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Ototoxicity of a New Glycopeptide, Norvancomycin with Multiple Intravenous Administrations in Guinea Pigs

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Time courses of plasma concentration of a new glycopeptide, norvancomycin (NVCM) after single intravenous (iv) and intraperitoneal (ip) injection, and the peak plasma concentrations (Cmax's) of this drug at various doses after single iv injection were determined in guinea pigs. There were significant differences in pharmacokinetic parameters between the two routes of administration and the Cmax linearly increased with the dose used increasing. Guinea pigs with normal hearing were then used to investigate ototoxic liability of NVCM after multiple intravenous administrations (54, 108, 216 mg/kg, qd for 14 days). Postrotatory vestibular nystagmus (PRN), auditory brainstem response (ABR) and electron microscopy (SEM TEM) results showed that, similarly to vancomycin, there was no functional or morphological evidence of ototoxicity of NVCM at the dose of 54, 108 mg/kg. In the high dose group (216 mg/kg), there was a 0～4 dB elevation of hearing threshold but no morphological changes. The results suggested that the ototoxicity of NVCM is absent or minimal after multiple iv administrations within this dose range.

Norvancomycin (NVCM), a new glycopeptide antibiotic, which has the same anti-microbial spectrum as vancomycin (VCM) is used extensively in the clinic to treat methicicillin-resistant *Staphylococcus aureus* (MRSA) infections[1]. This antibiotic differs from vancomycin only in that $-NHCH_3$ at the peptide amino terminal of vancomycin has been replaced by $-NH_2$. A few animal experiments on the ototoxicity of VCM have been reported but there is lack of data on ototoxocity of NVCM. It was found that there was no evidence of ototoxicity when VCM was administrated alone to gerbils or guinea pigs intraperitoneally (ip) at dose level of 75～150 mg/kg qd for 2 weeks. There was, however, a certain threshold elevation after click stimuli in the animals treated with a dose of 300 mg/kg VCM ip[2,3]. Some studies reported that the addition of a small dose of gentamicin (50 mg/kg qd, for 16 days) plus VCM (subcutaneous administration) resulted in a greatly augmented ototoxic effect[4]. NISHIHARA *et al.* however, failed to find such an increased effect in guinea pigs after intramuscular administrations. They ascribed this discrepancy to the difference in route of administration adopted in the experiments[5].

In clinic, patients usually receive NVCM intravenously. There is lack of data on pharmacokinetic parameters of NVCM after administrations with different routes. If there are differences in depositions of NVCM in plasma such as time courses of drug concentration after intravenous and non-intravenous administration, different results regarding ototoxicity might be obtained with various administration routes. Thus, it would be more reliable to draw data on ototoxicity of NVCM in animal experiments using intravenous administration route, as this is the clinically relevant dosing form.

In the present study, the time courses of plasma concentration of NVCM were determined after single iv and ip administration in guinea pigs. The peak plasma

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levels (Cmax) of this drug at various doses after single iv injection were also determined. Multiple intravenous administrations of NVCM (54, 108, 216 mg/kg, qd for 14 days) were then adopted in guinea pigs to explore potential ototoxicity. The same doses of VCM were administrated to other guinea pigs for comparison. After administrations, ototoxicity of NVCM and VCM were evaluated by behavioral, functional and morphological examinations.

Materials and Methods

Pharmacokinetic Study of NVCM

NVCM hydrochloride was obtained from the North China Pharmaceutical Co. (NCPC, China). Saline solutions of 10 mg/ml of drugs were prepared. Healthy guinea pigs were used as subjects. 10 mg/kg NVCM was injected iv (n=6) and ip (n=6) into animals respectively. Saline solution was injected iv and ip into control animals. Blood samples (0.5 ml) were collected immediately, 0.25, 0.5, 1, 1.5, 2, 4, 8, 12 hours after single iv and ip injection. Plasma concentrations of NVCM were determined with high-performance liquid chromatography (HPLC).

The peak plasma levels (Cmax's) of NVCM at various doses (10, 27, 54, 108, 216 mg/kg, six animals in each dose group) after single iv injection were measured with HPLC. The blood samples for the HPLC analysis were collected immediately after each intravenous injection.

Groups and Drug Administration

240 healthy albino guinea pigs with a positive Preyer's reflex, weighing 280～400 g, were used for ototoxic study. The animals were divided into seven different dosage groups (NVCM 54, 108, 216 mg/kg, VCM 54, 108, 216 mg/kg, control saline 1 ml). Each group contained 40 animals except 20 guinea pigs in the 54 mg/kg groups. VCM hydrochloride was from Eli Lilly & Co (USA). Saline solutions of 50 mg/ml of VCM and NVCM drugs were prepared. All drug and saline solutions were administrated intravenously into the left or right vena of pinna, at a rate of 0.1 ml/minute, qd for 14 days.

General Observations/Examinations for Ototoxicity

Each animal was observed daily for changes in physical appearance and behavior (gait and stance). Righting reflex was conducted by the technique described by WAITZ *et al.*[6] before and after administration. Body weights were determined weekly. Feed consumption was visually estimated every day.

Duration of Postrotatory Nystagmus (PRN)

Each animal was kept immobilized in its usual position by a restraining light-proof cage, rotating around a vertical axis at an angular speed of 180°/second. Nystagmus was elicited by interrupting the rotation of the cage after 10 full revolutions. The duration of the ocular nystagmus was the interval measured in seconds between the interruption of the rotation and the end of the zigzag movement of the eyes.

Auditory Brainstem Response (ABR)

Electrophysiologic tests were conducted in an anechoic and electrically shielded chamber. Modified Bravo/Sprit 2000 system (Nicolet Biomedical Inc) was used to record auditory brainstem response (ABR) thresholds of click and short tone burst of 2, 4, 6, 8, 16 and 32 kHz. Input - output functions for ABR after click stimuli were also recorded. Acoustic signals for ABR testing were presented through a TDH-39 earphone. Stimuli were 0.1 msecond click and 4 msecond tone burst (0.1 msecond rise-fall time) delivered at a rate of 11.1/second. Responses were band pass filtered between 100～3000 Hz and averaged 128 times. The stimulus intensity was attenuated in 5 dB steps starting with 90 dB and down to 0 dB nHL. The intensity at which a clear brainstem evoked response (wave III) could be still recognized was considered the threshold level.

Morphological Examination

Surface Preparation for Hair Cell Counting

After the last auditory test (28 days after starting administration), 5 animals of each group were sacrificed for inner and outer hair cell counting. The technique for this method was described previously elsewhere[7].

Scanning and Transmission Electron Microscopy

Five animals of each group were sacrificed for SEM examination and 5 animals for TEM respectively after the last auditory test (28 days after starting administration). The methods have been described previously elsewhere[8].

Statistical Analysis

Statistical analysis was performed with the Student t test, with P of 0.05 as the minimal level of significance. The Dunnett's test was used if the group variances were unequal.

Fig. 1. Time courses of plasma NVCM concentrations after single intravenous and intraperitoneal administration.

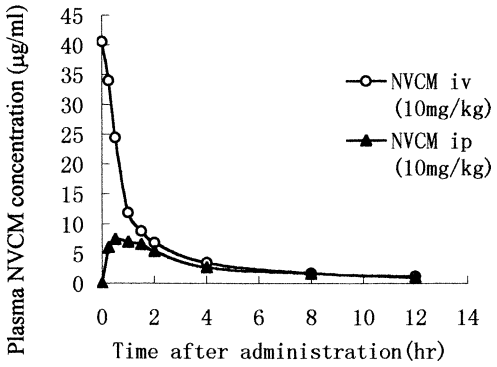
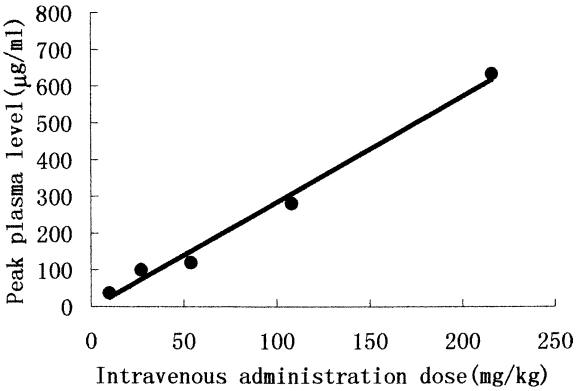


Fig. 2. Peak plasma NVCM levels after various single intravenous injections in guinea pigs.



Results

Pharmacokinetic Study

Time courses of NVCM concentration in plasma after single administration (iv and ip, 10 mg/kg) are shown in Fig. 1. After iv injection, the plasma drug level reached the peak (Cmax) immediately; the Cmax appeared in plasma about 0.23 hours after ip injection. The Cmax after ip was 18.41% of that after iv administration. The area under the drug concentration-time curves (AUC) after ip was 55.41% of that after iv injection.

Fig. 2 shows the average peak plasma concentrations of NVCM (Cmax's) of various dose groups after single iv injection in guinea pigs. At 10 mg/kg level, the Cmax was 40.58 μg/ml; at 54 mg/kg dose level, it reached to 120.1 μg/ml; the average Cmax was as high as 634.3 μg/ml when the high dose of 216 mg/kg was used. The Cmax increased almost completely linearly with the dose increasing ($r=0.9941$).

General Observations

During the injection period, 4 guinea pigs of NVCM 216 mg/kg, 5 animals of VCM 216 mg/kg groups died. Deaths were related to respiratory failure due to neuromuscular blockage. The rest of animals showed a normal walking behavior. They gained body weight and had positive righting reflex after administrations.

Postrotatory Nystagmus (PRN)

The duration of postrotatory nystagmus of each animal before administration arranged from 5 to 9 seconds. As in the saline group, there is no obvious change in average duration of PRN after multiple administrations with NVCM or VCM 54, 108 mg/kg. In the groups treated with 216 mg/kg, the average duration of PRN decreased slightly during and after the administration, however, there was no significant difference before (0 day) and after (14 days, 28 days) the treatment (Fig. 3).

Auditory Brainstem Response (ABR)

Input-output functions for ABR are presented in Fig. 4. In saline group, with stimulus intensity of click increasing, the output amplitude of wave III increased. In NVCM or VCM treated groups, the observed outputs were very close to or overlapping the outputs of the saline animals after multiple administrations.

Threshold shift (hearing loss) was defined as the difference in thresholds of ABR measured before and after administration. As shown in Table 1, the threshold shifts of saline group as well as of the groups treated with NVCM or VCM 56, 108 mg/kg were around 1 dB after administrations. There was no significant difference in hearing loss between the saline and the NVCM or VCM treated groups. In groups injected with NVCM or VCM 216 mg/kg, most animals displayed 0~4 dB hearing loss after administrations. Two weeks later, the hearing loss was still in this range. The threshold shift was more pronounced

Fig. 3. Average duration of PRN during and after the treatment period.

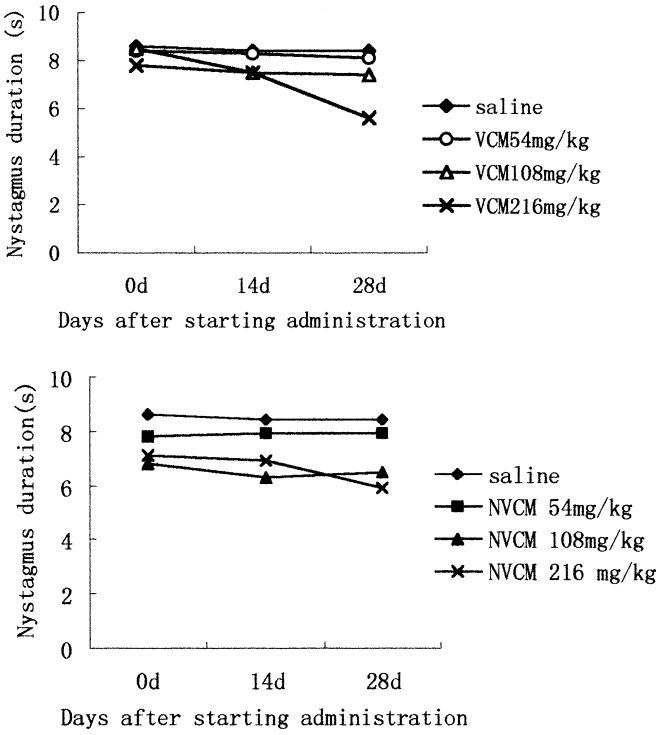


Fig. 4. Input - output functions for ABR of groups treated with NVCM and VCM.

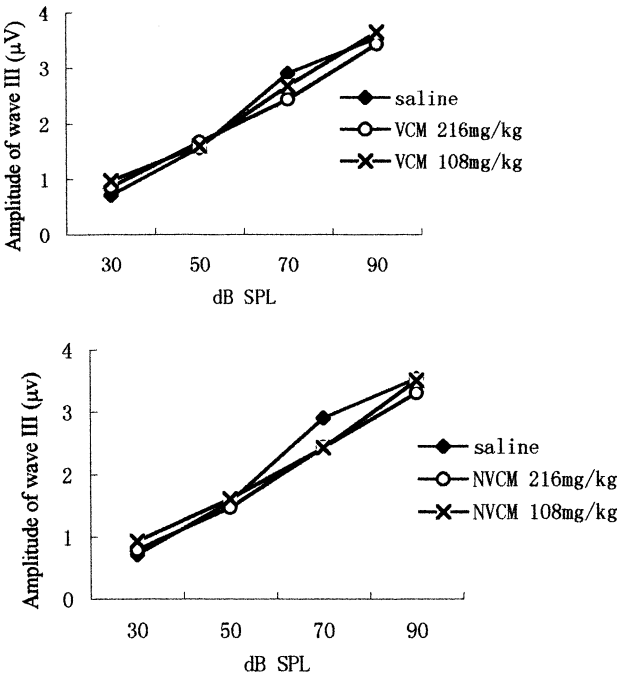


Table 1. Average threshold shift recorded by ABR after click and tone stimuli for different treatment modalities (dB) $\bar{X}$ (±S.D.).

Drugs	Dose mg/kg	Threshold Shift												
		14d*							28d					
		click	2kHz	4kHz	6kHz	8kHz	16kHz	32kHz	click	2kHz	4kHz	6kHz	8kHz	16kHz	32kHz
Control (saline)		0.2±1.1	1.0±2.0	0.5±1.5	1.0±2.0	0.7±1.8	0.1±1.1	0.2±0.8	0.1±1.1	0	0	0	0.2±0.2	0.8±1.1	0.6±1.2
NVCM															
	54	0.7±1.6	0.5±1.5	0.5±1.5	0.8±1.8	1.0±2.1	1.1±1.3	1.6±1.7	0.3±0.8	0	0.6±1.7	0.6±1.7	0.9±2.0	0.9±1.8	1.5±1.4
	108	0.8±1.7	0.3±1.2	0.6±1.7	0.6±1.7	0	0.5±1.3	0.7±0.8	0.8±1.6	0.4±1.4	1.3±2.3	0.4±1.4	0	0.9±1.6	1.9±1.2
	216	3.5±4.1	2.4±3.4	3.2±6.5	4.0±7.7	4.5±6.7	4.6±3.6	4.7±5.2	4.1±4.5	3.2±4.0	3.6±5.0	3.4±3.9	4.0±5.9	4.8±3.6	4.6±4.8
VCM															
	54	0.5±1.5	1.0±2.1	0	0.8±1.8	0.8±1.8	1.0±1.2	1.5±1.5	0	0.6±1.7	0	0.6±1.7	0.6±1.7	0.8±0.9	1.0±1.2
	108	0.5±1.8	1.1±2.7	0	0.3±1.1	0.3±±1.3	1.1±1.1	0.9 ±0.8	0	0	0	0.4±1.4	0.4±1.4	0.6±1.2	1.5±1.8
	216	3.0±3.0	1.0±2.1	4.0±4.2	3.7±3.7	3.0±3.2	4.1±4.6	4.7±2.4	1.6±3.0	1.3±2.3	3.8±3.5	1.9±3.7	1.3±2.3	4.2±1.9	3.9±2.6

* 14d 14days after starting administrations, 28d 28days after starting administrations

The results are means ± standard deviations.

at high-frequency. Statically, there were no significant differences in threshold shift between NVCM or VCM 216 mg/kg and the saline groups.

Morphological Examinations

Hair Cell Counting

Surface preparation showed a normal appearance of inner (IHC) and outer hair cells (OHC) in all groups after 14 day administration. No missing inner hair cells were found in the saline and all NVCM and VCM treated animals. Mean loss of outer hair cells was around 1～2% in all the drug treated groups, which was similar to that observed in the saline group.

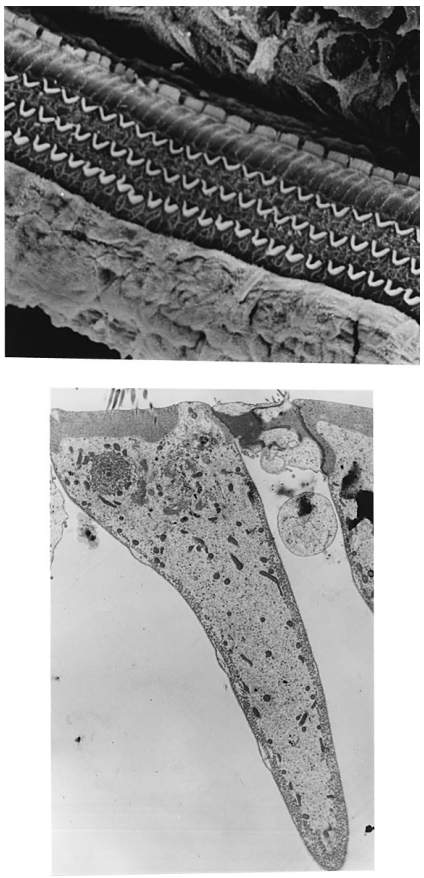
Scanning Electron Microscopy

As the appearance observed in the saline group, each turn of cochleas of all NVCM and VCM treated animals examined demonstrated normal surface structure. There was no sign of missing inner and outer hair cells or destruction. In high magnification, the stereocilia were arranged regularly. No disarray or fracture of hairs was found.

Transmission Electron Microscopy

In all the NVCM and VCM treated groups (56, 108 and 216 mg/kg), the hair cells examined displayed a normal intracellular structure. The cuticular plate was intact with stereocilia extruding outwards. Ample cell organelles such as mitochondria, Golgi bodies and ribosomes appeared in the cytoplasm, which had a normal structure. No swelling afferent or efferent synapses was found under the base of hair cells (Fig. 5).

Fig. 5.



(Upper) Scanning electron microscopy (SEM) of the first turn of organ of Corti after 14 day treatment of norvancomycin (216 mg/kg, iv, per day). There are no signs of missing inner or outer hair cells or destruction (×200).

(Low) Transmission electron microscopy (TEM) of one of OHCs from the first turn of the organ of Corti after 14 day treatment of norvancomycin (216 mg/kg, iv, per day). The hair cell shows a normal shape and size and contains normal cell organelles in the cytoplasm (×3000).

Discussion

In the present study, there was significant difference in the time courses of plasma NVCM concentration between single iv and ip administration. After single intravenous injection, the Cmax, which appeared immediately after administration, was approximately 6 times the Cmax after single ip injection. Although Tmax (time at which the peak concentration was reached) was about 0.23 hours after ip injection, indicating that NVCM was absorbed rather rapidly, the AUC from ip injection was much smaller than that from iv injection. The latter was about 2 times the former. These differences in Cmax and AUC between the two administrations indicated that the absorption process of NVCM from injection site to systemic circulation after ip injection was different from that after iv injection. Lower Cmax and smaller AUC after ip administration reflected reduced bioavailability.

Although there is no general agreement on the effect of pharmacokinetic parameters of drug on toxicity, many investigations reported that Cmax and AUC of VCM and its analogues were two important parameters affecting toxicity, especially ototoxicity[9~11]. Significant differences in Cmax and AUC between iv and ip injection found in the present study suggest that ototoxicity induced by NVCM may be

different at the same dose used between iv and ip administration. On the other hand, since the real fraction of absorbed NVCM from injection site into blood is not known after ip injection, it is not possible to evaluate the dose-dependent ototoxicity of NVCM. Relative low Cmax and small AUC after intraperitoneal injection with high dose might produce a false-negative result of ototoxicity. Thus, in order to obtain reliable data on ototoxicity of NVCM, the intravenous administration route, which is the same route used in clinic, has been adopted in the present animal study.

Low (54 mg/kg), middle (108 mg/kg) and high (216 mg/kg) doses of NVCM were used in the present study. In order to explore any potential ototoxicity of this drug and compare ototoxicity with exposure level, it is essential to ascertain the plasma concentrations of the drug at the various doses used. Peak plasma concentration of NVCM (Cmax) of each guinea pig from various dose groups was measured with HPLC after single iv administration. The results showed that the Cmax increased almost linearly with injection doses increasing ($r=0.9941$). This means that the resultant Cmax correlated to the iv injection dose completely. This is because that the guinea pigs had a relatively fixed and limited blood content, and 100% of iv injected NVCM entered and was added into the blood content immediately after injection. That the Cmax's were dose dependent even at high dose levels makes it possible to evaluate the dose-dependent ototoxicity of NVCM in the present study.

All the animals treated with NVCM in this study displayed normal walking behavior and normal righting reflex, demonstrating that the equilibrium function of these guinea pigs were perfect after administrations[12]. PRN is usually used to evaluate vestibular organ sensitivity[13]. Inhibition of PRN was often observed in the impairment of vestibular organs induced by aminoglycosides, lead exposure or alcohol administration[14,15]. In the present study, the average duration of PRN did not change significantly after administration in all NVCM or VCM treated groups. Thus, it is concluded that there was no evidence of vestibulotoxicity of NVCM in these dosages in this study.

The amplitude of evoked auditory potentials stimulated by a given intensity of sound is correlated to the number of living hair cells in the cochlea that were excited synchronously[16]. Like the results observed in saline and VCM treated groups, the amplitude of ABR of the animals in all NVCM groups increased with increasing intensity of click stimulation. Input-output functions of these drug-treated groups were close to or overlapping that in the saline group. From this finding, it is inferred that no obvious loss of hair cells occurred in these cochleas after NVCM administrations. Accordingly, hair cell counting with light microscopy showed that there was no significant inner or outer hair cell missing in all NVCM and VCM treated guinea pigs. Few studies investigated ototoxicity induced by VCM and analogues at electron microscopic level. In the present study, both SEM and TEM showed normal cochlear surface and intracellular structure of hair cells in all the NVCM and VCM treated animals. Based on these results, it is believed that multiple administrations of NVCM at the doses used in this study would not damage these hair cells or cause cell death.

In the NVCM 54 and 108 mg/kg groups (the doses used are 4~8 times the recommended therapeutic dose), there was less than 2 dB threshold shift immediately, and 2 weeks after the 14 day administration, which was similar to the threshold shift in VCM and saline groups. These results suggested that, similarly to VCM, there was no hearing impairment of NVCM at these dosages. In NVCM 216 mg/kg group (the dose used is 16 times the recommended therapeutic dose), there was 0~4.7 dB elevation of threshold after administration. SEM and TEM, however, showed normal structure of cochleas in this group. The non-correlation between threshold shift and morphology indicated that the hearing impairment might be only functional. Although there was no statically significant difference in hearing loss between NVCM and saline group, a certain slight elevation of threshold suggested that ototoxic dose of 216 mg/kg induced minimal damage.

As in many reported animal studies on ototoxicity of VCM[2,4,5], high dosage of NVCM (216 mg/kg, qd for 14 days) was used in this study. The purpose was to find potential dose-response ototoxicity of NVCM after systemic application. Considering safety in the clinic, high dose of this drug should be used in animal toxic experiment because there might be difference in ototoxic sensitivity to NVCM between guinea pig and human. Since there was no any evidence of ototoxicity at 54 and 108 mg/kg level, high level (216 mg/kg) has been adopted to evaluate the ototoxic dose of NVCM. The results showed that only at this dose level did minimal hearing damage appear. The very high ototoxic dose level in animals (216 mg/kg, much higher than the normal therapeutic dose) suggested that there is little ototoxicity of NVCM in the clinic.

There was no significant difference in hearing loss between all NVCM and VCM treated groups, indicating that the ototoxicity of the both drugs is similar. Minimal risk in oto- and vestibul-toxicity of NVCM in this study encourages the clinical use of this drug if precautions and

close monitoring are strictly maintained.

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References

1) YAN, H.; D. QI, X. CHENG, Z. SONG, W. LI & B. HE: Antibiotic activities and affinities for cell wall analogue of *N*-demethylvancomycin and its derivatives. J. Antibiotics 51(8): 750～756, 1998
2) LUTZ, H.; T. LENARZ, H. WEIDAUER, P. FEDERSPIL & S. HOTH: Ototoxicity of vancomycin: an experimental study in guinea pigs. ORL. J. Otorhinolaryngol. 53: 273～278, 1991
3) TANGE, R. A.; H. L. KIEVIET, J. V. MARLE, D. BAGGER-SJBÖÄCK & W. RING: An experimental study of vancomycin-induced cochlear damage. Arch. Otorhinolaryngol. 246: 67～70, 1989
4) BRUMMETT, R. E.; K. E. FOX, F. JACOBS, J. B. KEMPTON, Z. STOKES & A. B. RICHMOND: Augmented gentamicin ototoxicity induced by vancomycin in guinea pigs. Arch. Otolaryngol. Head Neck Surg. 116: 61～64, 1990
5) NISHIHARA, K.; T. SHIMIZU, H. KOTAKI, Y. SAWADA, T. OKUNO, K. KAGA, T. MUROFUSHI & T. IGA: Lack of effect of vancomycin and gentamicin on auditory function in guinea pigs. Antimicrob. Agents Chemother. 40:1098～1103, 1996
6) WAITZ, J. A.; E. L. MOSS & M. J. WEINSETEIN: Aspects of chronic toxicity of gentamicin sulfate in cats. J. Infec. Dis. 124: S125～S129, 1971
7) GAO, W. Y.; D. L. DING, X. Y. ZHENG & F. M. RUAN: Changes in the stereocilia and non-monotonic pattern of threthold shift after exposure to impulse noise. Hearing Research 54: 296～304, 1991
8) GAO W. Y.; M. L. WIEDERHOLD & R. HEJL: Ultrastructure of endolymphatic sac in the larva of the Japanese red-bellied newt *Cynops pyrrhogaster*. Cell and Tissue Research 291: 549～559, 1998
9) BEGG, E. J.; M. L. BARCLAY & C. M. KIRKPATRICK: The therapeutic monitoring of antimicrobial agents. Br. J. Clin. Pharmacol. 52 (Suppl. 1): 35S～43S, 2001
10) SACRISTAN, J. A. & J. SOTO: Vancomycin peak concentration and ototoxicity prevention. J. Antimicrob. Chemother. 30(6): 865～866, 1992
11) WOOD, M. J.: The comparative efficacy and safety of teicoplanin and vancomycin. J. Antimicrob. Chemother. 37: 209～222, 1996
12) GIANOLI, G. J.; B. DUFF, J. M. KARTUSH & K. R. BOUCHARD: Triple semicircular canal occlusion versus labyrinthectomy in the cat. Am. J. Oto. 18(1): 74～78, 1997
13) SHIMOGORI, H. & H. YAMASHITA: Efficacy of intracochlear administration of betamethasone on peripheral vestibular disorder in the guinea pig. Neurosci. Lett. 294: 21～24, 2000
14) PARRAVICINI, L.; A. FORLANI, M. MAZANATTI & A. ARPINI: Comparative ototoxicity of dibekacin and netilmicin in guinea pigs. Acta Pharmacol. et Toxicol. 53: 230～235, 1983
15) MAMELI, O.; M. A. CARIA, F. MELIS, A. SOLINAS, C. TAVERA, A. IBBA, M. TOCCO, C. FLORE & F. SANNA RAANDACCIO: Neurotoxic effect of lead at low concentrations. Brain Res. Bull. 55(2): 269～275, 2001
16) SALVI, R. & R. BURKARD: Auditory physiology. *In Eds.* B. F. MARPLE and W. L. MEYERHOFF, Hearing Loss. Thieme Medical Publishers Inc., New York, NY. pp. 27～53, 1997