Comparison of Gentamicin \(C_1\) and \((C_{1a}, C_2)\)-Levels in Patients

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Summary. A high performance liquid chromatographic (HPLC) assay method has been used to measure gentamicin serum concentrations in patients receiving gentamicin complex. The HPLC method resolves gentamicin \(C_1\) from the other two components, \(C_{1a}\) and \(C_2\); gentamicin \(C_{1a}\) and \(C_2\) co-chromatograph. In the analysis of 46 serum samples collected from 16 patients it was found that the mean ratio (PHR) of the peak height of gentamicin \(C_1\) to the height of the peak due to components \(C_{1a}\) and \(C_2\) was 0.53 ± 0.05; this value agreed well with the PHR's usually found from the HPLC analysis of aqueous solutions of gentamicin complex or of gentamicin dosage forms. In an additional two patients, the HPLC analysis of a sample of the gentamicin dosage form administered, a urine sample, and serum samples, resulted in almost identical PHR's for the respective patients. Finally, similar results were obtained from an experiment in a rabbit. It was concluded that the disposition of all three components of gentamicin complex are the same or very similar.

Key words: Gentamicin \(C_1\), \(C_{1a}\), and \(C_2\), disposition in patients, quantitative high pressure liquid chromatography.

Gentamicin, a broad spectrum antibiotic, has been shown to consist of three active components which have been designated \(C_1\), \(C_{1a}\), and \(C_2\) (Wilson et al., 1973). In a recent study (Mosegaard et al., 1975) the efficacy, tolerance and pharmacokinetics of gentamicin and gentamicin \(C_1\) were compared. Thirty elderly male patients were divided in a prospective randomized fashion into two groups of 15 patients. All patients received gentamicin or gentamicin \(C_1\) by intravenous injection according to the same dosage regimen. Biological fluid samples were assayed for antibiotic activity by a microbiological method. The results of that study indicated that the use of gentamicin may result in a greater incidence of renal function impairment than the use of gentamicin \(C_1\) alone. In addition, it was suggested that substantial differences existed between the two entities with respect to pharmacokinetic profiles. Serum concentrations of gentamicin were generally higher than those for gentamicin \(C_1\). The volume of distribution gentamicin \(C_1\) (25 ± 1.5% of body weight) was 47% greater than that of gentamicin (17 ± 1% body weight). The serum clearance of gentamicin \(C_1\) (55 ± 7 ml/min) was 72% greater than that of gentamicin (32 ± 3 ml/min). From these data one may conclude that the pharmacokinetics of the gentamicin components are not the same.

It is the purpose of this paper to present data which suggests that the pharmacokinetics of the gentamicin components are the same or very similar.

Materials and Methods

Human Subjects

Subjects were 18 patients in the University hospital who were receiving gentamicin complex (Garamycin Injectable®) either intramuscularly or intravenously for the treatment of infection. In total, 58 blood samples were collected during the course of their treatment with gentamicin for the purpose of measuring the serum concentration of gentamicin. Three
patients had one blood sample collected each, while for all other patients multiple samples were collected at various times during the course of administration of gentamicin. For each of two patients an aliquot of urine was collected during the multiple dose treatment with gentamicin. In addition, for these two patients, an aliquot of the gentamicin dosage form administered to the respective patients was retained for HPLC analysis.

**Rabbit Study**

Gentamicin sulphate, 200 mg in 5 ml 0.9% sodium chloride solution, was injected as a bolus into the medial vein of one ear of a 4 kg albino rabbit. Blood samples (0.5 ml) were collected from the marginal vein of the other ear before drug administration and at various intervals for a total of 5 h thereafter. The separated plasma from each sample was stored in a freezer pending drug analysis.

**Gentamicin Assay**

A slightly modified, recently developed HPLC assay (Peng et al., 1977) was used to quantitate gentamicin in the serum, urine, and gentamicin dosage forms. Samples of urine, collected from two patients, the dosage forms administered to those patients, and the plasma samples collected from the rabbit up to 2 h after gentamicin administration, were diluted prior to analysis to yield concentrations of gentamicin less than 20 μg/ml.

It is appropriate to give a brief description of the HPLC assay method. To a 0.1 ml aliquot of the sample to be analyzed, contained in a culture tube, were added 0.9 ml of basified phosphate buffer, and 2.5 ml of acetonitrile. After vortex mixing for 10 sec and centrifugation (2,000 rpm) for 1 min all the clear supernatant solution was directly poured into another culture tube which contained 2 ml of methylene chloride. After vortexing (10 sec) and centrifugation (1 min), 0.5 ml of the separated upper aqueous layer was pipetted into a new culture tube and mixed with 0.3 ml acetonitrile containing 4.0 mg of dansyl chloride. The culture tube was screwcapped and incubated in a water-bath at 75°C in darkness for 5 min and then cooled in ice water. Ethyl acetate (0.5 ml) and 6 ml carbonate buffer (pH 9.5) were added to the cooled reaction mixture, and the tube was then vortexed for 10–15 sec and centrifuged (1 min). An aliquot (5 μl) of the ethyl acetate phase was injected into the HPLC system. A reverse phase (μ-Bondapak C18®) column, and a mobile phase of acetonitrile: distilled water (95:5) at a flow rate of 1 ml/min were used for the chromatography. A fluorescence detector was used to monitor the column effluent.

**Results and Discussion**

The nature of the HPLC method for the analysis of gentamicin is such that it is possible to quantitate the gentamicin component C1 in a sample which contains the gentamicin complex. The chromatogram resulting from the HPLC analysis of a serum sample collected from a patient who was receiving gentamicin complex is shown in Figure 1. Two peaks attributed to gentamicin are observed with retention times of 5.1 min and 5.9 min. The analysis of spiked samples which contained the individual gentamicin components (C1, C1α, or C2) showed that the first peak in the analysis of samples which contained the gentamicin complex was due to components C1α and C2, while the second peak was due to component C1 (Peng et al., 1977). For the gentamicin complex the ratio of the peak height of the second peak to that of the first peak (PHR) is usually close to 0.55. Different batches of gentamicin complex may contain different proportions of the individual gentamicin components (Wilson et al., 1973). As a result the PHR from the HPLC analysis of different batches of gentamicin