Depletion of gentamicin and its major components from various tissues of turkeys

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Objective—To determine tissue depletion profiles for gentamicin and its 3 major components (C1, C1a, and C2) in turkeys.

Animals—Twenty 10-week-old male turkeys.

Procedure—4 birds were maintained as untreated controls. The remaining birds were treated with gentamicin sulfate at a dosage of 2 mg/kg, IM, once daily for 5 days. Treated birds were euthanatized 45, 60, 75, and 90 days (4 birds at each sample time) after the last dose of gentamicin was administered, and samples of muscle, liver, kidney, and skin and fat were collected. Control birds were euthanatized on day 45. Concentrations of the 3 major components of gentamicin were measured by means of reversed-phase high-performance liquid chromatography.

Results—Total gentamicin concentration (ie, sum of the concentrations of the 3 major components) was < 100 µg/kg for all muscle and skin and fat samples by day 45 and all liver samples by day 75. At all sample times, concentration of the gentamicin C1 component was higher than concentrations of the C1a and C2 components in all tissues.

Conclusions and Clinical Relevance—Results suggest that tissue depletion profiles of the 3 major components of gentamicin differ from each other. Withdrawal time, therefore, may depend on the ratio of the components in the pharmaceutical preparation used. (*Am J Vet Res* 2003;64:1234–1236)

Gentamicin is an aminoglycoside antibiotic used in the treatment of infections caused by gram-negative aerobic bacteria. Because gentamicin is nephrotoxic and ototoxic, the presence of drug residues in edible animal tissues is a public health concern. To produce animal products free from violative concentrations of gentamicin residues, it is necessary to determine profiles of drug depletion from tissues and establish adequate postadministration withdrawal times. Tissue depletion profiles for sheep,[1] pigs,[2] piglets, calves, and chicks[3] have been reported, and time for depletion of gentamicin from the kidneys has been found to vary widely depending on dosage, duration of administration, and analytical procedure used. Strong, practically irreversible tissue binding of gentamicin has also been reported.[4]

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Gentamicin is not a single compound but a mixture of 3 major components—C1, C1a, and C2—and a number of minor components. The major components differ in the degree of methylation in the 2-amino-hexose (purpurosamine) ring. Gentamicin C1a lacks methyl groups in this ring, while C1 and C2 have a methyl group in the 6′ position. Gentamicin C1 is also N-methylated in this position, while C1a and C2 have free amines instead. The C2 component consists of 2 stereoisomers (C2 and C2a). Radioimmunoassays, fluorescence polarization immunoassays, and microbiological assays have been used for quantitative determination of gentamicin concentrations in blood and tissues.[1,5] The **limits of quantification (LOQ)** for total gentamicin concentration vary with these methods but are generally in the range of 0.2 to 0.5 µg/mL. However, these methods lack the ability to identify and separately measure concentrations of the 3 major components of gentamicin, and it is not clear whether the performance characteristics of these methods are equivalent for the 3 components. If they are not, there may be some potential bias in the accuracy of total gentamicin concentrations, particularly if the composition of gentamicin in tissues differs from the composition of the analytical standard with which concentrations are measured. Differences in the ratios of the 3 components in pharmaceutical gentamicin preparations are also bound to increase the analytical bias.

The pharmacokinetics of various gentamicin components in Beagles[6] and horses[7] have been investigated, but pharmacokinetic variables and tissue depletion profiles can be meaningful only when each compound is analyzed separately. Tissue depletion profiles and withdrawal times are determined by linear regression of drug concentrations. Therefore, measuring concentrations of the 3 components of gentamicin is important in understanding its depletion from tissues.

To our knowledge, depletion of the 3 major components of gentamicin from various tissues in turkeys has not been investigated. The purpose of the study reported here was to determine tissue depletion profiles for gentamicin and its 3 major components; a novel reversed-phase **high-performance liquid chromatography (HPLC)** method was used to measure tissue concentrations of the components.

Materials and Methods

Turkeys—Twenty male turkeys obtained from a commercial breeder were used in the study. Turkeys were 10 weeks old at the beginning of the study and weighed between 5.5 and 7.0 kg. They were housed in a coop at the Experimental Institute (Faculty of Veterinary Science of Szent István University, ÜLLO-Dóra major, Hungary) and kept on the ground. Feed consisted of an antimicrobial-free commercial mash; feed and water were provided ad libitum.

Individual turkeys were identified with an identification number.

Experimental protocol—Four turkeys were maintained as untreated controls. The remaining 16 were all treated with gentamicin sulfate at a dosage of 2 mg/kg, IM, once daily for 5 consecutive days. Gentamicin was injected into the breast muscle; a different injection site was used each day. Gentamicin (200 mg/mL) was diluted with sterile saline (0.9% NaCl) solution to ensure accurate dosing. According to an analysis performed in our laboratory at the time of the present study, each 100 mg of gentamicin consisted of 29.7 mg of gentamicin C1, 22.3 mg of gentamicin C1a, and 48.0 mg of gentamicin C2 and C2a.

Four treated turkeys were euthanatized 45 days after the last dose of gentamicin was administered (ie, day 45), another 4 were euthanatized on day 60, another 4 were euthanatized on day 75, and the remaining 4 were euthanatized on day 90. Control turkeys were euthanatized on the same day that the first group of treated turkeys were euthanatized (ie, day 45). Samples of muscle, fat and skin, kidney, and liver were obtained after turkeys were euthanatized. Tissue samples were labeled and stored at –20°C until analyzed. All analyses were completed within 2 months after sample collection.

Analysis of gentamicin concentration—For determination of gentamicin concentration, 10 g of a homogenized tissue sample was placed in an Erlenmeyer flask, 20 mL of extraction solvent (1.7 g of $K_2H_2PO_4$ dissolved in 100 mL of 0.1M Na_2SO_4; pH, 8.8) was added, and the mixture was shaken vigorously for 30 minutes. The mixture was then centrifuged for 5 minutes at 4,500 × *g*, and the extract was transferred to a 50-mL Erlenmeyer flask. The extraction procedure was repeated again with 10 mL of extraction solvent, and the 2 extracts were combined. Proteins were removed by adding 0.3 mL of hydrochloride acid and centrifuging at 3,000 × *g* for 10 minutes. The pH was then adjusted to 8.8 with 25% potassium hydroxide solution. After centrifugation at 3,000 × *g* for 5 minutes, 20 mL of the solution was loaded on a cartridge[a] conditioned with 5 mL of twice-distilled water. The cartridge was washed with 5 mL of water, 5 mL of ethanol, and 5 mL of water again, and 2 mL (200 mg) of **ortho-phtaldialdehyde (OPA)** reagent dissolved in 1 mL of methanol and 0.4 mL of 2-mercaptoethanol was added. This solution was diluted to 20 mL with borate buffer solution and loaded on the cartridge for 2 minutes. The OPA was eluted with 2.5 mL of ethanol. Subsequently, 0.5 mL of distilled water was added to this solution, and the solution was vortexed for 10 seconds. Fifty microliters of this mixture was injected into the HPLC system.

The HPLC system consisted of an HPLC pump,[b] a programmable fluorescence detector,[c] an automatic injector,[d] and a data-module integrator.[e] The separation was performed with a reversed-phase column.[f] The mobile phase consisted of a 75:15:5 (vol/vol/vol) mixture of methanol, water, and acetic acid with 5.5 g of heptane-1-sulfonic acid sodium salt/L. The flow rate was 0.70 mL/min. Gentamicin components were detected with a fluorescence detector with a excitation wavelength of 330 nm and an emission wavelength of 440 nm. Limits of quantification of the components, defined as 3 times the **limit of detection (LOD)**, were 7.3 to 8.9 µg/kg for C1, 8.2 to 10.2 µg/kg for C1a, and 8.7 to 9.9 µg/kg for C2-C2a in all muscle, kidney, liver, and skin and fat samples. Recovery was 70%. Linear ranges were 25 to 200 µg/kg for muscle and skin and fat, 50 to 400 µg/kg for liver, and 150 to 1,500 µg/kg for kidney. The coefficient of correlation was 0.99. The within-laboratory repeatability coefficients of variation for gentamicin (ie, the sum of the 3 major components) were 7.9 to 10.7% for liver samples with concentrations of 100, 200, and 400 µg/kg; 7.3 to 7.9% for kidney samples with concentrations of 375, 750, and 1,500 µg/kg; and 9.4 to 17.1% for muscle and skin and fat samples with concentrations of 25, 50, and 100 µg/kg. The stability of gentamicin was measured with tissue samples spiked to a gentamicin concentration of 500 µg/kg and stored at –20°C for 8 weeks; gentamicin concentration in all spiked samples decreased by < 5%.

Statistical analyses—Natural logarithms of gentamicin and component concentrations in kidney and liver samples were used for linear regression.[8] The slopes obtained by means of linear regression were combined for covariance analyses to examine the rate of depletion from the tissues. Linear regression slopes could not be drawn for muscle and skin and fat samples because of a lack of detectable concentration in > 3 time points. The depletion profile was described by the equation:

$$\ln(Y) = \ln(A_{45}) - kt$$

where Y is the gentamicin or component concentration in the tissue at time t, t is the time in days after the first group

Table 1—Mean ± SD concentrations of gentamicin and its 3 major components in various tissues from turkeys treated with gentamicin at a dosage of 2 mg/kg, IM, once daily for 5 days

Time after gentamicin administration	Tissue	Concentration (µg/kg)			
		C1	C1a	C2	Total
45 days	Muscle	24 ± 6	14 ± 4	22 ± 7	60 ± 17
	Liver	105 ± 17	23 ± 18	69 ± 29	197 ± 64
	Kidney	283 ± 65	195 ± 52	199 ± 65	677 ± 182
	Fat and skin	19 ± 4	10 ± 2	15 ± 2	44 ± 8
60 days	Muscle	15 ± 3	10 ± 10	14 ± 4	39 ± 17
	Liver	62 ± 19	10	30 ± 7	102 ± 26
	Kidney	178 ± 22	110 ± 23	127 ± 15	415 ± 54
	Fat and skin	16 ± 3	9 ± 1	14 ± 3	39 ± 7
75 days	Muscle	< LOQ	< LOQ	< LOQ	< LOQ
	Liver	56 ± 11	ND	32 ± 9	88 ± 20
	Kidney	140 ± 19	91 ± 14	109 ± 24	340 ± 57
	Fat and skin	< LOQ	< LOQ	< LOQ	< LOQ
90 days	Muscle	< LOQ	< LOQ	< LOQ	< LOQ
	Liver	34 ± 6	ND	32 ± 14	66 ± 20
	Kidney	128 ± 23	69 ± 23	103 ± 23	300 ± 53
	Fat and skin	< LOQ	< LOQ	< LOQ	< LOQ

LOQ = Limit of quantification. ND = Not detected. For each sample time, values represent data for 4 turkeys.

Table 2—Results of linear regression of the natural logarithm of the concentration of gentamicin and its 3 major components as a function of time in kidney and liver samples from turkeys treated with gentamicin at a dosage of 2 mg/kg, IM, once daily for 5 days

	Kidney		Liver	
Component	Slope*	R^2	Slope*	R^2
C1	–0.0174	0.904	–0.0235	0.913
C1a	–0.0221	0.943	ND	ND
C2	–0.0141	0.931	ND	ND
Total	–0.0165	0.897	–0.0213	0.894

*Slope of the tissue depletion curve beginning 45 days after the last dose of gentamicin; mean value for 4 individual values is given. The regression equation was $\ln(Y) = \ln(A_{45}) - kt$ where Y is the gentamicin or component concentration in the tissue at time t, t is the time in days after the first group of turkeys was euthanatized (ie, day 45), A_{45} is the concentration on day 45, and k is the slope of the depletion curve following day 45. ND = Not determined.

of turkeys was euthanatized (ie, day 45), A_{45} is the concentration on day 45, and k is the slope of the depletion curve following day 45.

Results

Total gentamicin concentration (ie, sum of the concentrations of the 3 major components) was < 100 µg/kg for all muscle and skin and fat samples by day 45 and all liver samples by day 75 (**Table 1**). At all sample times, concentration of the gentamicin C1 component was higher than concentrations of the C1a and C2-C2a components. Gentamicin was not detected in samples from the control turkeys.

For kidney and liver samples, there were significant correlations between the natural logarithm of tissue concentrations and time from gentamicin administration (**Table 2**).

Discussion

Total gentamicin concentration has been used in previous studies assessing depletion from animals treated with gentamicin, and the fact that gentamicin is actually a combination of 3 major components—C1, C1a, and C2—that are separate chemical entities has gone practically unnoticed. Because the ratios of these 3 components in pharmaceutical preparations of gentamicin vary widely,[5,9,10] depletion of gentamicin may also vary.

Gentamicin is a polar entity that does not undergo metabolism in the body and is excreted mainly by glomerular filtration.[11] Knowledge of the ratio of gentamicin components and of their tissue depletion profiles is important in understanding whether residues of these components could contribute to consumer safety risk associated with gentamicin use in food animals. Gentamicin residues have been a subject of considerable interest because of the clinical importance of the drug and its toxic effects. The analytical problems associated with determining concentrations of the 3 components of gentamicin were identified in early studies of the pharmacokinetics of gentamicin[4,12] but subsequently overlooked.

In a previous study,[13] gentamicin was found in liver and kidney samples 73 days after administration at a dose of 4 to 7 µg/kg in sheep, and withdrawal times of 31 days for chickens and 46 days for pigs and calves have been reported.[3] In Beagles, the C1 component had a greater clearance and larger apparent volume of distribution than did the C1a and C2 components,[6] and C1a and C2 appeared to be less extensively bound to tissues than C1, suggesting that tissue binding affected the pharmacokinetics of these components. In the present study, the concentration of each of the components was determined in tissues.

In pharmaceutical preparations, gentamicin C1 consists of 25 to 50% of the total compound.[5,9,10] Withdrawal times are calculated from results of linear regression of tissue depletion profiles, but if depletion of the individual components varies, the withdrawal time may be affected by changes in the ratio of the components in the preparation used.

[a]Sep-pak silica cartridge, Waters, Milford, Conn.
[b]Waters 510, Waters, Milford, Conn.
[c]Hewlett Packard 1046, Hewlett Packard Inc, Palo Alto, Calif.
[d]712 WISP, Waters, Milford, Conn.
[e]Waters 745 B, Waters, Milford, Conn.
[f]C18 250 mm × 4.0 mm × 10 µm, BST Rutin, Budapest, Hungary.

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