Biological Half-Life of Gentamicin in Gopher Snakes

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SUMMARY AND CONCLUSIONS

A study was performed to determine the biological half-life and plasma concentrations of gentamicin in gopher snakes.

The snake plasma was assayed for gentamicin, using a microbiological agar well diffusion technique.

In the 1st study, 10 gopher snakes (groups A and B) were each given a single injection of gentamicin. They were anesthetized and samples of blood were collected each day to determine the antibiotic half-life. The average half-life was 82 hours (SD = 31) at an environmental temperature of 24 C. The variations in the results were probably due to metabolic differences within individual snakes.

In another study, 11 gopher snakes were treated with serial injections of gentamicin for about 2 weeks. Group C snakes were given gentamicin at a dose level of 2.5 mg/kg of body weight/72 hours, and those in group D were given a dose level of 2.5 mg/kg of body weight/day. Group E snakes were given gentamicin at a dose level of 5.0 mg/kg of body weight/72 hours, and group F snakes were given gentamicin at a dose level of 5.0 mg/kg of body weight/day. The snakes given gentamicin each day reached very high antibiotic concentrations in their plasma. With the long half-life of gentamicin in snakes, the gentamicin accumulation resulted from daily administration.

Plasma gentamicin concentrations in the snakes that were treated every 72 hours were lower than the concentrations in the daily treated snakes. However, these values were still higher than the therapeutic concentration recommended for human beings.

It was concluded that gentamicin should not be administered to snakes daily, but rather every 72 hours, and that a prolonged therapeutic dosing should be avoided.

Many pathogenic bacteria in snakes are gram-negative organisms (ie, *Aeromonas, Pseudomonas,* and *Klebsiella*[1-4]) which are resistant to most antibiotics. Gentamicin,[a] an aminoglycoside antibiotic, is effective against most of these gram-negative bacteria in vitro. However, gentamicin is nephrotoxic in mammals (including man) at high dosages. The published dose of gentamicin for dogs is 4.4 mg/kg of body weight 2 times/day for the first 2 days, then once a day thereafter.[5] There is some evidence that gentamicin, used in dosages extrapolated from mammals, can be toxic in snakes.

Jacobson reported visceral gout in 2 boid snakes,[6] thought to be due to renal damage induced by gentamicin. Experiences with treating reptiles with gentamicin at the National Zoological Park have shown that human dosages and treatment schedules may have resulted in excess accumulation of the drug, with resultant kidney damage and gout. In our preliminary study, 1 healthy black rat snake (*Elaphe obsoleta*) was given 5 mg of gentamicin/kg of body weight daily for 14 days. At 24 hours after the last dose, the plasma gentamicin concentration was greater than 100 μg/ml (recommended peak serum concentrations for human beings preferably are between 8 and 12 μg/ml).[7] Histopathologically, there was cloudy swelling of the proximal tubules, not present prior to treatment, as determined by renal biopsy. When snakes were given 10 times that dose (50 mg/kg/24 hr), the kidneys showed severe tubular necrosis and gout.

The purpose in the present study was to determine the biological half-life ($t_{\frac{1}{2}}$) of gentamicin in gopher snakes, and to determine the plasma gentamicin concentrations during and at the end of several treatment regimens and thereby to propose an appropriate dosage schedule for clinically treating captive snakes.

Materials and Methods

This study was performed on 21 gopher snakes (*Pituophis melanoleucus catenifer*). They were obtained from a commercial source and were housed in 38-L aquarium tanks at environmental temperature (24 C) for at least 1 month prior to the study. All snakes were in apparently good health and were fed mice each week; water was available ad libitum.

The gentamicin was administered subcutaneously to the hand-held snakes with a microliter syringe[b] as follows: In the 1st study, 10 snakes were each given a single injection of gentamicin. Five of the snakes (group A) were each given a dose of 5 mg/kg of body weight, and the other 5 snakes (group B) were each given a dose of 2.5 mg/kg of body weight. Samples of blood (0.5 ml of heparinized blood) were collected at about 6 hours after the injection, then daily for at least 4 days. The bleeding technique employed has been reported previously.[8] The blood samples were collected from the exposed caudal vena cava of snakes anesthetized with halothane,[c] nitrous oxide, and oxygen.

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[a] Gentocin, Schering Corp, Bloomfield, NJ.

[b] Hamilton Co, Reno, Nev.

[c] Fluothane, Ayerst Laboratories, New York, NY.

A microbiological method utilizing an agar well diffusion technique was used to assay gentamicin in snake plasma.[d] The susceptible organism utilized in this assay was a multiple antibiotic-resistant strain of *Staphylococcus epidermidis*.[e]

An *S epidermidis* suspension in nutrient broth (2 ml) containing 10^7 colony-forming units/milliliter were added to 20 ml of streptomycin assay agar[f] modified with the addition of 1.5% sodium chloride. The seeded agar was mixed and poured into a 150-mm plastic petri dish and allowed to solidify. Eighteen evenly distributed 3-mm wells were cut per petri dish.

A stock solution of 1,000 μg of gentamicin/ml was prepared by weighing gentamicin sulfate on an analytical balance and dissolving it in sterile distilled water. Gentamicin standards of 32, 16, 8, 4, and 2 μg/ml were prepared from blank snake plasma that had no antibacterial activity against *S epidermidis*, and from the stock gentamicin (1,000 μg/ml) solution. A plasma sample from a snake that had a calculated gentamicin value was used as a control with the daily assays. Plasma specimens and working standards were assayed by placing 5 μl of each sample into each of 3 wells by means of a microliter pipette.[g] The plates were then incubated overnight at 37 C, and the zones of inhibition were measured with a caliper to the nearest 0.1 mm. The specimen concentrations were determined by comparing them with a semi-log standard calibration plot that was based on concentration vs zone diameter. The calculated plasma gentamicin concentrations were used to determine the time necessary for the loss of one-half of antibiotic activity ($t_{\frac{1}{2}}$).

In the 2nd study, 11 healthy snakes (allotted to 4 groups; C through F) were given serial injections of gentamicin for about 2 weeks (Table 1). Group C snakes (n = 4) were treated with 2.5 mg of gentamicin/kg of body weight every 72 hours; group D snakes (n = 2), with 2.5 mg of gentamicin/kg/day; and group E snakes (n = 2), with 5 mg of gentamicin/kg every 72 hours. Samples of blood were collected every 72 hours from the snakes in groups C, D, and E prior to the day's gentamicin injection.

Group F snakes (n = 3) were given 5 mg of gentamicin/kg of body weight each day. Blood samples were collected from these snakes every 2 days, just prior to the day's gentamicin injection.

The blood samples were assayed for gentamicin as described in the 1st study.

[d] Special Microbiology Laboratory Manual, The Johns Hopkins Hospital, Baltimore, Md, pp 31-33.

[e] Clinical isolate, Agar Dilution Laboratory, The Johns Hopkins Hospital, Baltimore, Md.

[f] Streptomycin assay agar with yeast extract, pH 7.9 to 0.2. Difco Laboratories, Detroit, Mich.

[g] Eppendorf microliter pipette, Scientific Products, Columbia, Md.

TABLE 1—Plasma Concentrations of Gentamicin in Snakes Treated with Gentamicin (2nd Study)

Snake group (n = No. of snakes)	Dosage rate and schedule	Plasma concentration of gentamicin at 10 days after initial administration (μg/ml)	Plasma concentration of gentamicin at end of experiment (days = duration of experiment)
Group C (n = 4)	2.5 mg/kg/every 72 hours	10	11 (day 16)
		15*	12 (day 19)
		10*	13 (day 19)
		13*	22 (day 19)
Group D (n = 2)	2.5 mg/kg/day	27	27 (day 10)
		60	43 (day 26)
Group E (n = 2)	5 mg/kg/every 72 hours	22	21 (day 16)
		12	12 (day 22)
Group F (n = 3)	5 mg/kg/day	25	39 (day 22)
		46	84 (day 14)
		70	72 (day 12)

* Gentamicin concentration after 6 days' treatment.

Results

In the 1st study, the $t_{\frac{1}{2}}$ of gentamicin in 10 gopher snakes was 82 hours (SD = 31). There were no significant differences in the $t_{\frac{1}{2}}$ of group A and group B snakes (given single doses of 5 mg and 2.5 mg of gentamicin/kg of body weight, respectively).

In the 2nd study, the plasma gentamicin concentrations after 10 days of serial injections and at the end of the experiment are recorded in Table 1.

In group C snakes (2.5 mg/kg of body weight every 72 hours), a plasma concentration of 10 μg of gentamicin/ml was obtained 10 days after the initial dose; and at the end of the experiment, a concentration of 11 μg/ml was present in 1 snake. In 3 other snakes, at 6 days after administration, plasma concentrations of 15, 10, and 13 μg/ml were obtained, and at the end of the experiment (19 days), the concentrations were 12, 13, and 22 μg/ml, respectively.

In group D (2.5 mg/kg of body weight/day), plasma drug concentrations in the 2 snakes after 10 days were 27 and 60 μg/ml, and at the end of the experiment, plasma drug concentrations were 27 and 43 μg/ml.

After 10 days, the 2 snakes in group E (5 mg/kg of body weight every 72 hours) had gentamicin concentrations of 22 and 12 μg/ml. At the end of the experiment, plasma gentamicin concentrations were 21 and 12 μg/ml.

Concentrations of 25, 46, and 70 μg/ml were obtained after 10 days in group F snakes (5 mg/kg of body weight/day). At the end of the experiment, concentrations of 39, 84, and 72 μg/ml were reached.

The plasma concentrations of antibiotic in selected snakes given different gentamicin dosages and treatment schedules throughout the entire gentamicin treatment period are shown in Figure 1. These individual examples are representative of the other snakes studied.

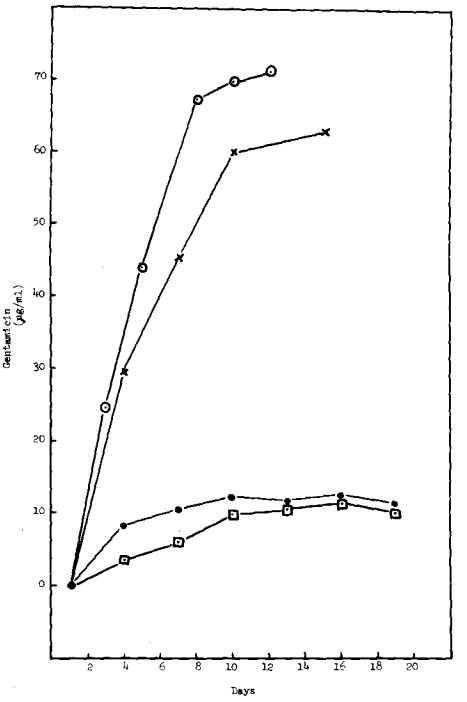

Fig 1—Plasma concentrations of gentamicin in selected snakes given different gentamicin dosages and treatment schedules throughout the gentamicin treatment period. Days refer to day 1; 2nd study. ⊡ = A group C snake (2.5 mg/kg of body weight/every 72 hours); X = A group D snake (2.5 mg/kg/day); • = A group E snake (5 mg/kg/every 72 hours); ⊙ = A group F snake (5 mg/kg/day).

Discussion

In the present studies, the snakes were handled similar to the way other captive snakes are handled in clinical situations.

The average $t_{\frac{1}{2}}$ of gentamicin in 10 gopher snakes (1st study) at ambient temperatures (24 C) was determined

to be 82 hours (SD = 31). The serum $t_{\frac{1}{2}}$ of gentamicin is about 2.3 hours in man[9] and about 2.7 hours in dogs.[10] The long $t_{\frac{1}{2}}$ of gentamicin in the snake is attributed in part to the low metabolic rate of reptiles. Mechanisms of drug metabolism in snakes, although not studied in these experiments, may also differ from those of mammals. The variation in the $t_{\frac{1}{2}}$ that occurred might have been due to metabolic differences within individual snakes.

In the 2nd study, a stairstep increase in the antibiotic value, ending with very high plasma concentrations, was observed in those snakes given injections of gentamicin each day (Fig 1). One possible explanation for such large concentrations is that the dosage exceeded the excretory capacity of the snake. Because the drug was administered more frequently than might be indicated from the $t_{\frac{1}{2}}$ calculated from these experiments, the gentamicin concentration increased.

In the snakes that were treated every 72 hours (groups C and E), the plasma concentrations of gentamicin reached a plateau and were lower than the concentrations reached with the snakes treated each day. However, the concentrations attained by treating the snakes every 72 hours were still higher than those recommended for human beings. In man, gentamicin trough levels (representing samples obtained just prior to subsequent dose) of 2 μg/ml of serum or greater have been associated with increased risk of toxicosis.[11]

With the long $t_{\frac{1}{2}}$ for gentamicin established in snakes, the daily administration of gentamicin has most likely led to the nephrotoxicosis and subsequent gout observed in some of the clinical cases at the National Zoological Park and by Jacobson.[6]

Although there is wide variation in physiologic factors between different species of snakes and other reptiles, the present study indicates that at relatively constant environmental conditions, which are similar to those in exhibited snakes, the therapeutic regimen recommended for man or other mammals is contraindicated in snakes and perhaps other reptiles. A safer schedule would be to administer gentamicin every 72 hours, along with other supportive measures, particularly adequate hydration, and to avoid prolonged course of therapy.

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