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References

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A Fluorescence Polarization Immunoassay Evaluated for Quantifying Astromycin, a New Aminoglycoside Antibiotic

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We evaluated a fluorescence polarization immunoassay (FPIA) procedure for measuring astromycin, a new aminoglycoside antibiotic. The calibration curve can be stored for at least 32 days without loss of accuracy. There were no interferences from hemoglobin (up to 4000 mg/L) or bilirubin (up to 200 mg/L), nor were there any cross-reactivities with amikacin, gentamicin, or tobramycin. Within-run precision (CV) for 20 replicates, each at concentrations of 5, 15, and 25 mg/L, was 1.25–2.11%. Between-run precision for these same concentrations, measured on five occasions, was 2.13–2.74%. Linear-regression analyses of data by FPIA (y) and HPLC (x) for plasma obtained from six healthy male volunteers who were given 200 mg of astromycin by 30-min intravenous infusion and for sera prepared by adding arbitrary amounts of astromycin showed highly significant correlations: r = 0.996 (n = 66), y = 0.96x − 0.48 mg/L and r = 0.981 (n = 59), y = 0.96x − 0.25 mg/L, respectively. This assay offers a rapid, efficient, and accurate system for therapeutic monitoring of astromycin in blood.

Additional Keyphrases: TDx system, HPLC compared - pharmacokinetics

Astromycin, a new aminoglycoside antibiotic developed in Japan, is expected to have a similar or better clinical efficacy for treating infections with Gram-negative bacteria, less nephrotoxicity than amikacin (1, 2), and less otoxicity than ribostamycin (3). The “therapeutic window” for astromycin concentration in plasma is reportedly almost the same as for amikacin, i.e., peak concentration 15–25 mg/L, trough concentration <5 mg/L (4).

Here we report our evaluation of the precision and accuracy of a fluorescence polarization immunoassay (FPIA) for astromycin in blood and compare results with those obtained by HPLC.

Materials and Methods

Assays. FPIA, a homogeneous immunoassay technique for drug measurement in serum or plasma, is based on the competitive binding between the drug in the patient’s sample and the tracer (drug labeled with fluorescein) for the antibody. For the present FPIA, we used the "TDx" system (Dainabot, Tokyo, Japan), an automated fluorescence polarization analyzer. The Dainabot reagent kits contained three vials: rabbit polyclonal antibody to astromycin, fluorescein bound to astromycin, and a pretreatment solution that is used to release the drug from protein. Six calibrators (0, 4, 10, 18, 30, and 50 mg/L) were used to construct the standard curve. Low (5 mg/L), medium (15 mg/L), and high (25 mg/L) controls were included in the daily assay runs. Emission and detection wavelengths were 485 nm and 525 nm, respectively. HPLC assay was performed according to the methods reported elsewhere (5). In brief, plasma samples or their dilutions were introduced into a CM-Sephadex C-25 mini-column and washed twice with 2-mL portions of sodium acetate buffer (pH 7, 0.4 mol/L). Drug adsorbed onto the column was eluted three times with 0.5 mL of 0.4 mol/L sodium acetate containing 10 mmol of sodium hydroxide (pH 11); 0.5 mL of micromycin sulfate solution (1 mg/L) was added as internal standard, and the mixture was diluted to 5 mL with the solution used for the mobile phase. We used a 150 × 6 mm (i.d.) column packed with YMC-PACK AM-312 (octadecysilane; Gasukurokogyo Inc., Tokyo, Japan). The mobile phase consisted of 28.5 g of anhydrous sodium sulfate, 1 mL of glacial acetic acid, 20 mL of paired-ion chromatography reagent (PIC-B7; Waters Associates, Milford, MA) and 50 mL of mercaptoethanol in 929 mL of distilled water (flow rate: 1 mL/min). For fluorometric detection after post-column reaction with o-phthalaldehyde.
at 45 °C we used emission and detection wavelengths of 340 nm and 430 nm, respectively.

Samples. Six healthy male volunteers [ages 23–44 (mean 34.7) years; weights 57.0–86.0 (mean 68.3) kg] with normal renal function participated in a pharmacokinetic study of astromicin after giving their informed consents. They were administered 200 mg of astromicin as an intravenous infusion during 30 min. Blood (5 mL) was sampled from each subject into a heparinized tube before (0 h), and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 12 h after this administration. The plasma was separated and stored at −70 °C until analyzed. At this same time we analyzed 59 serum samples that we had prepared by adding arbitrary amounts of astromicin to drug-free sera to give concentrations within the range 0 to 50 mg/L.

Data analysis. Concentrations of astromicin in the plasma from each subject were fitted to a two-compartment open pharmacokinetic model with use of a nonlinear least-squares method (LEAST) (6).

Results

Analytical Variables

Standard curve. The stability of a standard curve was determined as follows: The curve was constructed and stored on day 0 and the same controls (5, 15, and 25 mg/L) were measured as samples every four days up to 32 days, the stored standard curve being used. The controls were stored at <2 °C in the refrigerator throughout the study. Concentrations measured from day 0 through day 32 were all within ±10% of the expected values.

Dilution study. The linear assay range was determined by diluting the stock controls (5, 15, and 25 mg/L) with the buffer solution by two- to 16-fold. The results showed a linear relationship between assay response and drug concentration from 0.31 to 25 mg/L. The smallest concentration detectable was considered to be =625 µg/L, because the CV of five measurements at 625 µg/L was 10%, whereas that at 310 µg/L was 40%.

Analytical recoveries. Four serum samples for each of the three astromicin concentrations (5, 15, and 25 mg/L) were prepared by adding astromicin to drug-free sera. These samples were measured in duplicate with the TDx system and recoveries were calculated to be: 103.5 ± 1.9% (CV = 1.85%) for 5 mg/L, 102.3 ± 1.3% (CV = 1.22%) for 15 mg/L, and 101.3 ± 1.3% (CV = 1.24%) for 25 mg/L.

Interferences. The effects of bilirubin and hemolysis on FPIA were determined by adding unconjugated bilirubin and hemoglobin (both from Sigma Chemical Co., St. Louis, MO) to separate astromicin controls and assaying. Neither the former up to 4000 mg/L nor the latter up to 200 mg/L interfered with determination of astromicin concentrations by FPIA.

Cross-reactivity. The cross-reactivities of the astromicin reagents with other aminoglycosides used frequently—amikacin, gentamicin, and tobramycin—were evaluated by measuring the controls (5, 15, and 25 mg/L) of the respective FPIA systems for each of the aminoglycosides (Dainabot). At all concentrations we saw no cross-reaction of astromicin reagents with the other aminoglycosides.

Precision. Data that reflect the within-run precision for 1.0-µL aliquots of 50-µL samples of plasma were obtained by repeated analyses at 5, 15, and 25 mg/L (20 times at each concentration). As shown in Table 1, the CVs for these measurements ranged from 1.25% to 2.11%. Between-run precision was determined on five occasions by measuring three controls after constructing a standard curve on each occasion. The CVs ranged from 2.13% to 2.74% (Table 1).

Correlation study. The quantitative performance of the FPIA for astromicin was compared with that of an established HPLC assay. Linear regression analyses of the results were performed and highly significant correlations were observed between astromicin concentrations as measured by FPIA (y) and HPLC (x): y = 0.966 x + 0.19 mg/L for plasma samples obtained from healthy subjects and y = 0.96 x − 0.48 mg/L for sera supplemented with astromicin.

Pharmacokinetics in Healthy Subjects

The time–concentration curves for astromicin in each of six subjects after administration of the drug (200 mg, as an intravenous infusion during 30 min) fit well with a two-compartment open pharmacokinetic model. The pharmacokinetic parameters obtained were similar to the values reported elsewhere (5), as follows (mean ± SD): elimination constant at distribution phase (a) = 0.33 ± 1.57 h−1, that at elimination phase (b) = 0.437 ± 0.0666 h−1; intercompartment transfer constants k12 = 1.08 ± 0.745 h−1, k21 = 1.87 ± 0.772 h−1, volume of distribution of central compartment (V1) = 0.146 ± 0.0396 L/kg, and that at steady-state (Vss) = 0.212 ± 0.0229 L/kg.

Discussion

The total body clearance (Clb) of an aminoglycoside antibiotic is known to correlate well with the renal function expressed as endogenous creatinine clearance (Clcr), and the individualized dosage strategy with regard to the drug according to the renal function is recommended to achieve an optimal therapeutic effect (7). To re-adjust the dosage of the drug to optimum for an individual more precisely, therapeutic drug monitoring is essential (9). For astromicin, the Clb of the drug has been determined to be almost the same as Clcr of the patient (unpublished observation), by using the population pharmacokinetic analysis with NONMEM (9).

Several methods have been developed for measuring the concentrations of aminoglycosides in blood and other biological specimens: microbiological assays, enzymatic assays, radioimmunoassays, HPLC, and FPIA. Microbiological assays are inexpensive and can be performed in most laboratories without special equipment, but they are inherently unspecific, leaving much to be desired, perhaps because they are open to error at many steps (10). From the viewpoint of environmental pollution the method involving use of radioisotope is less desirable.

| Table 1. Within- and Between-Run Precision of the FPIA for Astromicin |
|-----------------|-----------------|---|
| Stated          | Measured, mean ± SD | CV, % |
| Within-run      |                 |     |
| 5.0             | 4.85 ± 0.10     | 2.11 |
| 15.0            | 14.43 ± 0.19    | 1.32 |
| 25.0            | 23.38 ± 0.29    | 1.25 |
| Between-run     |                 |     |
| 5.0             | 5.20 ± 0.14     | 2.74 |
| 15.0            | 14.90 ± 0.33    | 2.24 |
| 25.0            | 23.89 ± 0.51    | 2.13 |

*Each control serum measured 20 times.
*Each control serum measured in five different assay runs. For each run, the calibration curve was newly constructed.

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In this study we evaluated and compared FPIA with HPLC assay for measuring astromycin. The within- and between-assay precision of the FPIA are both excellent, with CVs of 1.25–2.11% and 2.13–2.74%, respectively. Results with the two assay methods correlated significantly. The FPIA offers advantages over the other method in its rapidity and simplicity. Moreover, this technique does not seem to be affected by either hemolysis, hyperbilirubinemia, or concomitant uses of other aminoglycoside antibiotics.

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References

Patterns of Lactate Dehydrogenase Isoenzymes in Serum of Patients with Acute Pulmonary Edema

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Total lactate dehydrogenase (LD; EC 1.1.1.27) activity in serum and proportions of LD isoenzymes were quantified on admission and discharge in 170 selected (from 240) patients with acute pulmonary edema (APE). The patients were divided into group A, 75 patients with normal LD values (<225 U/L); and groups B–E, with increased LD activity in serum: group B, 40 patients with increase in the proportion of LD-3 (>38%); group C, 12 patients with increased LD-5; group D, 36 patients with an isomorphic pattern of LD isoenzymes; and group E, seven patients with LD-1/LD-2 >0.76. Nine patients in group C (75%) had also signs of right-sided congestive heart failure, 30 in group D (83%) had hypotension on admission, and six in group E (86%) had signs of recent myocardial infarction. Evidently, half of patients with APE may show increased total LD activity in serum at the time of admission. LD isoenzyme proportions should be determined in such patients, because there is no one typical pattern of LD isoenzymes and some LD isoenzyme patterns may be associated with specific clinical situations.

Additional Keyphrases: congestive heart failure · acute myocardial infarction · shock

An enzyme abnormality commonly seen in patients with congestive heart failure is increased lactate dehydrogenase (LD; EC 1.1.1.27) activity in serum and disproportionately high isoenzyme LD-5, probably as a result of passive liver congestion and cellular hypoxia (I–5). Acute pulmonary edema (APE) is a part of the clinical entity of congestive heart failure in which the lungs are the main organ involved. In lung tissue the most prominent LD isoenzymes are LD-3 and LD-4 (6).

The aim of our study was to determine total LD activity and LD isoenzyme proportions in serum of patients with APE, to define characteristic patterns of LD isoenzymes in APE, and to find whether a certain pattern has any clinical significance.

Patients and Methods

We studied 240 patients with APE admitted to our department. The presence of APE was confirmed by the presence of pulmonary raules and pulmonary vascular congestion on roentgenography. The patient population included 170 men and 70 women. The mean age on admission was 72 years (range 60–85 years). Laboratory data for each patient included liver-function tests (bilirubin, alkaline phosphatase, aspartate and alanine aminotransferases, and proteins), creatine kinase (CK), and CK isoenzymes. Blood was sampled from each patient on admission and at discharge, for assay of LD activity and isoenzymes. Blood