

OTOTOXICITY STUDIES WITH BB-K 8, A NEW SEMISYNTHETIC AMINOGLYCOSIDE ANTIBIOTIC

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BB-K 8, a new semisynthetic aminoglycosidic antibiotic, was evaluated for ototoxicity, nephrotoxicity and neuromuscular blocking activity in the cat. As a reference, kanamycin was tested by the same experimental methods. Employing equivalent doses in terms of the free base and 7 days of intraperitoneal treatment, BB-K 8 and kanamycin were found to be similarly ototoxic and nephrotoxic. Acutely, both compounds also demonstrated a similar neuromuscular blocking activity after large intravenous doses.

Aminoglycosidic antibiotics are known to cause ototoxicity and nephrotoxicity as possible side effects. These toxicities, when detected by standard animal tests, are found to occur with greater or lesser frequency dependent on the particular antibiotic. Certain aminoglycosides may produce vestibular toxicity to a greater degree, while with others cochlear toxicity may be the more prominent effect. Examples of antibiotics in the first group are gentamicin and streptomycin^{1,2)} and in the latter group kanamycin³⁾.

This report will describe the ototoxic potential of a new aminoglycosidic antibiotic, BB-K 8, in cats. Comparisons of observed toxicity in relation to a dose response are made with data similarly obtained for kanamycin. The results were effected with large doses over a short 7-day period of antibiotic treatment. Neuromuscular blockade experiments were performed in acute preparations. The chemical preparation, microbiological evaluation and comparative pharmacokinetics are described in other BB-K 8 publications^{4,5,6)}.

Materials and Methods

Animals: Acclimated, healthy, adult cats of either sex were used. Weights ranged from 2.0 to 5.0 kg. All cats demonstrated an attention reaction to sound stimulation.

Drugs and Dose Selection: BB-K 8 free base and kanamycin sulfate were tested. All doses are stated in terms of the free base for both drugs. The conversion factor for the kanamycin salt was 0.83 or 83 % free base.

Drug solutions were prepared by dissolving the materials in acid as indicated in Table 1. The normality of the diluent was adjusted for each dose level to achieve a final pH of approximately 7.0. All doses were administered intraperitoneally in a standard volume of 1.0 ml/kg. Control animals received sterile saline at 1.0 ml/kg.

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The experimental objective was to demonstrate cochlear toxicity in response to a 7-day, high dose antibiotic treatment, while not unduly stressing the cats. The highest dose was established from a pilot study in which cochlear toxicity was produced without a marked change in the cat's weight or behavioral condition. Based on this dose, lower doses were determined by a factor of 1.21. To avoid acute toxicity on injection, the total antibiotic dose was administered in two parts. One half of each daily dose was given about 9 a.m., the other half about 4 p.m. for 7 consecutive days. A group totalling at least 5 animals was the standard requirement for each dose level.

Clinical Observations: All animals were examined before the study and daily during the dosing period, 1~2 hours before the second injection. The righting reflex was determined by dropping each cat from an inverted position-meter (2~3 feet) above floor level. Each day's performance was scored as satisfactory if the cat maintained its ability to land properly. Observations for ataxia were made while allowing each cat to walk about the room. A Galton whistle, hand clap or whistle sound was presented unobserved to the cat to test for a positive pinna reaction or attention response. In addition to positive responses, questionable or absent sound detection responses were noted.

Cochlear Microphonics: Sixteen to twenty hours after the last dose on day 7, each cat was anesthetized with pentobarbital sodium, 35 mg/kg intraperitoneally, and cochlear function was determined for both ears. After positioning in a stereotaxic apparatus with a separate head holder device that did not obstruct either ear, a cat's tympanic bulla was opened in its ventrolateral aspect. A ball-tipped, silver wire electrode (active) was positioned on the round window membrane with a second active electrode at the incision site. An indifferent electrode was also connected to the cat's head. Care was taken to duplicate electrode placements and to prevent accumulation of fluid in the middle ear. The sound stimulus was generated from a Hewlett Packard model 200 AB audio oscillator-impedance matched through a Utah model C8JC-3C free field speaker-5 feet from the ear. Sound frequency and intensity were adjustable and reproducible. Unfiltered peak to peak a.c. cochlear potentials (CM) were detected at the round window and amplified for display through a Tektronix type 502 dual beam oscilloscope.

Each ear, subjected to sound stimulation at $1,000 \pm 100$ Hz, $2,500 \pm 250$ Hz and $6,000 \pm 100$ Hz, was tested for the frequency within each range that produced the highest CM amplitude. Intensity at the selected sound frequency was increased within system limits to the point where maximum CM output occurred. Direct measurement of that maximum response was made from a Polaroid photograph of the oscilloscope display. The decibel (dB re: $\mu N/m^2$) level of stimulation intensity was determined with a General Radio type 1565 A sound survey meter subsequently brought to position near the cat's ear. All peak to peak measurements of the cochlear potentials were multiplied by oscilloscope amplification factors to determine the specific millivolt (mV) output.

Necropsy: Immediately after assessment of the cochlear microphonic function, the animals were sacrificed, necropsied and examined for gross pathologic lesions. Kidneys were submitted for histopathologic examination and definitive assessment of renal toxicity.

Serum Antibiotic Levels: Serum antibiotic levels were determined on days 1 and 7 of dosing, 1 hour after the first of the two daily injections. Blood withdrawn from the external jugular vein was allowed to clot and was centrifuged. The antibiotic content of the resulting serum was established by an agar diffusion technique with *Bacillus subtilis* (ATCC 6633) as the test organism.

Table 1. Drug doses (mg base/kg/day) and vehicle employed

Drug	Dose b.i.d.	Daily total dose	Vehicle
BB-K 8	166	332	1.0 N H ₂ SO ₄
	137	274	0.83 N "
	113	226	0.68 N "
	93	186	0.56 N "
Kanamycin	166	332	0.5 N HCl
	137	274	0.41 N "
	113	226	0.34 N "
	93	186	0.28 N "
	77	154	0.23 N "

Blood Urea Nitrogen Determinations: Blood urea nitrogen (BUN) was measured using the method described in the Technicon Autoanalyzer Manual. These determinations were carried out prior to the study and just before necropsy. No cat with an initial BUN of more than 35 mg % was used in the study.

Neuromuscular Blocking Activity: Adult cats of either sex were anesthetized with pentobarbital sodium, 35 mg/kg intraperitoneally. Isometric contractions of the tibialis anterior muscle were recorded with a Grass FTO 3 strain gauge and Beckman type R dynograph. The peripheral end of the severed sciatic nerve was connected through a bipolar electrode to a Grass S 8 square wave stimulator. Pulses, 10 milliseconds in duration at a rate of 6 per minute, were delivered with a voltage sufficient to produce a maximal muscle response. Arterial blood pressure and heart rate were monitored. All injections were by the intravenous route. When necessary, respiration was supported with a Harvard model 607 respirator.

Results

Clinical Observations

Data on the clinical observations are found in Table 2. Decreases in body weight of >200 g were seen in 2 of 20 cats receiving BB-K 8, in 2 of 30 receiving kanamycin and in 2 of 12 control cats. The BB-K 8 and kanamycin dose levels were generally well tolerated for the duration of the experiment.

During the course of treatment, cochlear toxicity was initially indicated in both BB-K 8 and kanamycin-treated cats by a dose-related reduction in the responsiveness of some cats (pinna search or attention) to varied sound stimuli. None of the control cats became less responsive to sound stimulation.

The symptoms of vestibular disorder were considered to be the appearance of ataxia or impaired righting ability. When these symptoms did occur, they were marginal and present in 2 cats or less per dose level with kanamycin (226, 274 and 332 mg/kg) and with BB-K 8 (332 mg/kg). In some cats demonstrating these suspected vestibular effects there were concomitant symptoms of weight loss, elevated BUN values and increased serum antibiotic levels. Because the behavioral symptoms associated with vestibular toxicity were marginal in severity with limited incidence, vestibular dysfunction as a primary manifestation of toxicity could not be supported.

Table 2. Clinical observations and indications of nephrotoxicity
(Number of cats exhibiting response)

Drug	Total dose mg base/kg	Total cats	Clinical observations				Mean serum* antibiotic level ($\mu\text{g/ml}$)		BUN >38 mg % Day 8	Microscopic nephrotoxicity
			Weight loss >200 g	Reduced pinna attention	Ataxia	Righting reflex impairment	Day 1	Day 7		
BB-K 8	332	5	0	3	1	2	376	416	0	1
	274	5	0	2	0	0	330	350	0	0
	226	5	2	1	0	0	232	266	0	0
	186	5	0	0	0	0	209	212	0	0
Kanamycin	332	10	1	6	2	1	364	413	1	4
	274	5	0	2	1	1	330	424	1	0
	226	5	1	3	1	1	294	322	1	1
	186	5	0	1	0	0	178	186	0	0
	154	5	0	0	0	0	184	161	0	0
Control	Saline	12	2	0	0	0	—	—	0	0

* 1 hour after the 1st injection.

Table 3. Microphonic potentials generated in response to sound stimulation

Drug	Total daily dose mg base/kg	Number of cats tested	Mean observed response mV \pm S.E.		
			1,000 Hz	2,500 Hz	6,000 Hz
Control (Saline)		12**	1.729 \pm 0.552	2.332 \pm 0.180	1.015 \pm 0.194
BB-K 8	332	5	0.265 \pm 0.077 *	0.347 \pm 0.022 *	0.090 \pm 0.025 *
	274	5	0.639 \pm 0.235	0.808 \pm 0.202	0.334 \pm 0.119
	226	5**	1.016 \pm 0.393	0.971 \pm 0.303	0.461 \pm 0.203
	186	5	1.458 \pm 0.424	1.494 \pm 0.286	0.822 \pm 0.267
Kanamycin	332	10**	0.128 \pm 0.027 *	0.285 \pm 0.041 *	0.074 \pm 0.017 *
	274	5	0.211 \pm 0.076	0.445 \pm 0.100	0.132 \pm 0.061
	226	5	0.300 \pm 0.116	0.862 \pm 0.335	0.147 \pm 0.049
	186	5	0.909 \pm 0.371	1.428 \pm 0.226	0.498 \pm 0.195
	154	5	1.427 \pm 0.433	1.551 \pm 0.239	0.813 \pm 0.262

* Significant regression ($P \leq 0.05$).

** Data from one infected middle ear is not included.

Clinical change was not detected in any of the cats receiving either BB-K 8 at a daily dose of 186 mg/kg or kanamycin at a daily dose of 154 mg/kg over the 7-day treatment period.

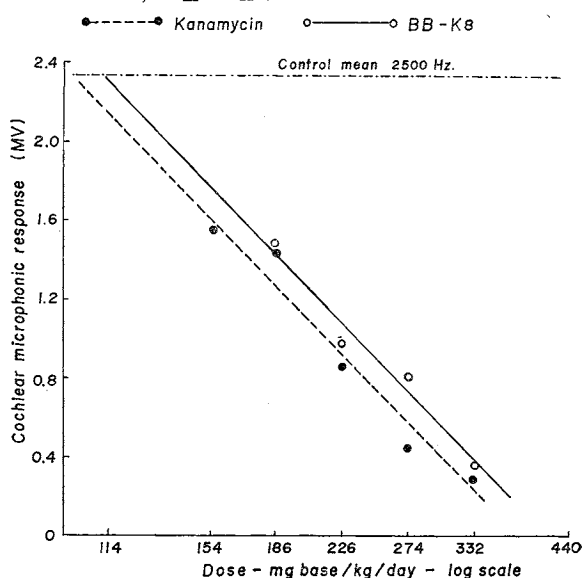
Cochlear Microphonics

The mean CM potentials from the round window membrane are shown in Table 3. All values are in mV (peak to peak) \pm S.E. Statistically significant regression at the $P \leq 0.05$ level is indicated by an asterisk in the column where applicable.

When the data are analyzed by means of a multivariate regression analysis, several conclusions may be drawn: (1) There is a very strong correlation between the responses at all three frequencies tested. Results were calculated at all three frequencies, but only the data at 2,500 Hz are illustrated (Fig. 1). (2) A significant log-dose regression was found with both BB-K 8 and kanamycin at all three frequencies ($P \leq 0.05$). (3) The regression coefficients for both antibiotics were found to be homogeneous, suggesting the possibility of a common mechanism of action.

The lines drawn in Fig. 1 use the common regression coefficients. As evidenced by the parallel lines, BB-K 8 produced a degree of cochlear toxicity similar to kanamycin. From the graph in Fig. 1, a dose can be estimated that would produce a decrease of 1.0 mV in CM output relative to the observed control value of 2.332 mV. Accordingly, a 1.0 mV reduction in CM output would require antibiotic dosage, stated

Fig. 1. Effect of BB-K 8 and kanamycin on cochlear microphonic potentials to sound stimulation at 2,500 \pm 250 Hz.



in terms of mg base/kg/day for 7 days, of 197 mg/kg for BB-K 8 and 179 mg/kg for kanamycin.

Nephrotoxicity

Evidence of nephrotoxicity is presented in Table 2. BUN and serum antibiotic levels were monitored during the dosing period as indirect evidence of nephrotoxicity. In this study, correlation between these indirect parameters and the more significant renal histopathology was not always consistent.

Marginal histopathologic signs of nephrotoxicity were noted in one of five cats receiving the highest dose of BB-K 8, 332 mg/kg; the kidneys of the other 4 animals were entirely normal. The same dose of kanamycin produced pathologic tubular changes in 4 of 10 animals. Three of 4 dose levels of BB-K 8 (186, 226, 274 mg/kg) and 3 of 5 dose levels of kanamycin (154, 186, 274 mg/kg) were without microscopic nephrotoxicity.

Duration of Cochlear Damage Induced

In a separate study, 5 additional cats received kanamycin, 332 mg base/kg/day for 7 days as described in the initial protocol. The animals were kept under normal conditions without dosing for an additional 14 days before determination of cochlear function. The following mean mV potentials \pm S.E. were obtained (day 8 values from the previous study are also shown in brackets): 1,000 Hz— 0.066 \pm 0.026 (0.128 \pm 0.027), 2,500 Hz— 0.202 \pm 0.023 (0.285 \pm 0.041), and 6,000 Hz— 0.028 \pm 0.004 (0.074 \pm 0.017). The values obtained 14 days after the last dose are not significantly different than those obtained in the 7-day experiment. Sustained loss of CM activity was indicated.

Supplementary Vestibular Toxicity Study

To compare the rate of appearance and the severity between cochlear toxicity and vestibular toxicity, BB-K 8 and kanamycin were dosed at 332 mg base/kg/day for 14 days instead of 7 days. Five cats were in each group. During this study, the attention response to sound stimulation became questionable in BB-K 8 cats at a mean of 10.2 days and undetectable at 13.6 days. The similar means for kanamycin were 8.0 and 11.4 days, respectively. Severe hearing losses affecting all cats were thereby indicated prior to termination of the 14-day high dose kanamycin and BB-K 8 treatment.

Ataxia or righting impairments were not observed in the group of BB-K 8 cats on or before termination after 14 days of dosing. One cat receiving kanamycin had questionable vestibular dysfunction on day 5, but there were definite ataxia and righting impairments on days 6 and 7 that continued until termination. The deteriorating condition of this cat was further evidenced by a BUN of 44 mg % on day 8, which progressed to a BUN of 144 mg % 18 hours after the last dose. The serum antibiotic level in this cat at this time was 72 mcg/ml. The BUN's in all the other cats were <35 mg %, and the serum levels of BB-K 8 and kanamycin were <5 mcg/ml 18 hours after the last dose.

Nephrotoxicity of similar degree and morphology was demonstrated with both compounds by microscopic examination of kidney tissues. With BB-K 8 4 of 5 cats and with kanamycin 5 of 5 cats exhibited nephrotoxicity.

With these two groups of cats BB-K 8 appeared slightly less vestibular toxic than

kanamycin. Both compounds were more cochlear and nephrotoxic rather than vestibular toxic. All toxicities were induced by extremely high multiples of the human dose.

Neuromuscular Blocking Activity

Data are summarized in Table 4. Large intravenous doses of BB-K 8 and kanamycin caused neuromuscular blockade. Both the effect on neuromuscular transmission and the lowering of blood pressure were reversible with time.

Table 4. Neuromuscular blocking activity after intravenous administration

Drug	ED ₅₀ * (mg base/kg)	95% Fiducial limits
BB-K 8	205	169~273
Kanamycin	158	129~198
α -Tubocurarine	0.10	0.08~0.11

* Dose producing 50% neuromuscular blockade.

Discussion

Several methods have been reported for determining ototoxicity in the cat, guinea pig, rat and monkey. Vestibular toxicity has been determined by subjective assessment of ataxia and impairment of the righting reflex^{2,7)}. Cochlear toxicity has been determined by the loss of PREYER's pinna reflex⁷⁾ or cochlear microphonic responses to sound stimulation^{2,8,9)}. Commonly, dosing has been for prolonged periods^{2,3)} and the amounts of drug required to produce ototoxicity vary with the species^{3,7)}.

In cats, a short, 7-day treatment at high doses can be used to assess cochlear toxicity without excessive complications of gross toxicity. For example, some drug-treated cats displayed severely reduced CM values without an abnormally elevated BUN, increased antibiotic serum level, nephrotoxicity or weight loss. In other of the drug-treated cats some or all of these symptoms occurred, but not with disabling severity.

The results are interpreted within the context of the methodology. The post-drug change in CM output could not be compared to a control within the same cat. Quantitation of effect is dependant on a statistical assessment of the mean values for drug groups as compared to untreated groups. Under standardized test conditions, the level of CM output varies between untreated cats. The age and physical condition of the ear are factors. These individual differences are also reflected by the sound intensities required to first detect or to induce maximum CM output at chosen frequencies. Therefore, in randomly selected cats, mean CM output values will likely include a large standard error. Our standard for measuring the cochlear function of an ear is the maximal amplitude of the CM in response to sound stimulation. Commonly, maximal intensities from the sound system were used for ears with low CM output. Sound levels near the ear did not exceed 106 dB at 1,000 and 2,500 Hz and 90 dB at 6,000 Hz (dB re: 20 μ N/m²).

The clinical observations after 7 days of drug treatment demonstrated that an extended dose period would improve evaluation of vestibular function with those compounds not primarily vestibular toxic. During the short-term drug treatment possible hearing losses, as determined by the attention response to sound, were not always detectable. Some cats reacted to sound when definite cochlear damage was later indicated by low CM output. Nevertheless, the CM determinations permitted dose response determination with respect to implied hearing loss without prolonging the treatment to complete deafness.

It was apparent from the presence or absence of cochlear, vestibular or nephrotoxic symptoms after identically stressing doses that some cats were individually tolerant to the toxic potential. Some cats with impairment of kidney function demonstrated early hearing loss. However, CM reductions were also evident without renal toxicity. The type and occurrence of toxic symptoms did not appear entirely dependent on the magnitude of serum antibiotic levels. The organ sites themselves seem more or less susceptible to the individual antibiotic's toxic activity.

Neither cochlear or vestibular histopathology were carried out to confirm the extent of ear damage. However, recent experiments did correlate hair cell lesions in the inner ear

with the severity and frequency pattern of hearing loss as determined in monkeys by operant conditioned audiometry techniques¹⁰. In the guinea pig the ability of the cochlea to generate the cochlear potential can be related, though not exclusively, to the number of hair cells present⁹. Our experiments demonstrated that the ear's CM activity had not recovered 14 days after 7 days of high dose kanamycin treatment.

In the literature, gentamicin has been reported to be ototoxic and nephrotoxic^{1,11}. Using our laboratory procedures, preliminary results indicate that gentamicin produces nephrotoxicity and vestibular toxicity prior to severe cochlear toxicity. This would be in contrast to BB-K 8 and kanamycin results.

BB-K 8 and kanamycin were primarily cochlear toxic. Doses causing significant cochlear impairment had no marked effect on vestibular performance. Based on BUN data, serum antibiotic levels and the degree of renal histopathology, it can be concluded that the nephrotoxic potential of BB-K 8 in cats was similar to kanamycin. Similar degrees of neuromuscular blocking activity were also seen with BB-K 8 and kanamycin.

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