Journal of
Bioequivalence & Bioavailability
ISSN: 0975-0851

Abu-Basha et al., J Bioequiv Availab 2013, 5:3
DOI: 10.4172/jbb.1000147

Research Article Open Access

Pharmacokinetics of Gentamicin C_1, C_{1a}, C_2 and C_{2a} in Broiler Chickens after IV, IM, SC and Oral Administration

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Abstract

The pharmacokinetics and bioavailability of four major gentamicin components (C_1, C_{1a}, C_2 and C_{2a}) in chicken plasma administered at 5 mg/kg body weight by different routes of administration (IV, IM, SC and oral) was determined using reversed-phase high performance liquid chromatography (RP-HPLC) and pre-column derivatization with Phenylisocyanate (PIC). All the components, except for C_{1a} were well absorbed (bioavailability of 60% or greater) following administration by the IM and SC routes. The bioavailability of C_{1a} was 58% and 35% following IM and SC administration, respectively. The apparent volume of distribution (V_{ss} and Vd_{area}) for the C_1 component was significantly smaller than for any of the other components individually or combined. In addition, the C_1 component had a significantly shorter t½β and MRT following intravenous administration and a higher C_{max}/Dose following intramuscular administration. This study showed significant differences in some pharmacokinetics parameters between four gentamicin components (C_{1a}, C_{2a}, C_1 and C_2) after administration of single mixture of gentamicin by different routes in chickens. The differences may have clinical and toxicological implications, and could explain the high variation in total gentamicin pharmacokinetics.

Keywords: Gentamicin components; Chickens; Pharmacokinetics; Bioavailability

Introduction

Gentamicin is a broad-spectrum bactericidal aminoglycoside antibiotic, produced by fermentation of *Micromonospora purpura* or *M. echinospora*. It is effective against wide variety of serious bacterial infections caused by susceptible gram-negative and some gram-positive aerobic bacteria [1,2]. Gentamicin is not a uni-molecule but a complex mixture of four major components, designated as C_1, C_2, C_{1a}, C_{2a}, and minor ones like C_{2b}. The components differ in their degree of methylation on the purpursamine ring [1].

It has been recognized that there is a wide variation in the major component ratio between different pharmaceutical gentamicin preparations [3,4] and therefore, the composition of the final product can vary considerably. Proportions of the different components in most commercial preparations fall within limits that are set and mentioned by the US pharmacopoeia [5] is 25-50% for C_1, 10-35% for C_{1a} and for sum of C_2 and C_{2a} are 25-55%. The British pharmacopoeia [6] limits are 25-50% for C_1, 15-40% for C_{1a}, and 20-50% for sum of gentamicin C_2 and C_{2a}. European Pharmacopoeia [7] determines the amount of C_1, C_{1a} and the sum of C_2 and C_{2a} were limited to 20-35, 10-30 and 40-60%, respectively.

Nephrotoxicity and ototoxicity are the most common side effects associated with the use of gentamicin. The severity of toxicity can vary depending on whether a single or multiple-daily administration plan is used [8]. Moreover, the available data reported remarkable differences in nephrotoxicity for gentamicin components in animals [9]. Consequently, the correlation between toxicity and pharmacokinetics of gentamicin components is important. Gentamicin C_1 has different disposition kinetics than the gentamicin complex when given separately to patients [10].

Several methods have been developed for determination of gentamicin. Only chromatographic methods are capable of identifying and quantifying the individual components of the gentamicin complex. Gentamicin has no UV or visible absorbing chromophores and therefore cannot be detected by traditional techniques without derivatisation [11,12]. This necessitates gentamicin derivatization to allow its detection with the required sensitivity. Either pre- or post- column derivatisation for fluorescence or UV detection can be implemented in this process. Gentamicin has been derivatised previously with O-phthalaldehyde (OPA), dansyl chloride, fluorescamine, 9-fluorenylmethyl chloroformate (FMOC-CI), 1-fluoro-2, 4-dinitrobenzene (DNFB) and 2,4,6-trinotrobenzenesulfonic acid (TNBS) [11].

The pharmacokinetics of individual gentamicin components were studied in dogs [13], turkeys [14] and the horse [15] after intravenous administration only. However, there is no available pharmacokinetics data in other species of animals including chicken. The purpose of this study was to determine and calculate the pharmacokinetics and bioavailability of four major gentamicin components in chicken plasma by different route of administration (IV, IM, SC and oral), using reversed-phase high performance liquid chromatography (RP-HPLC) and pre-column derivatization with phenylisocyanate (PIC).

Materials and Methods

Experimental animals

Fifty 2-2.5 kg body weight (bw) broiler chickens (Hubbard x

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Received March 16, 2013; **Accepted** April 15, 2013; **Published** April 22, 2013

Citation: Abu-Basha EA, Al-Shunnaq AF, Gehring R (2013) Pharmacokinetics of Gentamicin C_1, C_{1a}, C_2 and C_{2a} in Broiler Chickens after IV, IM, SC and Oral Administration. J Bioequiv Availab 5: 129-135. doi:10.4172/jbb.1000146

Hubbard) of 40-45 days old were used in this study. These chickens were purchased from a local poultry farm at 3 weeks old. They were placed in the Animal House at Jordan University of Science and Technology. The animals were monitored for 2 weeks for any apparent clinical signs and to ensure that they are free from antibiotics before drug administration. The Animal House temperature was maintained at 25 ± 2°C and humidity at 45-65%. All chickens had free access to water and antibacterial-free food.

Drug

Authentic standard powder of gentamicin sulphate with known amounts of gentamicin components (33, 25.5, 22 and 19.5% for C_{1a}, C_1, C_{2a} and C_2, respectively) was provided by North China Pharmaceutical Group (Hualuan Co. Ltd, Shijiazhuang, China), batch no. (040537). The drug (300 mg) was dissolved in sterile distilled water to a total volume of 15 ml to give a final concentration of 20 mg/ml prior drug administration.

Peak assignment

Peak assignments were made by elution of gentamicin sulphate standard solutions. The relative proportion described from the standard compositions has permitted peak identification. Gentamicin C_{1a}, C_1, C_{2a} and C_2 were identified by comparison of their retention time with those identified previously [16].

Chemicals and reagents

All the chemicals employed were of analytical grade. Acetonitrile and water were HPLC-grade (Frutarom, UK), Trifluoroacetic acid (TFA) and Triethylamine (TEA) were purchased from Scharlau, Spain. Phenylisocyanate (PIC) was purchased from Merck, Germany.

HPLC system

Chromatography was carried out on binary high pressure HPLC system (Shimadzu, Japan) which consisted of LC-10A DVP HPLC pump, SIL-10A DVP auto injector, SPD-10 AVP UV-vis detector, SCL-10 AVP system controller, DGV-12 A degasser, Shimadzu class-VP software Ver 6.12 SP4. Chromatographic separation was performed using Chromolith RP-18e (4.6 mm i.d. × 50 mm length, macropore 2 µm, mesopore 2 nm, Merck, Germany).

Experimental design

Chickens were individually weighed before drug administration and doses were calculated accordingly. The chickens were divided into 5 equal groups (10 chickens / group) in a parallel design. Chickens of group 1 did not receive any drug and served as a control group. Chickens of group 2, 3, 4 and 5 were received total gentamicin (5 mg/kg) body weight as a single IV, IM, SC and oral administration, respectively. Gentamicin was given in the right brachial vein, pectoral muscle, under the skin of the neck and directly by thin plastic syringe into the crop for IV, IM, SC and oral administration, respectively. Food was withheld for 12 h before drug administration and was offered 6 h after drug administration to exclude any influence of feed on the absorption of the drug. Water was given freely to all groups. Blood samples (1- 1.5 ml) were collected from the left brachial vein into heparinized tubes at 0 (pretreatment), 5, 15 and 30 min and 1, 2, 4, 6, 8, 12, 24 and 48 h after drug administration. The samples were directly centrifuged at ~1000 g for 10 min to obtain clear plasma and stored at –20°C until analysis.

Preparation of standard curve

Daily fresh calibration curves were prepared by dissolving dried gentamicin mixture powder in HPLC-grade water, in a measuring flask to obtain a concentration of 1000 µg/ml. The stock solution was added to HPLC-grade water or chicken plasma to produce 1, 10, 25, 50 and 100 µg/ml. Four calibration curves were made for each concentration according to their ratios. The calibration curves were obtained by plotting the peak height as a function of the respective concentrations for each component and the linear regression was calculated for each component.

Sample preparation

Concentrations of gentamicin components in plasma were assayed according to previously described method [16] with slight modification. The calibration and plasma samples of gentamicin were prepared by adding 200 µl of plasma to 300 µl of triethylamine solution (5 mg/ml in 90% acetonitrile and 10% water) to precipitate plasma protein. The mixture was shaken for 15 second by vortex mixer and centrifuged at ~1000 g for 5 min. The supernatant (400 µl) was transferred to clean glass tubes and 200 µl of phenylisocyanate (5 mg/ml in acetonitril) was added as derivatizing agent. The mixture was shaken by vortex mixer for 10 seconds and transferred to a shaker water-bath. The water path was set at 65°C for 30 min with slow shaking. The mixture was then transferred to Eppindorf tubes and centrifuged at ~1000 g for 5 min to ensure complete precipitation and clearness of the resultant. The clear supernatant was injected directly into the HPLC system using special glass vials.

Chromatographic condition

Gentamicin was eluted with a mobile phase consisting of acetonitrile-water (36:64, v/v) and 0.1% of triflouroacetic acid was added in aqueous acetonitrile. The mobile phase was filtered through a 0.45 µm membrane filter and degassed. The flow rate was performed at 2 ml /min and the UV detector set at a wavelength of 240 nm. The volume of injection was 100 µl.

HPLC methods for gentamicin C_{1a}, C_{2a}, C_1 and C_2 were validated by measuring the specificity, accuracy, precision, linearity, sensitivity and recovery in chicken plasma. The specificity of this method was assured since there were no interfering peaks present in chromatograms corresponding to the retention time of gentamicin –PIC derivatives. The accuracy of the method was 98.6, 100.2, 99 and 99.2% for gentamicin C_{1a}, C_{2a}, C_1 and C_2, respectively. The intra-day coefficients of variation (CV) for 4 major components ranged from 3.2 to 6, whereas, the inter-day CV ranged from 2 to 7. The limit of quantification (LOQ) was 0.3, 0.25, 0.25 and 0.2 µg/ml for gentamicin C_{1a}, C_{2a}, C_1 and C_2, respectively based on signal-to-noise ratio of 6:1. Moreover the mean percentage recoveries of gentamicin C_{1a}, C_{2a}, C_1 and C_2 from the plasma were 93%, 95%, 94% and 98%, respectively. Gentamicin derivatives C_{1a}, C_{2a}, C_1 and C_2 were stable during 24 h from preparation.

Pharmacokinetics analysis

The pharmacokinetic analysis of the data was performed using non-compartmental analysis based on statistical moment theory (SMT) according to previously described methods (Gibaldi and Perrier, 1982), with the help of a commercially available software program (WinNonlin®, Pharsight Corporation, Cary, NC, USA).

The parameters calculated were: area under plasma concentration-time curve (AUC) using linear trapezoid method; area under the first moment curve (AUMC); mean residence time (MRT), where MRT= AUMC/AUC; volume of distribution (Vd_{area}), where Vd_{area}=(dose/AUC × β); total body clearance (CLB), where CLB = dose/AUC; and apparent volume of distribution at steady state (V_{ss}), where V_{ss}=MRT × CLB. The absolute bioavailability (F) was calculated as (AUCnon IV/AUCIV) ×100. The maximum plasma concentration (C_{max}) and time to maximum concentration (T_{max}) following extravascular administration were determined empirically directly from the time-concentration curve. Doses of the individual components were calculated by multiplying the administered dose (5 mg/kg) by the percentage of the component contained in the gentamicin formulation. C_{max} and $AUC_{0-\infty}$ were normalized by dose prior to comparison.

Statistical analysis

One-way analysis of variance (ANOVA) was used to test the hypothesis of no differences between the average pharmacokinetic parameter values between the 4 major gentamicin components. If the means were different, a multiple comparison of the means was performed using the Fisher's Least Significant Difference test (LSD). The data were log-transformed prior to analysis, since they were not normally distributed on a linear scale. Some parameters (C_{max} and T_{max}) were not normally distributed, even after log-transformation, and the Kruskall-Wallis ANOVA on ranks and Tukey test were used for these data. The differences were considered significant when $P<0.05$. All data are expressed as the geometric mean ± SD of the log data, the latter being an approximation of the co-efficient of variation [17]. For C_{max} and T_{max}, the median, 25th percentile and 75th percentile are reported.

Results

A representative HPLC chromatogram of blank chicken plasma containing PIC and TEA without gentamicin and the separation of the gentamicin components in chicken plasma are illustrated in figures 1A and 1B, respectively. The calibration curves of gentamicin components, spiked in chicken plasma, were linear (data not shown). Close correlation with the linear regression equations were observed for all four components (r_2=0.999, 0.998, 0.998 and 0.997 for C_{1a}, C_{2a}, C_1 and C_2, respectively). The peak heights were proportionally related to gentamicin component concentrations.

The mean concentrations ± SE of gentamicin C_{1a}, C_{2a}, C_1, C_2 and total gentamicin (determined by summation of the concentrations of 4 major components) after single IV, IM and SC administration of a single dose of gentamicin (5 mg/kg bw) are shown in figures 2, 3 and 4, respectively. The pharmacokinetics parameters of gentamicin C_{1a}, C_{2a}, C_1, C_2, and total gentamicin following single IV, IM and SC administration of a single dose of gentamicin (5 mg/kg bw) are shown in tables 1, 2 and 3, respectively.

The apparent volume of distribution (V_{ss} and Vd_{area}) for the C_1 component was significantly smaller than for any of the other components individually or combined. In addition, the t½β and MRT were significantly shorter for C_1 following intravenous administration (Table 1). The data collected after intramuscular administration also suggests that C_1 has a smaller apparent volume of distribution (Vd_{area}/F). This is also the most likely reason for C_1 having a significantly higher C_{max}/Dose following intramuscular administration. All gentamicin components were rapidly and extensively absorbed following intramuscular and subcutaneous administration with the exception of C_{1a}, which had a bioavailability of 58 and 35%, respectively. This component also had a lower C_{max}/Dose and $AUC_{0-\infty}$/Dose as well as a higher Vd_{area}/F and CLB/F. Gentamicin was not detected in chicken plasma, after a single oral administration of gentamicin (5 mg/kg bw).

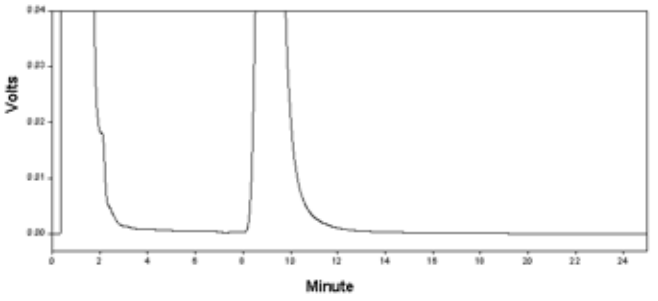

Figure 1a: A representative HPLC chromatogram of chicken plasma containing containing PIC and TEA without gentamicin.

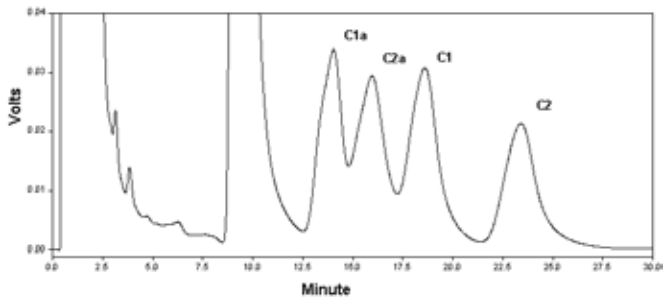

Figure 1b: A representative HPLC chromatogram of chicken plasma containing gentamicin (C_{1a}, C_{2a}, C_1 and C_2) showing the separation.

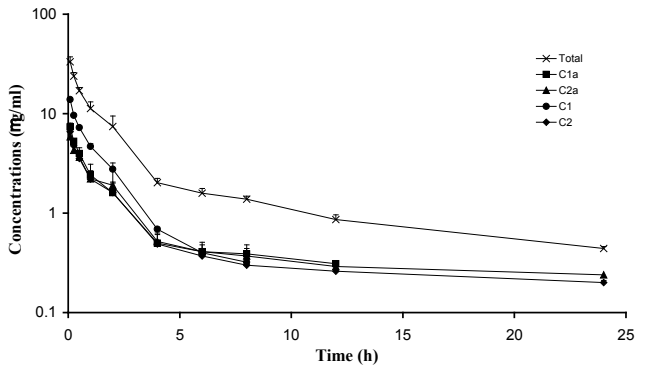

Figure 2: Semilogarithmic plot showing the plasma concentration-time profile of gentamicin C_{1a}, C_{2a}, C_1 and C_2 and total gentamicin after IV (5 mg/kg bw) administration. Values are mean ± SE (n=10).

Discussion

Gentamicin is a polarized water-soluble compound; it is excreted un-metabolized via the kidney and has very poor intestinal membrane permeability [12,18]. There are numerous reports of gentamicin pharmacokinetics in human and animals. With few exceptions, these consider gentamicin to be single molecule and describe the

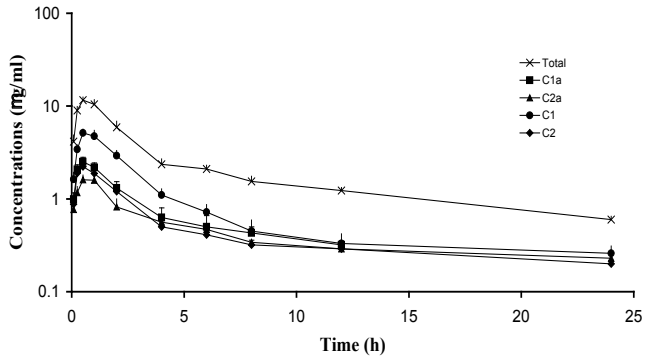

Figure 3: Semilograthimic plot showing the plasma concentration-time profile of gentamicin C_{1a}, C_{2a}, C_1 and C_2 and total gentamicin after IM (5 mg/kg bw) administration. Values are mean ± SE (n=10).

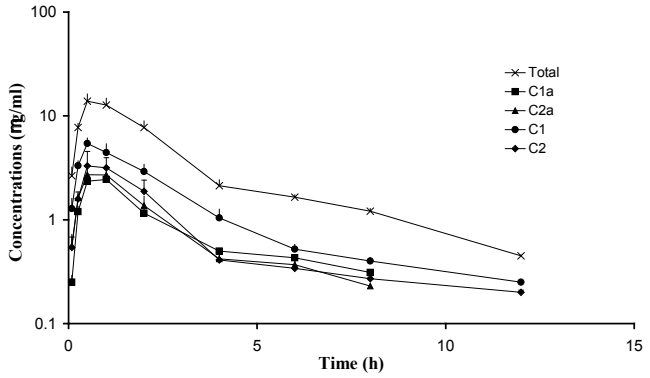

Figure 4: Semilograthimic plot showing the plasma concentration-time profile of gentamicin C_{1a}, C_{2a}, C_1 and C_2 and total gentamicin after SC (5 mg/kg bw) administration. Values are mean ± SE (n=10).

Parameters	C_1 (1.28 mg/kg)	C_{1a} (1.65 mg/kg)	C_2 (0.98 mg/kg)	C_{2a} (1.10 mg/kg)	Total (5.00 mg/kg)
$t_{1/2\beta}$ (h)	1.69 ± 0.56[a]	6.45 ± 1.12[b]	3.87 ± 0.62[b]	5.24 ± 0.90[b]	4.00 ± 0.49[b]
MRT (h)	1.66 ± 0.44[a]	6.76 ± 1.05[b]	4.02 ± 0.41[b]	6.05 ± 0.77[b]	3.37 ± 0.36[b]
Vd_{area} (ml/kg)	193.64[a] ± 0.45	741.74[b] ± 0.96	629.55[b] ± 0.47	627.66[b] ± 0.86	557.80[b] ± 0.41
V_{ss} (ml/kg)	132.42[a] ± 0.50	539.15[b] ± 0.87	453.50[b] ± 0.42	502.70[b] ± 0.70	325.06[b] ± 0.43
CL_B (ml/hr/kg)	79.60 ± 0.56	79.76 ± 0.49	112.73 ± 0.39	83.10 ± 0.46	96.35 ± 0.30
$AUC_{0-\infty}$ /Dose (µg.h.kg/ml/mg)	12.57 ± 0.56	12.54 ± 0.49	8.86 ± 0.39	12.04 ± 0.46	10.38 ± 0.30
Extrapolated AUC (%)	5 ± 0.66	31 ± 0.65	10 ± 0.45	25 ± 0.73	5 ± 0.69

[a,b] Different superscripts indicate statistically significant differences

Table 1: The pharmacokinetics parameters of gentamicin C_{1a}, C_{2a}, C_1, C_2, and total gentamicin following single IV administration. Values are geometric mean ± SE of the log-transformed data (n=10).

Parameters	C_1 (1.28 mg/kg)	C_{1a} (1.65 mg/kg)	C_2 (0.98 mg/kg)	C_{2a} (1.10 mg/kg)	Total (5.00 mg/kg)
$t_{1/2\beta}$ (h)	2.67 ± 0.76	3.39 ± 1.15	4.70 ± 0.63	6.61 ± 0.94	3.78 ± 0.35
MRT (h)	3.45 ± 0.54	4.85 ± 0.98	5.93 ± 0.53	8.76 ± 0.90	4.43 ± 0.24
Vd_{area}/F (ml/kg)	304.30[a] ± 0.57	674.52[b] ± 0.74	972.63[b,c] ± 0.50	1242.65[c] ± 0.62	679.26[b] ± 0.33
CL_B /F (ml/hr/kg)	79.04 ± 0.43	137.68 ± 0.90	144.46 ± 0.40	130.32 ± 0.68	124.59 ± 0.37
$AUC_{0-\infty}$ /Dose (µg.h.kg/ml/mg)	12.65 ± 0.43	7.26 ± 0.90	6.92 ± 0.40	7.68 ± 0.68	8.03 ± 0.37
Extrapolated AUC (%)	10 ± 0.69	23 ± 0.69	22 ± 0.37	40 ± 0.35	12 ± 0.64
Bioavailability (%)	101 ± 0.65	58 ± 0.88	78 ± 0.48	64 ± 0.79	77 ± 0.42
C_{max}/Dose Median (25th-75th percentile) (µg/mL)	4.48[a] (3.81-5.82)	2.49[b] (1.59-3.05)	1.94[b] (1.51-2.37)	1.73[b] (1.25-2.05)	2.51[a,b] (2.19-2.30)
T_{max} Median (25th-75th percentile) (h)	0.50 (0.50-1.00)	0.50 (0.50-1.00)	0.50 (0.50-0.50)	0.50 (0.50-1.00)	0.50 (0.50-1.00)

Values are mean ± SE (n=10)

[a,b] Different superscripts indicate statistically significant differences

Table 2: The pharmacokinetics parameters of gentamicin C_{1a}, C_{2a}, C_1, C_2, and total gentamicin following single IM administration.

Parameters	C_1 (1.28 mg/kg)	C_{1a} (1.65 mg/kg)	C_2 (0.98 mg/kg)	C_{2a} (1.10 mg/kg)	Total (5.00 mg/kg)
$t_{1/2}$ (h)	1.54 ± 0.86[a]	2.37 ± 0.65[a,b]	3.32 ± 0.59[b]	3.54 ± 0.45[b]	2.21 ± 0.27[a,b]
MRT (h)	2.35 ± 0.56[a]	3.26 ± 0.48[a,b]	3.81 ± 0.35[b]	4.09 ± 0.32[b]	2.71 ± 0.20[a,b]
Vd_{area}/F (ml/kg)	217.89[a] ± 0.77	785.25[b] ± 0.50	586.99[b,c] ± 0.24	603.05[b,c] ± 0.35	443.63[c] ± 0.40
CL_B/F (ml/hr/kg)	98.20[a] ± 0.48	229.29[b] ± 0.34	122.49[a] ± 0.53	118.04[a] ± 0.36	139.07[a] ± 0.34
$AUC_{0-\infty}$/Dose (µg.h.kg/ml/mg)	10.18 ± 0.48[a]	4.36 ± 0.34[b]	8.16 ± 0.53[a]	8.47 ± 0.36[a]	7.20 ± 0.34[a]
Extrapolated AUC (%)	11 ± 0.84	18 ± 0.44	15 ± 0.64	18 ± 0.56	3 ± 0.39
Bioavailability (%)	81 ± 0.66[a]	35 ± 0.65[b]	92 ± 0.52[a]	70 ± 0.43[a]	69 ± 0.29[a]
C_{max}/Dose Median (25th-75th percentile) (µg/mL)	4.74[a] (4.01-7.06)	1.79[b] (1.35-1.87)	2.81[a] (1.91-3.27)	2.78[a] (2.08-3.80)	2.85[a] (2.70-3.62)
T_{max} Median (25th-75th percentile) (h)	0.50 (0.50-1.00)	1.00 (0.50-1.00)	0.75 (0.50-1.00)	0.50 (0.50-1.00)	0.50 (0.50-1.00)

[a,b] Different superscripts indicate statistically significant differences

Table 3: The pharmacokinetics parameters of gentamicin C_{1a}, C_{2a}, C_1, C_2, and total gentamicin following single SC administration. Values are mean ± SE (n=10).

pharmacokinetics of total gentamicin only. It is interesting that gentamicin consists from 4 major components (C_{1a}, C_{2a}, C_1 and C_2) and other minors. The pharmacokinetics of individual gentamicin components has not been targeted by many researchers, but this does not detract from its importance. On the contrary, the pharmacokinetics of gentamicin components has been a subject of interest due to clinical and toxicological considerations. The study of the individual components has been hampered by the lack of a suitable calibrated method of detection and the analytical problems associated with gentamicin derivatization.

Direct UV detection of gentamicin is not possible because gentamicin has no UV or visible chromophores and cannot be detected by traditional techniques [19,20]. Therefore, derivatization of gentamicin to allow its detection with suitable sensitivity is necessary. Pre-column derivatization with a suitable fluorescent reagent allows for the simplest, accurate and most sensitive analysis. Derivatization of gentamicin by O-phthalaldehyde (OPA) [21], dansyl chloride [21], fluorescamine [22], 9-fluorenylmethyl chloroformate [1], 1-fluoro-2, 4-dinitrobenzene [21] and 2,4,6-trinitrobenzenesulfonic acid [23,24] were reported. However, these reagents are with drawbacks related to reagent stability, time consuming and detection sensitivity. For example, OPA is unstable and the elution of the OPA derivatives has been found to affect by the concentration of inorganic cations in the HPLC mobile phase [19,11].

In our previous study, the concentrations of gentamicin in chicken plasma after IV, IM, SC and oral administration were determined using microbiological assay [25]. This bioassay method is simple and inexpensive, but unable to quantify of the individual components of gentamicin [12,25]. High correlation (r_2=0.97) was found between HPLC and bioassay methods in determining the mean plasma gentamicin in chickens. However, there were significant differences in some pharmacokinetics parameters when HPLC and bioassay were compared [25].

We modified a simple and rapid liquid chromatographic method for determination of gentamicin components in plasma using Phenylisocyanate (PIC) as pre-column derivating reagent. This method shows good specificity, accuracy, stability, precision and linearity. To our knowledge, this is the first study that determined and calculated the pharmacokinetics of each gentamicin component (C_{1a}, $C_{2a,}$ C_1 and C_2) separately in chicken plasma using RP-HPLC and phenylisocyanate as derivatizing reagent.

After single IV administration, gentamicin C_1 has the shortest t1/2β (1.69 h) followed by C_2 (3.87), C_{2a} (5.24 h) and C_{1a} (6.45 h). These differences are attributable to differences in both clearance and apparent volume of distribution between the components. The estimated value for CLB is highest for C_2 (112.73 ml/hr/kg), followed by 79.60, 79.76 and 83.1 ml/hr/kg for C_1, C_{1a} and C_{2a} respectively. In contrast, the estimated value for V_{ss} is highest for C_{1a} (539.15 mL/kg) followed by 502.70, 453.50 and 132.42 mL/kg for C_{2a}, C_2 and C_1 respectively.

After a single IM administration of 5 mg/kg bw of gentamicin the C_{max}/Dose were 4.48, 2.49 1.94 and 1.73 µg/ml all occurring at 0.5 hours for gentamicin C_1, C_{1a}, C_2 and C_{2a}, respectively. The higher C_{max}/Dose for C_1 can be ascribed to the smaller apparent volume of distribution for this component. The calculated value for Vd_{area}/F was 304.30, 674.52, 972.63 and 1242.65 ml/kg for components C_1, C_{1a}, C_2 and C_{2a} respectively. The shortest t1/2β was noted for gentamicin C_1 (2.67 h), whereas 3.39, 4.70 and 6.61 h were calculated for gentamicin C_{1a}, C_2 and C_{2a}, respectively. Once again, this can be accounted for by the smaller apparent volume of distribution for this component. Gentamicin C_1 had the highest bioavailability (F=101%), while gentamicin C_{1a} had the lowest (F=58%). The bioavailabilities of gentamicin C_2 and C_{2a} were 78 and 64%, respectively.

After a single SC administration of gentamicin at a dose of 5 mg/kg bw, C_{max}/Dose were 4.74, 1.79, 2.81 and 2.78 µg/ml at 0.50, 1.00, 0.75 and 0.50 h for gentamicin C_1, C_{1a}, C_2 and C_{2a}, respectively. The shortest t1/2β was once again noted for gentamicin C_1 (1.54 h), while gentamicin C_{1a}, C_2 and C_{2a} had higher t1/2β values 2.37, 3.32 and 3.54 h, respectively. Gentamicin C_{1a} had the lowest bioavailability (35%), resulting in a significantly lower C_{max}/Dose (1.79 µg/ml) compared with the other components. The C_{max}/Dose was 4.74, 2.81 and 2.78 µg/ml for components C_{1a}, C_2 and C_{2a}, respectively.

The extrapolated percentages of the $AUC_{0-\infty}$ for components C_{1a} and C_{2a} were greater than the generally accepted 20% following both intravenous and intramuscular administration (IV: 31% and 25% respectively; IM: 23% and 40%). This may have affected the accuracy with which some of the pharmacokinetic parameters were estimated for these components.

Our results showed significant differences between the 4 major components in some pharmacokinetics parameters after IV, IM and SC administration of gentamicin at a dose of 5 mg/kg bw in broiler chickens. Specifically, the apparent volume of distribution was smaller and the t½β was shorter for component C_1. Also notable was that C_{1a} seemed to have been poorly absorbed following SC administration. Differences have also been reported in the pharmacokinetic parameters of the different gentamicin components in dogs (larger apparent volume of distribution and slower clearance of component C_1) [13] and horses (faster clearance of C_{1a}) [15]. The differences in PK were mainly attributed to higher tissue binding for C_1 compared with others [13]. This supports the hypothesis that there are significant differences in the pharmacokinetics of gentamicin components, but these differences are not consistent across species.

Other study conducted by Shem-Tov et al. [14] found significant differences in the pharmacokinetics profiles between major components in body tissue when the drug was given to turkeys. Therefore, the differences in total gentamicin pharmacokinetics and nephrotoxicity reported in the previous studies may result from the differences in the pharmacokinetics behavior of the different component.

After oral administration of gentamicin at a dose of 5 mg/kg bw, no component could be detected in plasma samples in all tested chickens. The oral bioavailability (F) was 0.0%. These finding are in consistent to those described previously [23]. This may due to high polarity and cationic nature of the drug that result in scant absorption from gastrointestinal tract.

In conclusion, our results showed significant differences in some pharmacokinetics parameters between four gentamicin components (C_{1a}, C_{2a}, C_1 and C_2) after administration of single mixture of gentamicin by IV, IM, SC and oral routes. The differences may have clinical and toxicological implications, and could explain the high variation in total gentamicin pharmacokinetics. A modified rapid and simple method was developed for the determination of gentamicin components in chicken plasma. This RP-HPLC method used pre-column derivatization of gentamicin with Phenylisocyanate (PIC). This method is able to measure 4 major components of gentamicin in plasma at low concentrations. It is therefore, well suited for performing pharmacokinetics and analytical studies. Further studies are needed to determine the minimum inhibitory concentration for each gentamicin component against susceptible microorganisms of interest. In addition, toxicological profile for each of these components should be studies in different animal species. Based on these studies, a determined ratio of gentamicin components should be recommended to avoid the wide variation in pharmaceutical preparations.

Acknowledgments

This study was funded by the Deanship of Scientific Research at Jordan University of Science and Technology (grant no. 121/2004).

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