Original

Ototoxic Effects of 2',3'-Dideoxy-1-N-Isoseryl Kanamycin A (ISDK) on the Inner Ear in Guinea Pigs

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Abstract: 2',3'-Dideoxy-1-N-isoseryl kanamycin A (ISDK) is one of the derivatives synthesized from 2',3'-dideoxy kanamycin A. In the present study, the ototoxic effect of ISDK on the inner ear of guinea pigs was investigated by comparing it with the effect of amikacin. The antibiotics were administered intramuscularly to male guinea pigs for 3 weeks at dosages of 100 or 200 mg/kg/day. The ototoxic effect of ISDK is weaker than that of amikacin, which is synthesized from kanamycin A by substitution of a 1N-(s)-4-amino-1-hydroxy-butyl moiety.

Key words: 2',3'-dideoxy-1-N-isoseryl kanamycin A, ototoxicity, guinea pigs, amikacin

Introduction

Aminoglycoside antibiotics, such as gentamicin (GM), kanamycin A (KM), and streptomycin have potent antibacterial activity against a wide range of Gram-positive and -negative bacteria, including *Pseudomonas aeruginosa*¹⁻³⁾. Recently, semisynthetic antibiotics, such as arbekacin (HBK), dibekacin (DKB), netilmicin (NTL), and amikacin (AMK), with remarkable activity against resistant bacteria, have been developed commercially to treat resistant infections^{1,4-7)}. Ototoxicity, the main adverse effect of aminoglycoside antibiotics, has been investigated in humans and animals⁸⁻¹³⁾.

In the present study, we compared the ototoxic effects of two KM derivatives, 2',3'-dideoxy-1-N-isoseryl kanamycin A (ISDK)¹⁴⁾ and AMK, in guinea pigs.

Materials and Methods

The Chemical structure of ISDK, shown in Fig. 1, was discovered by Kobayashi *et al.*¹⁴⁾ Male guinea pigs weighing about 320 g received intramuscular injection of ISDK or AMK which were dissolved in saline at dosages of 100 or 200 mg/kg/day for 3 weeks; the dose was expressed in terms of mg (potency). Physiological saline was administered to the control group for 3 weeks at a dosage of 10 ml/kg/day. Six male guinea pigs that showed a normal Pryer's reflex (pinna reflex response) were assigned to each experimental group. During treatment, body weights were measured twice a week, and pinna reflex responses were measured twice a week with an audiometer (Nagashima Co., Ltd., Tokyo) at frequencies of 10, 15 and 20 KHz (90 db to 120 db). After the final administration, all animals were sacrificed under anesthesia. Whole bodies were perfused with physiological

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Fig. 1. Chemical structures of ISDK, AMK, and KM.

saline and fixed with Wittmack's solution to isolate the inner ears. Serial horizontal sections 18 μm thick were prepared and stained with hematoxylin and eosin according to the standard celloidin embedding method for examination under a light microscope.

Results

Mortality and mean body weights

No animals died during treatment with physiological saline (control), ISDK or AMK (Table 1). There were no statistically significant differences between the dosages of ISDK 100 mg/kg, AMK 100 mg/kg, AMK 200 mg/kg and the control. Guinea pigs given ISDK at a dosage of 200 mg/kg/day had a significantly lower average increase in body weight than the control animals after 15 days (Fig. 2); their mean body weights on day 22 were 80% of the control.

Pinna reflex test

There was no loss of pinna reflex in animals receiving 100 mg/kg/day of ISDK or AMK. However, one animal at 10 KHz, 3 animals at 15 KHz, and 5 animals at 20 KHz receiving 200 mg/kg/day of ISDK and 3 animals at 10 KHz, 4 animals at 15 KHz, and 6 animals at 20 KHz receiving 200 mg/kg/day of AMK showed loss of pinna reflex on day 22 (Table 1).

Fig. 2. Average growth curves of male guinea pigs given ISDK and AMK for 3 weeks.

Table 1. Incidence of pinna reflex loss and light microscopic findings of inner ear damage in guinea pigs treated with ISDK and AMK at doses of 100 or 200 mg/kg/day for 3 weeks.

Drug	Dose (mg/kg/day)	No. of dead animals/No. of experimental animals	Incidence of pinna reflex loss on 22nd day**	Cochlea damage*,**	
				Loss of outer hair cell	Loss of inner hair cell
ISDK	100	0/6	{ 10 KHz 15 KHz 20 KHz }	Slight (2)	
	200	0/6	{ 10 KHz (1) 15 KHz (3) 20 KHz (5) }		{ Slight (1) Moderate (2) Severe (3) }
AMK	100	0/6	{ 10 KHz 15 KHz 20 KHz }	Severe (6)	
	200	0/6	{ 10 KHz (3) 15 KHz (4) 20 KHz (6) }		

* Slight: Outer hair cell loss at hook. Moderate: Outer hair cell loss from basal end to the first turn. Severe: Outer hair cell loss from basal end to above the second turn.

** Arabic numbers in parentheses indicate the number of abnormalities.

Histopathological findings in the Cochlea (Table 1 and Fig. 3~7)

No animals receiving 100 or 200 mg/kg/day of ISDK or AMK showed abnormalities of the inner hair cells. Four animals receiving 100 mg/kg/day of ISDK showed no abnormal outer hair cells (Fig. 3). Two animals receiving 100 mg/kg/day of ISDK showed a slight

Fig. 3. Effect of ISDK 100 mg/kg/day for 3 weeks on the outer hair cells (OHC) and inner hair cells (IHC) of Corti's organ in the guinea pig, showing no abnormality of the OHC and IHC at the basal end. ($\times 100$)

Fig. 4. Effect of ISDK 100 mg/kg/day for 3 weeks on the outer hair cells of Corti's organ in the guinea pig, showing intact outer hair cells at the first turn. ($\times 100$)

Fig. 5. Effect of ISDK 200 mg/kg/day for 3 weeks on the outer hair cells of Corti's organ in the guinea pig, showing loss of outer hair cells at the first turn (arrow). ($\times 100$)

Fig. 6. Effect of ISDK 200 mg/kg/day for 3 weeks on the outer hair cells of Corti's organ in the guinea pig, showing loss of outer hair cells at the third turn (arrow). ($\times 100$)

Fig. 7. Effect of AMK 200 mg/kg/day for 3 weeks on the outer hair cells of Corti's organ in the guinea pig, showing loss of outer hair cells from the fourth turn to the apex of the cochlea (arrow). ($\times 100$)

loss of outer hair cells (Fig. 4). Slight (1 animal), moderate (2 animals) and severe (3 animals) changes of outer hair cells were observed in animals receiving 200 mg/kg/day of ISDK (Fig. 5 and 6). Six animals receiving 200 mg/kg/day of AMK showed severe changes of outer hair cells (Fig. 7).

Discussion

ISDK is a KM derivative synthesized from 2',3'-dideoxy kanamycin A which exhibits a high antibacterial activity against aminoglycoside resistant bacteria (Table 2). We confirmed that the LD_{50} values of ISDK, KM, and AMK for male mice are 235 mg/kg, 240 mg/kg and 267 mg/kg i.v., respectively (Table 3). In a previous study, we reported the ototoxic effect of AMK in guinea pigs^{12,13)} and confirmed a slight loss of outer hair cells at a dosage of 100 mg/kg/day and a severe loss of outer hair cells at a dosage of 200 mg/kg/day i.m. Because of these results, dosages of ISDK and AMK of 100 and 200 mg/kg/day were used in this study. Previous studies of the ototoxicity of aminoglycoside antibiotics have demonstrated that the outer hair cells regularly disappear from the basal end to the upper turns of the cochlea. At the same time, the pinna reflex response regularly shows a loss from a high frequency (20 KHz) to a low frequency^{12,15,16)}.

In the present study, we observed cochlear damage in guinea pigs receiving 100 or 200 mg/kg/day of ISDK. The ototoxic effect of ISDK is weaker than that of AMK synthesized from KM by substitution with 1N-(s)-4-amino-1-hydroxybutyl moiety at the same dosage.

We previously reported that the ototoxicity of aminoglycoside antibiotics in guinea pigs increases in the order of gentamicin, sisomicin, tobramycin > dibekacin, AMK > KM, butirosin, micromomicin, HAPA-B > habekacin, netilmicin > ribostamycin, dactimicin^{10,13)}.

Table 2. Minimal inhibitory concentration (mcg/ml $\times 10^{-1}$) of ISDK, KM, and AMK.

Test organisms	KM	AMK	ISDK
<i>Staphylococcus aureus</i> FAD 209	1.56	0.78	0.78
<i>Staphylococcus aureus</i> Smith	0.39	0.39	<0.20
<i>Staphylococcus aureus</i> ApO1 (G)	25.00	3.12	1.56
<i>Bacillus subtilis</i> PCI 219	0.78	0.39	0.20
<i>Escherichia coli</i> K-12	0.78	0.20	0.39
<i>Escherichia coli</i> K-12 ML 1629 (A)	>100.00	0.78	0.78
<i>Escherichia coli</i> K-12 LA290 R55 (D)	50.00	3.12	0.78
<i>Escherichia coli</i> W677	1.56	1.56	1.56
<i>Escherichia coli</i> JR225 (E)	3.12	0.39	0.78
<i>Escherichia coli</i> JR66/W677 (B, D)	>100.00	1.56	3.12
<i>Mycobacterium smegmatis</i> ATCC 607	6.25	0.78	0.78
<i>Proteus rettgeri</i> GN 311	0.78	0.78	1.56
<i>Pseudomonas aeruginosa</i> A3	6.25	<0.20	0.78
<i>Pseudomonas aeruginosa</i> T113 (A)	50.00	1.56	3.12
<i>Pseudomonas aeruginosa</i> GN315 (G)	>100.00	25.00	50.00
<i>Proteus vulgaris</i> OX19	0.78	0.39	0.78
<i>Klebsiella pneumoniae</i> PC1602	1.56	1.56	1.56
<i>Klebsiella pneumoniae</i> 22 #3038 (B, D)	>100.00	6.25	3.12
<i>Providencia</i> sp. Pv16 (F)	3.12	1.56	3.12
<i>Serratia marcescens</i>	12.50	6.25	6.25

Letters in parentheses show the resistant bacteria producing 3'-phosphotransferase-I (A), 3'-phosphotransferase-II (B), 4'-adenylyltransferase (C), 2'-adenylyltransferase (D), 3-acetyltransferase (E), 2'-acetyltransferase (F) and 6-acetyltransferase (G) (Kobayashi Y. et al., Bull Chem Soc Jpn, 1987).

Table 3. The LD₅₀ value of ISDK, AMK and KM for male mice.

Drug	LD ₅₀ value (mg/kg)	Animal species
ISDK	235 (221~252)	Jcl-ICR
AMK	267 (253~286)	Jcl-ICR
KM	240 (221~261)	Jcl-ICR

(Yokota M., 1994)

- The LD₅₀ value was calculated according to the method of Litchfield-Wilcoxon.
- (): The value in parentheses indicates 95% confidence limits.
- Route of administration was intravenous.

Considering the weakness of its ototoxicity, ISDK may be classified as a mildly ototoxic aminoglycoside antibiotic together with tobramycin, dibekacin, and AMK. However, ISDK has stronger ototoxicity than KM, its mother compound.

Acknowledgement

We appreciate the helpful advice and information of Professor Masatoyo Akiyoshi (Pathological Laboratories, Institute for Chemotherapeutics, Chiba) and Dr. Masayuki Yokota (Meiji Seika Kaisha, Ltd.) concerning histopathological examination.

References

- 1) Kawaguchi H, Naito T, Nakagawa S and Fujisawa K: BB-K8, a new semisynthetic aminoglycoside antibiotics. *J Antibiot (Tokyo)*, **25**: 695–708 (1972) (in Japanese)
- 2) Welles JR, Emmerson JL, Gibson WR, Nickander R, Owen NV and Anderson RC: Preclinical toxicology studies with tobramycin. *Appl Pharmacol*, **25**: 398–409 (1973)
- 3) Akiyoshi M: Ototoxicity. In: The aminoglycosides. Ueda Y (Ed). Nankodo, Tokyo, pp 123–140 (1985) (in Japanese)
- 4) Umezawa S, Tsuchiya T, Muto R, Nishimura T and Umezawa H: Synthesis of 3'-deoxykanamycin effective against kanamycin-resistant *Escherichia coli* and *Pseudomonas aeruginosa*. *J Antibiot (Tokyo)*, **24**: 274–275 (1971) (in Japanese)
- 5) Kondo S, Iinuma K, Yamamoto H, Maeda K and Umezawa H: Synthesis of 1-N-[(s)-4-amino-2-hydroxybutyryl]-kanamycin B and 3',4'-dideoxykanamycin active against kanamycin-resistant bacteria. *J Antibiot (Tokyo)*, **26**: 412–415 (1973) (in Japanese)
- 6) Wright JJ: Synthesis of 1-N-ethylsisomicin: a broad-spectrum semisynthetic aminoglycoside antibiotics. *J Chem Soc Chem Commun*, 206–208 (1976)
- 7) Umezawa S and Tsuchiya T: Total synthesis and chemical modification of the aminoglycoside antibiotics. In: Aminoglycoside Antibiotics, Umezawa H and Hooper IR (Eds). Handb Exp Pharm, Springer-Verlag, Berlin, Vol. 62, pp 37–110 (1982)
- 8) Akiyoshi M, Sato K, Nakada H and Tajima T: Audiometrical and histopathological evaluation of ototoxicity of 3',4'-dideoxykanamycin B (DKB), a new semisynthetic aminoglycoside antibiotic in guinea pigs. *Jpn J Antibiot*, **27**: 15–26 (1974) (in Japanese)
- 9) Akiyoshi M, Sato K, Nakada H, Tajima T, Suzuki K and Kishimoto K: Evaluation of ototoxicity of amikacin (BB-K8) by animal test. *Jpn J Antibiot*, **28**: 288–304 (1975) (in Japanese)
- 10) Akiyoshi M, Yano S, Nakada S, Kurebe M, Yokota M, Sakamoto K and Kobayashi F: Ototoxicity of HBK in adult guinea pigs. *Clin Report*, **20**: 7987–8014 (1986) (in Japanese)
- 11) Rober EB and Kaye EF: Studies of aminoglycoside ototoxicity in animal models. In: The Aminoglycosides, Microbiology, Clinical use and Toxicology. Andrew W and Neu HC (Eds). Marcel Dekker Inc, New York, pp 419–452 (1982)
- 12) Yokota M, Sakamoto K, Inouye S, Akiyoshi M and Kitasato I: Ototoxic effects of a pseudodisaccharide aminoglycoside antibiotic, dactimicin, on the inner ears of guinea pigs. *Chemotherapy*, **35**: 431–440 (1989)
- 13) Kitasato I, Yokota M, Inouye S and Igarashihi M: Comparative ototoxicity of ribostamycin, dactimicin, dibekacin, kanamycin, amikacin, tobramycin, gentamicin, sisomicin and neotilmicin in the inner ear of guinea pigs. *Chemotherapy*, **36**: 155–168 (1990)
- 14) Kobayashi Y, Tsuchiya T, Umezawa S, Yoneta T, Fukatsu S and Umezawa H: Synthesis of 2',3'-dideoxykanamycin A, 2',3'-dideoxyamikacin and related substances. *Bull Chem Soc Jpn*, **60**: 713–720 (1987)
- 15) Yokota M: Ototoxic effects of some aminoglycoside antibiotics on the inner ear in guinea pigs. *J Showa Med Assoc*, **37**: 535–544 (1977) (in Japanese)
- 16) Koeda T, Umemura K and Yokota M: Toxicology and pharmacology of aminoglycoside antibiotics. In: Aminoglycoside Antibiotics. Umezawa H and Hooper IR (Eds). Handb Exp Pharm, Springer-Verlag, Berlin, Vol. 62, pp 293–356 (1982)

[Received December 15, 1995: Accepted January 10, 1996]