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Effect of Isepamicin Dosing Scheme on Concentration in Cochlear Tissue†

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To investigate the possible effect of the dosing scheme of aminoglycosides on their concentration in the cochlear tissue, we gave two groups of 12 guinea pigs subcutaneous doses of 45 mg of isepamicin (ca. 30 mg of active product) per kg of body weight daily for eight consecutive days. The first group received the drug by continuous infusion, while the second group received it by single daily injection. On the final day of administration, the animals were sacrificed and the cochlear tissue was removed. The tissues from the cochleas of pairs of guinea pigs were pooled. The isepamicin concentrations in the cochlear duct tissue (organ of Corti plus lateral wall) and the cochlear nerve tissue were determined separately. Hearing levels before and after treatment were assessed by means of frequency-specific auditory brain stem responses (ABR). The creatinine level in serum was determined on the last day of the administration. None of the animals in either group showed signs of renal insufficiency or of hearing impairment. The median isepamicin concentration in the cochlear duct was 2.40 μg/mg of protein after continuous administration and 2.50 μg/mg of protein after once-daily administration, compared with the concentration in the cochlear nerve, where it was 1.93 μg/mg of protein after continuous administration and 2.59 μg/mg of protein after once-daily administration. These differences are statistically insignificant. The results give evidence for linear uptake kinetics of isepamicin in the inner ear tissue and may be directly relevant to the clinical dosing of the drug.

Major side effects of the otherwise very useful and often life-saving aminoglycoside drugs are their nephrotoxicity and ototoxicity. Several aspects of their ototoxicity have recently been reviewed in detail elsewhere (15). The thesis that the target specificity of the aminoglycosides for the inner ear is due to their accumulation in the inner ear fluids is being abandoned. Instead, it is now believed that a specific pharmacodynamic phenomenon is responsible for the toxic effect. Active uptake of the drug followed by interference with the phosphoinositide metabolism in the cells of the inner ear (presumably the outer hair cells) has been postulated as the basic mechanism of the ototoxicity (18). The focus of attention is therefore moving from the inner ear fluids to the inner ear tissue.

In analogy with the renal toxicity, there is interest in the saturability of the uptake of aminoglycosides in the inner ear tissue. The saturable uptake kinetics of some of the drugs (gentamicin, netilmicin, and amikacin) in the renal cortex have already been established (11, 13). These findings, which are based on animal experiments, were confirmed in humans (6, 6a, 22). Their clinical relevance is that a once-daily administration of the drug results in lower cortical concentrations than those produced by repetitive injections or even continuous infusion. In addition, high levels of the drug in serum, exceeding by far the MICs for 50 and 90% of organisms (MIC_{50}s and MIC_{90}s) of the various pathogens, are improving their antibiotic effect; this also supports the once-daily administration (17). The consequent question is whether the dosing scheme also plays a role in the process of ototoxicity and, more precisely, whether the uptake of the drug in the inner ear tissue is saturable.

Because of the very small amount of tissue available in the inner ear, it is hardly feasible to establish biochemical saturation curves, which consist of some dozens of points. The computer simulation of the uptake kinetics as explained in the first paragraph of the Results section shows that an alternative way of investigating possible saturation is to compare the drug levels in tissue after continuous infusion with those after discontinuous administration (e.g., once daily). Isepamicin is a new semisynthetic aminoglycoside antibiotic derived from gentamicin B. Its resistance to aminoglycoside-inactivating enzymes is superior to that of all other available aminoglycosides. In vitro and in vivo experiments showed comparable spectra and potency to those of amikacin (14). It has been used as the test drug in the present study.

(Part of this study was presented at the 27th Workshop on Inner Ear Biology, Stockholm, Sweden, 19 June 1990.)

MATERIALS AND METHODS

Study design. Twenty-four male pigmented guinea pigs were divided into two groups and received isepamicin (a gift from Schering-Plough, Bloomfield, N.J.) according to one of two dosing schemes. Hearing levels were assessed before and at the end of the treatment. The creatinine levels in serum were determined on the last day of the administration. The proper functioning of the pumps was verified by determining the levels of isepamicin in serum at the end of the treatment and comparing these levels with the expected values. For assessment of the hearing levels and for the implantation of the pumps, the animals were anesthetized with ketamine (Ketalar; Parke Davis), ca. 60 mg/kg of body weight, by intramuscular injection. After 7 days of adminis-

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tration, the concentration of isepamicin in the inner ear tissue was determined and the levels produced by the two different dosing schemes were compared.

Administration of the drug. All animals received isepamicin, 45 mg of powder (ca. 30 mg of active product) dissolved in saline per kg of body weight daily for seven consecutive days. The body weight was determined just before the first administration, and the dose was not corrected for the gain in body weight during the experiment. The first group of 12 animals received the drug by daily subcutaneous injection under short ether anesthesia. The second group of 12 animals was implanted with a miniosmotic pump (Alzet model 2ML1; Scientific Marketing Associates, London, England), which released the drug at a constant rate. The pumps were not primed before implantation. Daily short ether anesthesia was applied to match the experimental conditions of the first group.

Evoked-response audiometry. The hearing level of each animal was evaluated before and at the end of the treatment by auditory brain stem responses. Acoustic broadband clicks (80 μs) were generated by an ERA MK III Stimulator (Amplaid) and were filtered by means of a set of filters (multichannel programmable filter instrument model 9016; Frequency Devices Inc.) to a narrow-band noise with a bandwidth of one-third of an octave centered at 1, 2, 4, 8, 16, and 32 kHz, the slopes of the filters being 90 dB per octave. An amplifier (model M-505; Onkyo) and a speaker (model SB-F4F; Technics) were used. The guinea pigs were put at a distance of 50 cm from the speaker. The system was calibrated with a sound level meter (Brüel & Kjaer type 2218 micro UA 0196). Electrical responses were registered by means of three subcutaneous electrodes at the vertex (+), left ear (−), and right ear (mass). A time window of 10 ms was used. The signal was averaged over 200 cycles. The resulting image was interpreted only in terms of the hearing threshold, defined as the minimal sound level that could yield a reproducible peak III or peak V. Neither latency nor shape of the peaks was taken into consideration.

Collection of inner ear tissue. For the removal of the inner ear tissue, the animals were killed at day 8 with T61(R) (a composite drug for animal euthanasia, based on the narcotic hydroxybutyramide [Hoechst AG, Frankfurt, Germany]) 4 h after the last injection. After decapitation, the bulla was rapidly removed and opened. The stapes was removed, and the round window membrane was perforated. The cochlea was flushed once with saline. By using microdissection techniques under the operation microscope, the inner ear tissue of each cochlea was carefully removed and collected in plastic tubes containing 200 μl of 0.01 M phosphate buffer solution. The tissue of the cochlear nerve was kept separate from that of the cochlear duct (essentially the organ of Corti plus the lateral wall). The tissues of pairs of animals (i.e., four cochleas) were pooled. Every two animals therefore yielded two tubes, one containing the cochlear duct tissue and the other containing the cochlear nerve tissue of four cochleas. Consequently, the initial 24 animals yielded 12 cochlear duct tubes and 12 cochlear nerve tubes. Each tube will be referred to as a sample. The tubes were stored at −20°C. All further manipulations were carried out with plastic equipment.

Determination of concentrations in tissue. After the tissues were stored in buffer solution at −20°C, they were homogenized by ultrasonication (Soniprep 150; M.S.E., 60 s). As indicated for the extraction procedure of Giuliano et al., 20 μl of 50% trichloroacetic acid was added to each test tube (12). After 15 min, the tubes were centrifuged (12,000 × *g* for 5 min). The supernatant, containing the hydrophilic drug, was removed and weighed. The isepamicin concentration was determined by a radioimmunoassay (^{125}I-isepamicin radioimmunoassay kit [Clinetics Corp.]; a gift from Schering-Plough). The minimal detectable concentration in this assay is 0.2 μg of isepamicin per ml (95% confidence limits). The intra-assay variation ranges from 2.89% (8.14 μg/ml) to 4.37% (120.14 μg/ml). The interassay variation ranges from 4.29% (8.24 μg/ml) to 6.13% (119.22 μg/ml). The recovery of isepamicin from cochlear duct tissue was 101% (standard deviation [SD], 10%), and the recovery from cochlear nerve tissue was 101% (SD, 8%). The protein content of the pellet was determined by a colorimetric assay (BCA protein assay reagent; Pierce, Rockford, Ill.) (20). The ratio of isepamicin content (micrograms) to protein content (milligrams) yielded the isepamicin concentration in tissue.

Data analysis. (i) Hearing levels. Paired values, comparing the shift of hearing thresholds between the first and last days at each frequency, were analyzed separately within the continuous-infusion and once-daily groups by means of Wilcoxon matched-pairs signed-rank test. To obtain an overall level of significance of 5% per animal, according to the Bonferroni principle we used a 1% level of significance to test each frequency separately. The tests were carried out two tailed at the 1% level of significance. The hearing shifts between the once-daily and the continuous-infusion groups were compared by a two-tailed Mann-Whitney U test at a 1% level of significance (according to the same Bonferroni principle).

(ii) Concentrations in tissue. Three samples were lost during the processing. The remaining 21 samples can be grouped as follows: 5 cochlear duct samples and 5 cochlear nerve samples in the continuous-infusion group, and 5 cochlear duct samples and 6 cochlear nerve samples in the once-daily group. The differences between the continuous-infusion and the once-daily groups were compared within the cochlear duct and cochlear nerve groups by both a Mann-Whitney U test and a separate-variance *t* test. These tests were carried out one tailed at the 5% level of significance. Similarly, the differences between the cochlear duct and the cochlear nerve groups were compared within the continuous-infusion and the once-daily groups by both a Wilcoxon matched-pairs signed-rank test and a *t* test for paired variables. These tests were carried out two tailed at the 5% level of significance. All statistics were performed by a computer running CSS/pc software (release 2.1; Statsoft Inc.).

RESULTS

Figure 1 shows a computer simulation of the expected drug levels in tissue after two administration schemes, assuming a half-life of the drug in tissue of 7 days, which corresponds to the estimated value of Tran Ba Huy et al. (21), which was obtained with gentamicin but is the only available value in the literature. Absence of saturation is simulated in Fig. 1a, and increasing degrees of saturation are simulated in Fig. 1b, c, and d, respectively. If the uptake of the drug in the tissue is saturable, it is not likely that this would interfere with the levels in tissue after continuous administration, in which the drug is presented to the uptake mechanisms virtually molecule by molecule. On the other hand, when the drug is given in a once-daily administration, the total daily amount is presented to the uptake mechanisms all at once; it is thus very conceivable that these uptake mechanisms might become saturated, which would result in decreased drug levels in tissue. These two situations are

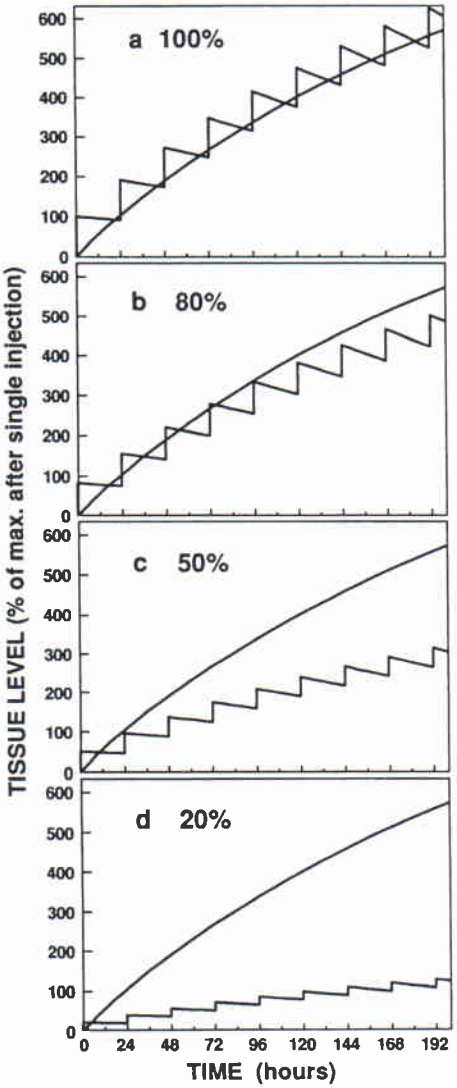

FIG. 1. Simulation of the levels of a drug in inner ear tissue with an assumed half-life of 7 days in tissue. The figure shows a comparison of the levels in the inner ear tissue after continuous and once-daily administration, assuming saturation thresholds of 100% (no saturation) (a), 80% (b), 50% (c), and 20% (d).

simulated for different saturation thresholds in Fig. 1b to d. It can be inferred from these figures that the ratio of the peak level in tissue after discontinuous administration to the corresponding level in tissue after continuous administration at, for instance, day 8, reflects the degree of saturation. This ratio is smaller than the one representing saturation but equal to or larger than the one representing no or almost no saturation. This parameter has been used in the present study to investigate whether the uptake of an aminoglycoside in the inner ear tissue is saturable.

Control parameters. Of the 12 animals receiving continuous infusion, 2 died during one of the daily ether anesthesias and 1 died of unknown causes. The remaining nine had mean body weights (±SD) of 290 ± 31 g at the beginning of the treatment and 355 ± 19 g at the end. The mean isepamicin level in serum (±SD) at the end of the treatment was 2.9 ± 0.6 μg/ml, with a range of 1.9 to 3.6 μg/ml. The mean urea level in serum (±SD) was 52 ± 6 mg/dl, with a range of 45 to 62 mg/dl, which was within the normal range as determined in 24 normal guinea pigs (51 ± 29 mg/dl). The mean creatinine level in serum (±SD) was 0.48 ± 0.05 mg/dl, with a range of 0.41 to 0.56 mg/dl, which was also within the normal range (0.50 ± 0.20 mg/dl).

Of the 12 animals receiving daily injections, 1 died during one of the daily ether anesthesias. The remaining 11 had mean body weights (±SD) of 340 ± 16 g at the beginning of the treatment and 385 ± 18 g at the end. The mean urea level in serum (±SD) was 42 ± 9 mg/dl, with a range of 32 to 56 mg/dl, which was within the normal range as determined in 24 normal guinea pigs (51 ± 29 mg/dl). The mean creatinine level in serum (±SD) was 0.43 ± 0.05 mg/dl), with a range of 0.40 to 0.50 mg/dl, which was also within the normal range (0.50 ± 0.20 mg/dl).

Hearing levels. For the continuous-infusion group, the differences in hearing thresholds between the beginning and the end of the treatment are shown in Table 1. No significant hearing loss occurred at any frequency.

For the once-daily group, the differences in hearing thresholds between the beginning and the end of the treatment are shown in Table 2. No significant hearing loss occurred at any frequency, although the median hearing loss at 32 kHz is 5 dB ($P = 0.10$). If the test is carried out one tailed (meaning that only losses [no enhancements] of hearing are expected to occur after isepamicin treatment), P is 0.05, which is borderline. When the hearing shifts of the

TABLE 1. Shifts of hearing thresholds between days 1 and 8 at different fequencies in the continuous-infusion group

Animal	Difference in hearing threshold (dB) at frequency[a] of:						
	Spectrum[b]	1	2	4	8	16	32
1	0	−20	20	0	−5	−15	−20
2	−5	−15	5	−15	−5	−10	−30
3	10	−10	0	5	−5	0	0
4	0	−5	0	−5	5	0	−10
5	5	−15	0	−5	10	0	0
6	10	10	5	10	0	0	5
7	−5	−5	0	−5	0	−5	10
8	5	5	0	20	0	−5	20
9	5	5	0	5	0	5	0

[a] Frequencies expressed in kilohertz.
[b] Broadband 0 to 40 kHz.

TABLE 2. Shifts of hearing thresholds between days 1 and 8 at different frequencies in the once-daily group

Animal	Difference in hearing threshold (dB) at frequency[a] of:						
	Spectrum[b]	1	2	4	8	16	32
1	0	0	−20	−5	−10	0	−5
2	5	15	10	5	0	5	5
3	−5	−5	−10	−5	−5	−5	5
4	−5	5	5	0	−5	5	0
5	−5	0	5	5	0	0	40
6	−5	−5	25	−5	0	15	0
7	−5	15	−10	0	5	−10	10
8	5	10	20	−10	10	0	10
9	−10	5	0	−5	0	5	10
10	5	5	0	−5	−5	−5	−5
11	5	−10	5	5	5	0	0

[a] Frequencies expressed in kilohertz.
[b] Broadband 0 to 40 kHz.

TABLE 3. Concentrations of isepamicin in inner ear tissue after 7 days of continuous administration

Sample[a]	Protein content (μg)	Supernatant wt (g)	Drug concn (μg/ml)	Drug amt (μg)	Concn in tissue (μg/mg)
D1	347	0.2585	3.22	0.83	2.40
D2	404	0.2734	2.61	0.71	1.77
D3	326	0.2945	3.71	1.09	3.36
D4	562	0.2707	2.61	0.71	1.26
D5	263	0.2847	2.97	0.85	3.22
N1	378	0.2848	3.05	0.87	2.30
N2	378	0.2731	2.33	0.64	1.68
N3	462	0.2851	2.42	0.69	1.49
N4	488	0.2985	3.15	0.94	1.93
N5	236	0.2707	3.04	0.82	3.48

[a] D1 to D5, cochlear duct samples; N1 to N5, cochlear nerve samples.

continuous-infusion and the once-daily groups were compared, no significant differences existed at any frequency.

Drug concentration in tissue. (i) Continuous administration. Five tubes with cochlear duct tissue and five tubes with cochlear nerve tissue were investigated for their concentrations of isepamicin. The results are shown in Table 3. The median isepamicin levels in tissue were 2.40 μg/mg of protein in the cochlear duct and 1.93 μg/mg of protein in the cochlear nerve. No statistically significant difference was found between the drug levels in the cochlear duct tissue and those in the cochlear nerve tissue.

(ii) Once-daily administration. Six tubes with cochlear duct tissue and six tubes with cochlear nerve tissue were investigated. One of the cochlear duct tubes was lost during processing. The results are shown in Table 4. The median isepamicin levels in tissue were 2.50 μg/mg of protein in the cochlear duct and 2.59 μg/mg of protein in the cochlear nerve. No statistically significant difference was found between the drug levels in the cochlear duct tissue and those in the cochlear nerve tissue.

When the continuous-infusion and once-daily groups were compared, no statistically significant difference between them was found, either at the cochlear duct level or at the cochlear nerve level (Table 5).

TABLE 4. Concentrations of isepamicin in innear ear tissue after 7 days of once-daily administration

Sample[a]	Protein content (μg)	Supernatant wt (g)	Drug concn (μg/ml)	Drug amt (μg)	Concn in tissue (μg/mg)
D1	179	0.2717	1.84	0.50	2.80
D2	320	0.2718	2.19	0.60	1.86
D4	310	0.2547	3.04	0.77	2.50
D5	294	0.2420	2.91	0.70	2.39
D6	368	0.2809	45.42	12.76	34.72
N1	315	0.2726	3.15	0.86	2.72
N2	273	0.5393	1.25	0.67	2.46
N3	452	0.2608	1.31	0.34	0.75
N4	299	0.2789	1.71	0.48	1.59
N5	184	0.2847	2.74	0.78	4.25
N6	236	0.2687	8.69	2.34	9.89

[a] D1 to D6, cochlear duct samples; N1 to N6, cochlear nerve samples.

DISCUSSION

In contrast to the renal uptake kinetics of aminoglycosides, the kinetics in the inner ear tissues are not well understood (15). This lack of understanding is due to the particular difficulties in handling inner ear tissues, i.e., their inaccessibility and their very small volume.

As far as we were able to ascertain, only three reports have been published on levels of aminoglycosides in inner ear tissue (8, 10, 21), and only two of these studied saturation (10, 21). Tran Ba Huy et al. found saturation of the uptake of gentamicin in rat inner ear tissue at very high doses (100 mg/kg/day) (21) and Dulon et al. found no saturation of gentamicin uptake in guinea pigs at up to 300 mg/kg/day (10). It has to be mentioned that both studies used glass vials for processing the specimens, although it is known that the cationic aminoglycosides tend to stick to these surfaces (7). Moreover, no special extraction procedure was used.

The half-life of aminoglycosides in inner ear tissue is estimated to be about 7 days (21); this causes the drugs to accumulate in the tissue. If the uptake of the drug is saturated, the accumulation will not proceed as rapidly as in the unsaturated situation. This difference in drug levels in tissue increases with time and can be used as a valid estimate of saturation. To mimic the clinical situation, we administered the drug at a dose in the high therapeutic range for 7 days. The drug was given subcutaneously, either once daily or continuously by an implanted pump. Drug levels in tissue were determined shortly after the occurrence of the peak concentration, i.e., at a moment when the concentration was still nearly maximal. This peak has been shown to occur 2.5 h after the peak in serum in rats (21). In a preliminary study we found the peak in serum in guinea pigs to occur between 30 and 50 min after subcutaneous injection, which was in agreement with the data obtained with rats. The inner ear tissue was removed 4 h after the last injection, corresponding to some 3 to 3.5 h after the peak concentration in serum. The functioning of the osmotic pumps was checked by determination of the levels in serum on the final day and was proved to be good. No renal failure occurred. The hearing levels of all the animals were evaluated before and after treatment. The technique of frequency-specific auditory brain stem responses that was used for this purpose is able to discern an isolated high-frequency hearing loss, which would be the earliest sign of ototoxicity (9). It is therefore considered a very sensitive method. All thresholds were within the normal range for all frequencies. No change occurred between the first and last days of treatment. The median 5-dB hearing loss at 32 kHz in the once-daily group ($P = 0.05$) might be a first sign of ototoxicity, although our preset cutoff P value of 0.01 renders this alleged hearing loss insignificant. All these results confirmed the many reports that therapeutic dosing for 1 week is not likely to cause serious toxic side effects (see, e.g., reference 1).

Aminoglycosides are cationic molecules with a high electrostatic affinity to anionic substances. Only plastic equipment and materials were therefore used. In a pilot study we found gentamicin levels in tissue of less than 0.5 μg/mg 4 h after a single subcutaneous injection of 80 mg of gentamicin per kg. This was in agreement with the results of Tran Ba Huy et al. (21), who found similar concentrations in tissue both after single injection and after a 4-h continuous infusion and who reported these levels to be the peak levels in tissue. The results of the present study show much higher concentrations of isepamicin in tissue after 7 days (although still not exceeding the levels in serum), thus yielding evidence for

TABLE 5. Statistical parameters of the concentrations of isepamicin in the inner ear after 7 days of continuous or once-daily administration

Infusion method and sample	No. of samples	Concn of isepamicin (μg/mg)					
		Mean ± SD	Minimum	25th percentile	Median	75th percentile	Maximum
Continuous							
Duct	5	2.40 ± 0.91	1.26	1.77	2.40	3.22	3.36
Nerve	5	2.18 ± 0.79	1.49	1.68	1.93	2.30	3.48
Once daily							
Duct	5	8.85 ± 14	1.86	2.39	2.50	2.50	34.7
Nerve	6	3.61 ± 3.3	0.75	1.59	2.59	4.24	9.89

accumulation of the drug. There was no difference in concentrations in tissue between the once-daily and the continuous-infusion groups. For both cochlear duct and cochlear nerve tissues, once-daily administration yielded higher, although statistically insignificant, levels. As discussed above, saturation would give the opposite effect. Taking into account an alpha error of 0.05 and a beta error of 0.20, the present study design would have been able to demonstrate a difference of at least 50% in levels in tissue. Hence, only Fig. 1c and d would have been significant if such differences had occurred. These data therefore give evidence that no major saturation takes place at the given dosages and levels in serum. In contrast, the uptake shows linear kinetics. It is noteworthy that isepamicin uptake in the kidneys also follows linear kinetics (6b).

It is of course tempting to speculate on the clinical implications of these findings. If no saturation occurs within the therapeutic dosing range, a higher peak level in tissue can be expected to occur after a single high-dose injection than after multiple smaller injections or even after continuous infusion of the same total dose. These higher peak levels could induce a higher degree of toxicity and therefore should be avoided. On the other hand, the presumably very long half-life of the drug in tissue implies that the levels in tissue do not follow the levels in serum but rather the mathematically integrated function of the levels in serum (the area under the concentration-time curve). This is confirmed by our findings after 7 days, which show that the differences between the two schemes are minimal. It also is in agreement with the finding that the degree of ototoxicity does not depend on the single dose or peak level in serum, but on the total dose or total area under the curve during the whole treatment (2). We therefore tend to believe that the actual administration scheme does not play a critical role in the eventual ototoxicity. A recent confirmation comes from the work of Bamonte et al., who indeed found no difference in ototoxicity in guinea pigs between two dosing schemes consisting of one or three daily injections of netilmicin and amikacin (1).

An interesting point is the finding of equal concentrations in the cochlear duct and cochlear nerve tissues. As reviewed elsewhere, the neural degenerations occur only after outer hair cell lesions and are believed to be a result of these lesions (15). The present findings might, however, suggest that there is an intrinsic effect on the neurons. Direct toxicity of aminoglycosides to cochlear or vestibular neurons has been mentioned in earlier studies (16, 19), although it is difficult to differentiate between primary effects on the neurons and secondary retrograde degeneration. Alternatively, the outer hair cells might be more susceptible to the effects of aminoglycosides. Intrinsic pharmacodynamic phenomena might explain this susceptibility. The high concentration of phosphatidylinositol diphosphate in the outer hair cells, to which the aminoglycosides bind with great affinity, is one of these phenomena.

In conclusion, the present study contributes to our understanding of the uptake kinetics of the aminoglycoside isepamicin in inner ear tissues. It is the first to report linear uptake kinetics of this drug. The possible clinical relevance of this finding is discussed.

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