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Are Aminosugars Ototoxic?*

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Sind Aminozucker ototoxisch?

Zusammenfassung. Die Ototoxizität des Kanamycin-Fragmentes 3-Amino-3-deoxy-D-glukose (3-Aminoglukose, Kanosamin) wurde via perilymphatischer Perfusion am Meerschweinchen untersucht. Im Gegensatz zu früheren Befunden (Owada 1962) konnte bei Konzentrationen von 10 oder 28 mM kein Einfluß auf das Mikrophonpotential (CM) festgestellt werden. Kanamycin erniedrigte CM signifikant bereits bei 1 mM, und dieser Effekt war wenigstens teilweise reversibel.

Schlüsselwörter: Aminoglykosid-Antibiotika – Aminozucker – Ototoxizität – Perilymphatische Perfusion – Mikrophonpotentiale

Summary. The ototoxicity of the kanamycin fragment 3-amino-3-deoxy-D-glucose (3-aminoglucose, kanosamine), was investigated by perilymphatic perfusion in the guinea pig. Concentrations of 10 or 28 mM of this compound had no effect on cochlear microphonic potentials (CM), contrasting with previous observations (Owada 1962). Kanamycin at 1 mM decreased CM significantly under otherwise identical conditions. The action of kanamycin was at least partially reversible.

Key words: Aminoglycosides – Aminosugars – Ototoxicity – Perilymphatic perfusion – Cochlear microphonic

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Table 1. Ototoxicity in the guinea pig of aminoglycosides and their fragments

Drug	Route of application	Concentration mM	Ototoxicity	Reference
Dihydrostreptomycin	Perfusion	1.4	++	[a]
Streptamine	Perfusion	3.8	−	[a]
Streptidine	Perfusion	3.0	−	[a]
N-methyl-L-glucosamine	Perfusion	4.4	−	[a]
Neomycin	Perfusion	10	++	Lodhi et al. 1980
Neamine	Perfusion	10	−	Lodhi et al. 1980
Methyl-neobiosamine	Perfusion	10	−	Lodhi et al. 1980
Kanamycin	Systemic	[b]	+	Owada 1962
2-desoxystreptamine	Systemic		−	Owada 1962
3-amino-3-deoxyglucose	Systemic		++	Owada 1962
N-acetyl-3-amino-3-deoxyglucose	Systemic		−	Owada 1962
3-amino-3-deoxy-methyl-glucoside	Systemic		−	Owada 1962
6-amino-6-deoxyglucose	Systemic		−	Owada 1962

Ototoxicity as determined by measurement of cochlear microphonic potentials is indicated as (+) or (++)

[a] W. Jung, unpublished pilot studies

[b] s.c. injections of 200 mg drug (Kanamycin-equivalents)/kg body weight daily for 6–40 days

The question whether aminosugars or fragments of aminoglycoside antibiotics have ototoxic properties seems of considerable importance for any molecular theory of ototoxicity of these antibiotics.

We have previously demonstrated that aminoglycosides interact in a ligand-and-receptor-like fashion with polyphosphoinositides (Lodhi et al. 1980; Schacht 1979). The structural prerequisites that we suggested for this interaction (Schacht et al. 1977) are not met by aminosugars. We would thus predict that these compounds do not show aminoglycoside-like toxicity. In contrast, ototoxicity of aminosugars could lend support to a hypothesis of aminoglycoside interference with glucose transport (García-Quiroga et al. 1978).

Owada (1962) reported that 3-amino-3-deoxy-D-glucose (3-aminoglucose, kanosamine), a fragment of kanamycin was more ototoxic by parenteral injection than the parent drug. A survey of other antibiotic fragments (Table 1) shows that 3-aminoglucose is unique in this regard.

We re-investigated the toxicity of 3-aminoglucose using perilymphatic perfusion of the drug in the guinea pig.

Materials and Methods

The methods of cochlear perfusion have been described in detail by Jung and Schön (1979) (Table 2, Exp. 1) or by Nuttall et al. (1977) and Lodhi et al. (1980) (Fig. 1 and Table 2, Exp. 2). Basically, the

Table 2. Effect of drugs on cochlear microphonic potential

Drug	Concentration (mM)	Change of CM (%/min)		*N*	Significance of drug effect (*p*-value)
		Before drug	With drug		
Experiment 1					
Kanamycin A	1.6	−0.29 ± 1.45	−0.78 ± 0.21	4	0.08
Kanamycin A	7.9	−0.28 ± 0.94	−1.04 ± 0.67	5	< 0.01
Kanamycin A	12.5	−0.61 ± 1.96	−1.34 ± 0.59	7	< 0.01
Kanamycin A	18.7	−0.29 ± 0.96	−1.54 ± 1.15	4	< 0.01
3-amino-3-deoxy-glucose	28	−0.56 ± 0.76	−0.42 ± 0.30	5	n.s.
Experiment 2					
No drug	0	−0.26 ± 0.21	−0.31 ± 0.20	6	n.s.
Kanamycin A	10	−0.46 ± 0.36	−1.56 ± 0.90	6	< 0.01
3-amino-3-deoxy-glucose	10	−0.45 ± 0.43	−0.22 ± 0.27	3	n.s.

Perilymphatic spaces were perfused for 15–30 min with artificial perilymph only ("before drug") followed by artificial perilymph containing drug ("with drug"). Numbers are means ± SD Significance of difference was determined by comparing loss of cochlear microphonic potential before and after addition of the drug to the perfusion fluid (Student's *t*-test)

procedure involves opening of the bulla in the anesthetized animal and implantation of capillaries into scala tympani and scala vestibuli of the basal turn of the cochlea. Perfusion is carried out with "artificial perilymph" to which the drug is added. Cochlear microphonic potentials are measured in response to defined sound stimuli. Ototoxicity was determined as the rate of loss of cochlear microphonic potential (%/min) during perfusion with drug as compared to a preceding perfusion period (15–30 min) without drug.

Kanamycin A was obtained as the sulfate from Bristol Laboratories, Syracuse, NY (USA) or as "Kanamycin Grünenthal" or "Resistomycin Bayer". Pure 3-amino-3-deoxy-D-glucose (kanosamine) was a kind gift from Prof. H. H. Baer, Ottawa (Canada).

Results

During perilymphatic perfusions, stable cochlear microphonic potentials occasionally can be maintained for several hours (Fig. 1). The addition of 10 mM 3-aminoglucose (Fig. 1, "B") did not significantly alter CM even after more than 1 h of application. A total of eight perfusions at 10 and 28 mM drug, respectively (Table 2), failed to indicate an effect of 3-aminoglucose. In constrast, kanamycin A, both when added in place of 3-aminoglucose (Table 2) or following this compound (Fig. 1), caused a rapid and reliable decrease of CM, its magnitude being dependent on the concentration of the drug.

In most experiments, the drug treatment was followed by perfusion with "artificial perlymph" without drug. This usually resulted in a decrease of the rate of CM loss (e.g., −0.20%/min ± 1.47 following −1.34%/min for 12.5 mM kanamycin) or an actual increase of CM (e.g., +0.53%/min ± 1.02 following −1.54%/min for 18.7 mM kanamycin) indicating some reversibility of the toxic effect.

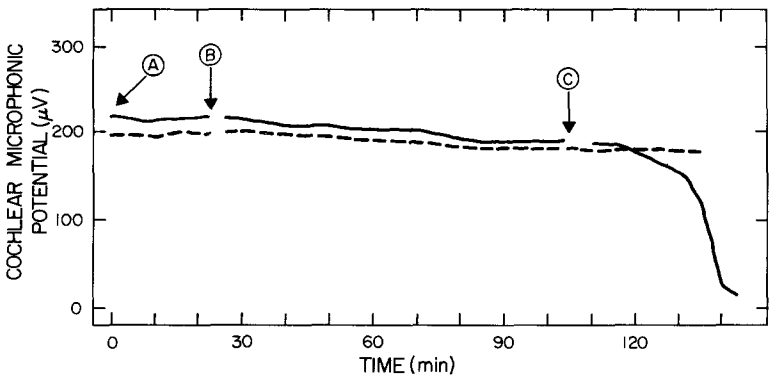

Fig. 1. Cochlear microphonic potential during perfusions. Solid line: Perfusion was started at "A". If a stable response was obtained, drug was added to the perfusion fluid ("B"), here 10 mM 3-amino-3-deoxyglucose. At "C", aminoglucose was replaced by 10 mM kanamycin A. Dashed line: Control perfusion, no drug added at B or C

Discussion

Kanosamine, 3-amino-3-deoxy-D-glucose is clearly not ototoxic when administered by perilymphatic perfusion. Perilymphatic perfusions provide reliable measurement of drug toxicity: they differentiate between aminoglycosides of different toxicity essentially in agreement with systemic application of the drugs (Lodhi et al. 1980), and show concentration dependence of the drug action and high reproducibility as demonstrated here. It therefore seems unlikely that the difference in ototoxicity measured in this and a previous study (Owada 1962) can be explained by the difference in route of administration. This supports our previous conclusions that certain structural features on the intact antibiotics are the carriers of their ototoxic properties.

Reversibility of the suppression of microphonic potentials by aminoglycosides had been demonstrated by Wersäll and Flock (1964) at the lateral line organ. While such reversibility is potentially interesting in antibiotic therapy it is difficult to investigate in conventional animal experimentation where the drugs are administered perenterally. Perilymphatic perfusions may provide a useful tool to study the kinetics of aminoglycoside toxicity.

Fragments of aminoglycoside antibiotics show little or no antibacterial activity. Our results lend support to the notion that it is the intact antibiotic molecule that possesses both ototoxic and antibacterial properties. However, the number of compounds tested is not exhaustive and the question remains whether other small molecules or combinations of fragments may be toxic. Preliminary studies with a kanamycin hydrolysate consisting of a 1 : 1 mixture of 3-amino-3-deoxyglucose and 6-amino-6-deoxyglucose indicated ototoxic properties in perfusions (Jung, unpubl. data). Thus, the toxicity of aminoglycoside derivatives may deserve further careful examination. It seems, however, that 3-amino-3-deoxyglucose is less toxic than previously assumed.

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