] Parenteral tobramycin products that contain preservatives such as phenol and bisulfites can also cause airway irritation and bronchospasm if administered by inhalation.[4,5]

To avoid many of these problems, PathoGenesis Corporation now markets a preservative-free product called Tobi that contains 60 mg/mL of tobramycin in a solution with pH of 6.0, osmolality in the range of 158 to 183 mOsm/kg, and chloride concentration of 75 mmol/L.[6] However, this product is expensive. Alternatively, reconstitution of another preservative-free tobramycin formulation (Nebcin, Eli Lilly; 1.2-g pharmacy bulk vial) with half-normal saline (0.45% sodium chloride) should produce a solution with ideal inhalation characteristics (pH between 4.5 and 8.7, osmolality between 150 and 550 mOsm/kg, and chloride concentration between 31 and 300 mmol/L). However, the stability of this product is unknown.

The objective of this study was to evaluate, by means of a validated, stability-indicating liquid chromatographic method with mass spectrum detection, the stability of tobramycin over 6 months' storage at room temperature and in the refrigerator.

A stability-indicating liquid chromatographic method with mass spectrum detection for the parent molecular ion (M+1, m/z = 468) was validated before the study. Degradation products created by accelerated degradation of tobramycin at a pH of 1 and a temperature of 79°C were readily identified by differences in mass. The primary degradation products had m/z ratios of 135 and 155 and were tentatively identified as hydrolysis products. Validation demonstrated that tobramycin could be quantified accurately and reproducibly. On the basis of the mean of duplicate determinations of standards over the 5-day assay validation period, the maximum deviation from the nominal concentration of standards was 4.28% and the average deviation was 1.25%. The maximum deviation from the expected concentration for quality control samples was less than 7.50% and the average deviation was 2.51%. Analytical reproducibility within days (as measured by coefficient of variation) averaged less than 1.40% for each of the 6 standards. Similar reproducibility was observed for quality control samples.

On study day zero, each of twenty-four 1.2-g vials of Nebcin (lot 4MF91N, expiry August 1, 2003) were reconstituted with 15 mL of half-normal saline. Pairs of reconstituted vials were pooled, and the 12 resulting 30-mL samples, each containing 80 mg/mL of tobramycin, were stored in the original manufacturer's 50-mL vials. Six of these vials were stored at room temperature and 6 were stored at 4°C for the duration of the study. Immediately after preparation and on study days 4, 5, 7, 22, 28, 35, 42, 47, 63, 96, and 195, the vials were sampled and the tobramycin concentration determined. Physical inspection was also completed on each of the study days, and the pH was determined on days 0 and 195. Also on study day zero, three 1.2-g vials of Nebcin (lot 4MF91N, expiry August 1, 2003) were reconstituted with sterile water, half-normal saline, or normal saline (0.9% sodium chloride), respectively, and the resulting solutions were further diluted as necessary to prepare 40 and 80 mg/mL tobramycin samples. The osmolality of these 40 and 80 mg/mL tobramycin solutions was also determined to confirm the suitability of the formulation.

Solutions of Nebcin reconstituted with half-normal and normal saline all had pH, osmolality, and chloride concentration within the ideal ranges[1] (Table 1).

During the storage period, all study samples (80 mg/mL in half-normal saline) retained more than 90% of their initial concentration of tobramycin. Linear regression indicated that the amount remaining on day 195 was 96.5% of the initial concentration for samples stored at 4°C and 100.2% for samples stored at room temperature. This variation between days was within assay error. Samples were monitored for degradation products (m/z of 135 and 155) on study days 0 and

Table 1. Properties of Various Solutions of Tobramycin Immediately after Preparation

Tobramycin Concentration and Solvent	pH	Osmolality (mOsm/kg)	Chloride (mmol/L)
80 mg/mL			
Normal saline (0.9% NaCl)	6.85	380	154
Half-normal saline (0.45% NaCl)	6.91	251	77
Sterile H_2O	6.99	125	0
40 mg/mL			
Normal saline (0.9% NaCl)	6.82	334	154
Half-normal saline (0.45% NaCl)	6.77	203	77
Sterile H_2O	6.88	72	0
60 mg/mL			
Tobi (from PathoGenesis Corporation)*	6.01	158–183	75
Ideal	4.5–8.7	150–550	31–300

*Based on information in the product monograph.[6]

195. There was essentially no difference over time in the amount of degradation products found (less than 0.1% in all samples).

All solutions were initially clear and light yellow. Solutions stored at 4°C remained clear and light yellow for the duration of the study, whereas solutions stored at room temperature became slightly more yellow. No visible particles were observed in any solution during the study period. Throughout the study period there was no significant change in pH.

These results agree with the previously reported stability of tobramycin.[7-9] It is recommended that, after consideration of sterility and contamination rate in an IV additive admixture program, an expiry date not exceeding 195 days at 4°C or 25°C be established for 80 mg/mL solutions of tobramycin dissolved in half-normal saline. Such solutions are in the ideal range for pH, osmolality, and chloride concentration.

References

1. Weber A, Morlin G, Cohen M, Williams-Warren J, Ramsey B, Smith A. Effect of nebulizer type and antibiotic concentration on device performance. *Pediatr Pulmonol* 1997;23:249-60.
2. Eschenbacher WL, Boushey HA, Sheppard D. Alteration in osmolarity of inhaled aerosols causes bronchoconstriction and cough, but absence of a permeant anion causes cough alone. *Am Rev Respir Dis* 1984;129:211-5.
3. Godden DJ, Borland C, Lowry R, Higenbottam WA. Chemical specificity of coughing in man. *Clin Sci* (*Lond*) 1986;70:301-6.
4. Nikolaizik WH, Jenni-Galovic V, Schoni MH. Bronchial constriction after nebulized tobramycin preparations and saline in patients with cystic fibrosis. *Eur J Pediatr* 1996;155:608-11.
5. Gilligan PH. Microbiology of airway disease in patients with cystic fibrosis. *Clin Microbiol Rev* 1991;4:35-51.
6. PathoGenesis Corporation. Tobi (tobramycin solution for inhalation) technical monograph, international edition. Seattle (WA); 2000. Available (as PDF file) at: http://www.abouttobi.com/healthcareprofessionals/monograph.html (accessed 2001 Aug 24).
7. Walker SE, Madden MJ. Thermal stability of tobramycin in a tobramycin/phenylephrine inhalation solution. *Can J Hosp Pharm* 1986;39:92-5.
8. Krekorian LS. The effect of temperature and solution type on tobramycin stability [residency project]. Toronto (ON): University of Toronto; 1983.
9. Stiles ML, Allen LV Jr. Stability of nafcillin sodium, oxacillin sodium, penicillin G potassium, penicillin G sodium, and tobramycin sulfate in polyvinyl chloride drug reservoirs. *Am J Health Syst Pharm* 1997;54:1068-70.

Shirley Law, DipPharmTech
Research Assistant
Quality Control Laboratory
Department of Pharmacy
Sunnybrook and Women's College Health Sciences Centre
Toronto, Ontario

Acknowledgements
This study was funded by Eli Lilly Canada.