Spectrofluorimetric method for aminoglycoside antibiotics

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This study presents a chemical method for the quantitative determination of aminoglycoside antibiotics in biological fluids. The method is based on an ion-exchange chromatographic separation of aminoglycosides in serum and urine. The clearcut separation of aminoglycosides is achieved by eluting the column with sulphuric acid solution. The aminoglycosides thus separated are determined by a modification of the procedure reported by Pesez & Bartos (1967).

Antibiotics. Tobramycin sulphate (Biogal), neomycin sulphate (Roussel Uclaf), sisomicin sulphate (Shering Corporation), kanamycin sulphate (USSR), amikacin (Bristol Laboratories).

For the column chromatography Amberlite IRC 50, was used. The resin was treated successively with 2 M hydrochloric acid (5 to 6 times volume of the resin) and distilled water and then was converted to the Na-form by eluting with 1 M sodium hydroxide. After this the resin was washed with distilled water to neutral pH. The resin thus prepared was kept refrigerated.

Reagents. All chemicals used were of analytical grade.
A. Reagents for ion-exchange chromatography. 1. Sulphuric acid, 0·1 M aqueous solution. 2. Sulphuric acid, 0·5 M aqueous solution.
B. Reagent for quantitative determination. 1. Acetylacetone. 2. Formaldehyde, 30 w/v aqueous solution.

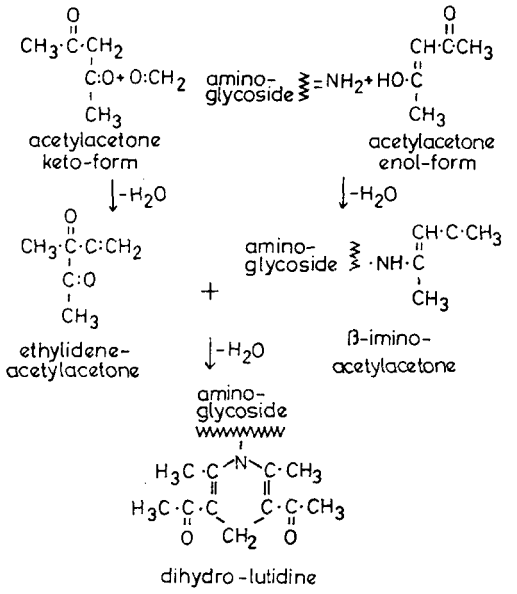

FIG. 1. Reaction sequence for spectrofluorimetric determination of aminoglycoside.

3. Britton-Robinson buffer (pH = 2·6 preparation: Solution I: 0·2 M phosphoric acid, 0·2 M acetic acid and 0·2 M boric acid. Solution II: 1·0 M sodium hydroxide. To 100 ml solution I was added 15 ml solution II). To 10 ml of the buffer, 0·8 ml acetylacetone and 2·0 ml formaldehyde were added. After shaking the mixture the volume was made up to 30 ml with buffer.

Aminoglycoside determination. Aminoglycosides can be determined spectrofluorimetrically by means of a fluorescent dihydro-lutidine derivative developed by condensation of the primary amino groups with acetylacetone and formaldehyde, under acidic conditions (pH = 2·6).

Separation of aminoglycoside antibiotics from biological fluids. A glass column (13·9 mm diam. i.d. × 290 mm length Labor MIM, Hungary) was packed with 3 ml of Amberlite IRC 50 in the Na-form as described above. Human urine (2 ml), or human serum (5 ml), diluted to 10 ml with distilled water was applied to the column. First 20 ml of distilled water was applied to the column, and then the impurities were eluted with 20 ml of 0·1 M sulphuric acid. The aminoglycoside antibiotics were eluted with 20 ml 0·5 M sulphuric acid; 2·0 ml of this eluant was used for the determination of aminoglycoside antibiotics as described below. The elution here was about 0·5 ml min^{-1}.

Spectrofluorimetric procedure. Into a 15 ml glass test tube was placed a 2·0 ml sample of the eluate, 2·0 ml Reagent B was added and the mixture was heated for 10 min at 100 °C in a water bath. 10 min later

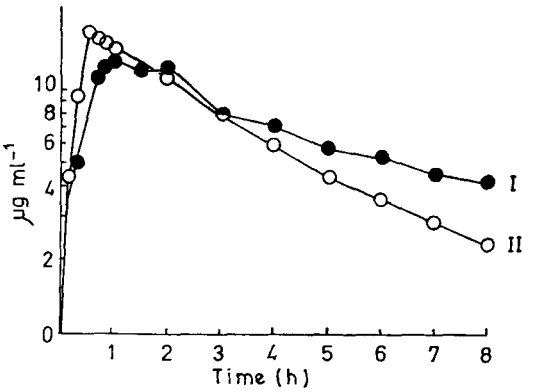

FIG. 2. Antibiotic concentration in serum, I and II representing different subjects.

emission was determined at 488 nm using a wavelength of 421 nm for excitation in a Hitachi MPF-4 fluorimeter with tubes with a 1 cm light path.

Calibration curves proved a linear correlation between the concentration of the drugs and the intensity of fluorescence.

The method was calibrated in the range of 0–5 μg ml^{-1} and had a sensitivity of 0·05 μg ml^{-1}.

In two patients receiving 1 mg kg^{-1} tobramycin, serum concentrations were determined every 10 min for 1 h after the injection of tobramycin and hourly up to 8 h. Fig. 2 shows the serum concentration curves.

June 26, 1978

REFERENCE

Pesez, M., Bartos, J. (1967) Talanta 14: 1097–1108

Physiological pharmacokinetics of β-lactam antibiotics: penicillin V distribution and elimination after intravenous administration in rats

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To clarify the physiological action and behaviour of a drug in man and to establish a dose scheduling for therapeutics, conventional pharmacokinetics based on the curve fits of the time-course of a drug concentration in blood have been widely used. The two-compartment open model is generally used for β-lactam antibiotics administered to man and other species (e.g. Dittert et al 1970; review by Nightingale et al 1975). However, due to the lack of anatomical or physiological meaning for transfer rate constants derived from this method, the time-course of antibiotic concentration in the particular target organ, under normal and diseased states, appears difficult to predict.

The present communication describes the physiologically based pharmacokinetics for penicillin V to predict tissue concentrations in rats. This approach by physiological perfusion model has been used to describe the pharmacokinetics of several drugs (for examples, Bischoff & Dedrick 1968; Bischoff et al 1971; Benowitz et al 1974; Harrison & Gibaldi 1977; Tterlikkis et al 1977) and has the intrinsic possibility of being scaled up for application to man from animal results.

Scheme 1 represents the flow diagram of various compartments used in the present analysis. This model assumes that (1) each tissue acts as a well-stirred compartment, (2) the antibiotic distribution is limited by the blood flow rate, and (3) tissue-to-blood concentration ratio of penicillin V is independent of the antibiotic concentration. A typical mass balance equation is given for the total drug in the liver:

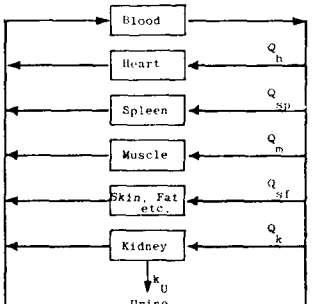
$$V_l\frac{dC_l}{dt} = (Q_l - Q_g)C_b - Q_l\frac{C_l}{K_l} + Q_g\frac{C_g}{K_g} - \frac{T_B(C_l/K_l)}{K_B + (C_l/K_l)} - \frac{T_M(C_l/K_l)}{K_M + (C_l/K_l)} \quad \ldots \quad (1)$$

where $V_l(dC_l/dt)$ is the accumulation of the drug in the liver, $(Q_l - Q_g)C_b$ is the rate of inflow with blood, $Q_l(C_l/K_l)$ and $Q_g(C_g/K_g)$ are the rates of outflow from the liver and inflow from the gut wall, respectively, with blood, and the last two terms represent capacity-limited biliary secretion and metabolism possibly occuring in the liver.

The mass balance equation for the blood is written by:

$$V_b\frac{dC_b}{dt} = M\,I(t) + Q_h\frac{C_h}{K_h} + Q_{sp}\frac{C_{sp}}{K_{sp}} + Q_m\frac{C_m}{K_m} + Q_{sf}\frac{C_{sf}}{K_{sf}} + Q_k\frac{C_k}{K_k} + Q_l\frac{C_l}{K_l} - (Q_h + Q_{sp} + Q_m + Q_{sf} + Q_k + Q_l)C_b \quad \ldots \quad (2)$$

where b, h, sp, m, sf, k, and l signify blood, heart, spleen, skeletal muscle, skin-fat, kidney, and liver, respectively, V_i is the compartment volume, C_i is the total antibiotic concentration bound and unbound to any protein, Q_i is the blood flow rate to the compartment, K_i is the tissue-to-blood partition coefficient, M is the total dose (μg), and I(t) is the injection function, which is a short pulse to simulate an intravenous injec-



Scheme 1. Pharmacokinetic model for distribution and elimination of penicillin V in the rat.

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