ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1983, p. 344–346
0066-4804/83/020344-03$02.00/0

Vol. 23, No. 2

Gentamicin Persistence in Rat Endolymph and Perilymph After a Two-Day Constant Infusion

P. TRAN BA HUY,* A. MEULEMANS, M. WASSEF, C. MANUEL, O. STERKERS, AND C. AMIEL

Université Paris VII (Hôpital Lariboisière and Faculté Xavier Bichat) and Institut de la Santé et de la Recherche Médicale (Unités 13 and 251), Paris, France

Received 23 August 1982/Accepted 30 November 1982

The kinetics of gentamicin in the inner ear fluids of rats were studied up to 15 days after cessation of a 2-day constant infusion of 10 μg/min. In endolymph, the concentration of gentamicin persisted at about 1 μg/ml for up to 15 days, precluding the determination of the half-life of the drug in this fluid. In perilymph, gentamicin cleared more slowly than after a shorter period of infusion. These results suggest that the tissues of the inner ear could bind the aminoglycoside and then slowly release it into the surrounding fluids.

Endolymph, which plays a major role in the physiology of hearing, must also be considered as a possible route for penetration of aminoglycoside antibiotics to the sensory structures of the inner ear. Nonetheless, there have been few investigations of aminoglycoside pharmacokinetics in this fluid (4, 6). In a previous study of rats receiving a constant infusion of gentamicin (15 μg/min) for up to 6 days, we found that gentamicin entered perilymph slowly and cleared from this fluid with a half-life of 3 h and entered endolymph even more slowly (5). The present study was designed in an attempt to determine the half-life of gentamicin in endolymph on the basis of its disappearance from this fluid after the cessation of a 2-day constant infusion given to rats.

Seventeen male Sprague-Dawley rats weighing 180 to 220 g each were given a 2-day constant infusion of gentamicin. This infusion was produced by two minipumps (Alzet; Alza Corp., Palo Alto, Calif.) implanted subcutaneously while the animals were under brief anesthesia. Each minipump delivered a constant rate of 1 μl of a 300-mg/ml solution per h, yielding a total infusion rate of 10 μg of gentamicin per min.

In three rats, samples of cochlear endolymph and perilymph were collected from one side, concomitantly with plasma, after 48 h of gentamicin infusion. At the end of the sampling procedures, the two mimipumps were withdrawn (t = 0). The animals were maintained under anesthesia and, during the fourth hour following the cessation of the infusion, samples from the contralateral ear were collected concomitantly with plasma. In these rats, the glomerular filtration rate was measured by the determination of the clearance of [^{3}H]inulin before any sampling was initiated.

In 14 rats, the minipumps were withdrawn at 48 h while the animals were under brief anesthesia (t = 0). Concomitantly, in eight animals, a sample of about 300 μl of blood was collected by cutting the tail to ensure that these animals had been infused in a manner comparable to that of the first three rats. In all cases, the plasma concentrations of gentamicin were in agreement with those expected, based on the pumping rate according to the specifications of the supplier. Therefore, for the rest of the animals, plasma concentrations were not assayed at the end of the infusion period. Samples of labyrinthine fluids from both sides and one sample of plasma were collected from each animal at a time from 8 h to 15 days after cessation of the infusion.

Preparation of animals and sampling procedures were identical with those detailed in an earlier report (5). Because of technical difficulties, the sampling of perilymph from one side failed in five instances: at 4 and 8 h, 6 days, and twice at 7 days. The sampling of endolymph also failed in five instances: at 4 h, 6 days, twice at 7 days, and 10 days. The gentamicin assays were performed with a modified commercial radioimmunoassay kit (2). Its sensitivity was 100 pg of gentamicin per tube, so that the sensitivity achieved was 0.5, 0.02, and 0.001 μg/ml in endolymph, perilymph, and plasma, respectively.

The mean values ± standard error of the mean of sodium and potassium concentrations in endolymph samples were 1.9 ± 0.2 and 155.7 ± 5.3 mM (n = 29), respectively, attesting to their purity.

The glomerular filtration rates determined at 2 days in the first three animals were 1.146, 1.014, and 0.813 ml/min, which are in the range of normal values for our laboratory.

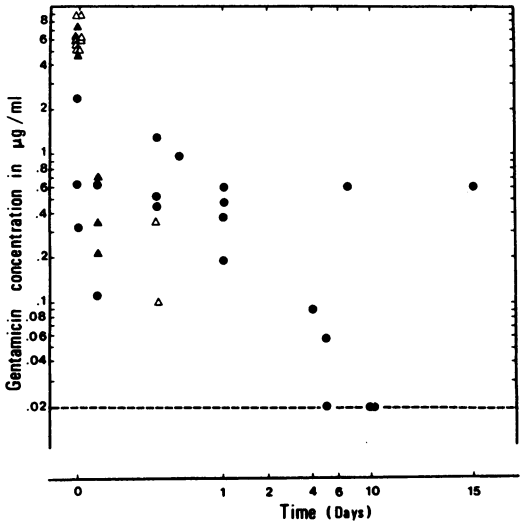

FIG. 1. Log-log curve of the disappearance of gentamicin from plasma (▲, plasma group I; △, plasma group II) and perilymph (●) of rats during the 15-day period after cessation of a 2-day constant infusion of gentamicin (10 µg/min). The dashed line indicates the limit of sensitivity of the radioimmunoassay in perilymph. The limit of sensitivity in plasma (0.001 µg/ml) is not represented on the figure.

At $t = 0$, the gentamicin concentration in plasma was 6.2 ± 0.7 µg/ml in the first three animals and 6.5 ± 0.6 µg/ml in the next 8 animals. At the same time, the gentamicin concentration was 1.5 ± 0.7 µg/ml ($n = 3$) in perilymph and 1.2 ± 0.3 µg/ml ($n = 3$) in endolymph. The perilymph-to-plasma and endolymph-to-plasma ratios were 0.24 and 0.19, respectively.

Gentamicin disappeared rapidly from plasma after terminating the infusion. Only two of three samples had detectable levels of gentamicin at 8 h. After that time, the drug was no longer detectable (Fig. 1).

The decline of the gentamicin concentration in perilymph was much slower than that in plasma. Although there was considerable animal-to-animal variability and some samples did not contain any detectable amount of drug, the decay curve suggested a slow disappearance of gentamicin. However, one 15-day sample contained a significant concentration (0.62 µg/ml).

In endolymph, gentamicin concentrations did not decline appreciably during the 15-day postinfusion observation period. However, in 3 of 26 samples collected during this interval, concentrations were below the limit of sensitivity of the radioimmunoassay (Fig. 2).

In our previous study (5), using a constant infusion rate of 15 µg of gentamicin per min, we had observed that the gentamicin concentrations reached a plateau in endolymph at 2 days but that there were evidences of renal insufficiency by day 4. Accordingly, in the current study, we adopted a 2-day infusion period so as to load the endolymphatic compartment, but with 10 µg of gentamicin per min to minimize risks of renal failure, which would have biased a decay study. The normal glomerular filtration rate measured on the first three animals indicates that this approach was successful.

As would be expected, the concentrations of gentamicin achieved at 2 days in plasma are

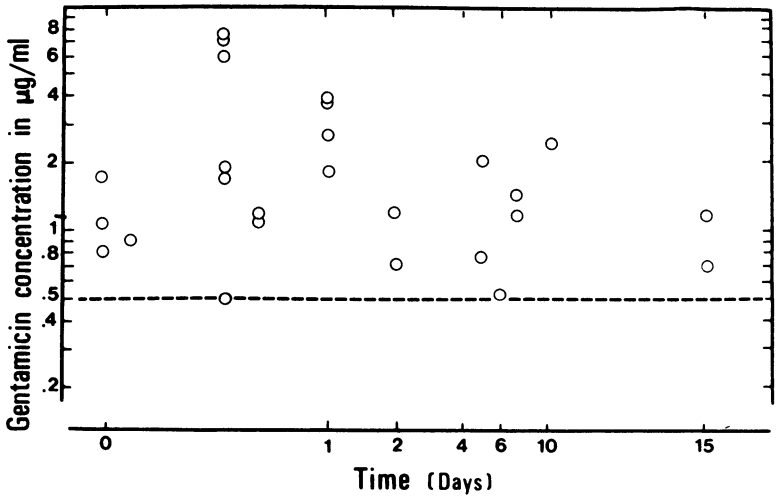

FIG. 2. Log-log representation of the concentrations of gentamicin in endolymph as a function of time following cessation of the 2-day constant infusion of gentamicin (10 µg/min). The dashed line indicates the limit of sensitivity of the radioimmunoassay in endolymph.

lower than those found in our previous study (6.4 ± 0.4 μg/ml [n = 11] versus 12.5 ± 1.3 μg/ml [n = 3]). However, the ratios of the mean perilymph to plasma concentrations (0.24) and endolymph to plasma concentrations achieved at the same time (0.19) remained in the range of those measured previously (0.19 and 0.08, respectively). The rapid disappearance of gentamicin from plasma was in agreement with the half-life of 40 min found previously.

With respect to the kinetics of gentamicin in labyrinthine fluids, this agent was detectable in endolymph up to 15 days after the end of infusion. This persistence precluded determination of the half-life of gentamicin in this fluid. The elimination rate of gentamicin from perilymph was much slower than that observed in our previous study after cessation of a 4-h constant infusion of 15 μg of gentamicin per min (half-life, 3 h) (5). This slow elimination of gentamicin from these fluid compartments might be explained by loading of the compartments which line the endolymphatic space, especially the stria vascularis and the organ of Corti, by the greater total amount of gentamicin delivered in the present experiment (14.4 mg/100 g of body weight) than in the previous one (1.8 mg/100 g of body weight). The above structures do have the capacity to bind the aminoglycoside antibiotics (3), at first in a reversible manner (7). Once the infusion has been stopped, the drug would be slowly released to perilymph and endolymph. The identification of the compartments involved and evaluation of their respective influences upon the kinetics of gentamicin in perilymph and endolymph would require a simultaneous study of fluids and tissues of the inner ear during a longer period of observation. It is possible that clearance of gentamicin from both fluids was decreased as a result of the toxic action of the aminoglycoside antibiotic upon the boundary membranes of the endolymphatic compartment. This is unlikely since the toxic action is supposed to increase rather than to decrease the membrane permeability (1, 3).

The mean concentration of gentamicin achieved in endolymph by the end of the 2-day infusion period (1.2 ± 0.3 μg/ml) appeared to be close to that observed in our previous study after 6 days of a 15-μg/min constant infusion rate (1 ± 0.5 μg/ml [n = 5]), although the total amount of infused drug differed widely (14.4 mg versus 65 mg/100 g of body weight). This could be explained either by a large capacity of the surrounding tissues to bind the aminoglycoside and thus to limit the increase of the concentration in endolymph or by the saturation of a possible transport mechanism of the drug towards the endolymph developing between days 2 and 6.

The authors are indebted to J. E. Hawkins and J. Schacht from the Kresge Hearing Research Institute, Ann Arbor, Mich., for invaluable discussions about the manuscript, to S. Couette and C. Coureau for technical assistance, and to F. Carlier for secretarial help.

This investigation was supported by grant 816048 from the Institut National de la Santé et de la Recherche Médicale and by a grant from the Université Paris VII (Unité d'Enseignement et de Recherche Xavier Bichat).

LITERATURE CITED

1. **Lodhi, S., N. D. Weiner, and J. Schacht.** 1979. Interactions of neomycin with monomolecular films of polyphosphoinositides and other lipids. Biochim. Biophys. Acta **557**:1–8.
2. **Meulemans, A., C. Manuel, and P. Tran Ba Huy.** 1981. Radioimmunoassay of gentamicin in microliter and nanoliter samples of biological fluids. Chemotherapy **27**:29–33.
3. **Schacht, J.** 1979. Isolation of an aminoglycoside receptor from guinea pig inner ear tissues and kidney. Arch. Otorhinolaryngol. **224**:129–134.
4. **Stupp, H. F.** 1970. Untersuchung der Antibiotikaspiegel in den Innenohrflüssigkeiten und ihre Bedeutung für die spezifische Ototoxizität der Aminoglykosidantibiotika. Acta Otolaryngol. (Stockh.) **262**(Suppl.):1–85.
5. **Tran Ba Huy, P., C. Manuel, A. Meulemans, O. Sterkers, and C. Amiel.** 1981. Pharmacokinetics of gentamicin in perilymph and endolymph of the rat as determined by radioimmunoassay. J. Infect. Dis. **143**:476–486.
6. **Watanabe, Y., R. Nakajima, R. Oda, M. Uno, and T. Naito.** 1971. Experimental study on the transfer of kanamycin to the inner ear fluids. Med. J. Osaka Univ. **21**:257–263.
7. **Wersäll, J., and A. Flock.** 1964. Suppression and restoration of the microphonic output from the lateral line organ after local application of streptomycin. Life Sci. **3**:1151–1155.