高神大學醫學部 論文集 第2卷 第1號
The Journal of Kosin Medical College
Vol. 2, No. 1, March, 1986

EFFECTS OF GENTAMICIN ON THE SHORT-CIRCUIT CURRENT ACROSS THE ISOLATED FROG SKIN

고신대학 의학부 의예과 및 생리학교실

홍성원, 박양생

Gentamicin 이 개구리 피부를 통한 short-circuit current 에 미치는 영향

Sung Won Hong and Yang Saeng Park

Departments of Premedical Sciences and Physiology
Kosin Medical College, Pusan, Korea

= 국문요약 =

상피 조직의 Na 이동에 미치는 gentamicin(GM)의 영향을 연구하기 위하여 Ussing과 Zerahn의 방법에 따라 적출된 개구리 피부에서 short-circuit current(SCC)와 산소 소모능에 대한 GM의 영향을 조사하였다. GM을 투여하지 아니했을 때 SCC는 초기에 서서히 감소하다가 40-60분 후에 249.1㎂/3.14㎠ 내외로 안정되었다. 피부 내측에 GM을 0.1, 0.2, 0.5, 그리고 1.0㎎/㎖ 농도로 각각 투여했을 때 초기(10분)에는 SCC가 증가하였으나 그후 서서히 감소하여 약 60분 후에는 각각 약물투여 전의 88, 84, 82 및 75%가 되었다. 그러나 GM을 피부 외측에 투여했을 때는 0.1㎎/㎖에서 51%, 0.2㎎/㎖에서 37%, 그리고 0.5-1.0㎎/㎖에서 16-17% 정도 SCC가 증가하였다. 한편 피부 내측과 외측에 GM을 0.1㎎/㎖ 농도로 동시 투여했을 때는 약물투여 전보다 SCC가 15% 정도 증가했는데, 이러한 증가는 상피세포 외측 막의 Na이동에 대한 GM의 영향과 내측막의 Na이동에 대한 GM의 영향이 합쳐진 결과 나타난 것으로 보여진다.

SCC에 대한 영향이 상대적으로 가장 큰 GM농도(0.1㎎/㎖)에서 개구리 피부의 산소 소모율은 대조군과 별다른 차이를 보이지 아니했다.

이상의 성적으로 보아 GM은 개구리 피부 상피세포의 외측막에서 Na 투과도를 증가시키며 내측막에서는 능동적 Na이동을 감소시키는 것으로 사료된다.

I. INTRODUCTION

Although gentamicin is a prototype aminoglycoside antibiotic which is effective in treating Gram-negative bacterial infections, its clinical

use has been limited mainly due to the development of nephrotoxicity.[1,13,21] Numerous studies conducted in the past indicated that the gentamicin-induced renal toxicity is confined to the cortical segment of the proximal renal tubule.

When gentamicin is administered into an animal it is accumulated in the renal cortex and not in the renal medulla and liver.[20,26] Following glomerular filtration, gentamicin is significantly reabsorbed and accumulated within the cells of the proximal convoluted tubule.[3,17,32] Furthermore histopathologic lesions induced by gentamicin administration are specifically localized in the proximal tubule.[11,15,21]

Various studies on the mechanism of aminolycoside nephrotoxicity indicated that early events of pathogenesis involve 1) alterations in plasma membrane structure and function,[18,38,39] 2) interference with mitochondrial energy production,[33,37] 3) lysosomal dysfunction including phospholipidosis and/or changes in phospholipid metabolism,[5,6,12,19,22,31] and 4) inhibition of the membrane associated enzyme, Na-K-adenosine triphosphatase.[38,39,40]

With respect to renal function, one of the earliest abnomalities in aminoglycoside treated animals is a decrease in urine concentrating ability.[15,21] Although the mechanism of this phenomenon has been a controversial issue,[13] recent studies by Lee and Park[16] suggested strongly that it involves reduction in Na reabsorption in the proximal tubule. In rats chronically treated with gentamicin, the above authors observed that despite the urine concentrating ability was significantly reduced in the absence of changes in glomerular filtration rate, the negative free water clearance was not reduced below the control level, indicating that both the activity of the countercurrent multiplication system in the Henle's loop and the ADH-dependent water reabsorption in the distal nephron were not impaired by the gentamicin treatment. They, therefore, concluded that gentamicin-induced polyuria and urine concentrating defect are primarily attributed to the osmotic diuresis associated with proximal tubular Na rejection

The present study was therefore undertaken to investigate the mechanism(s) with which gentamicin impairs epithelial Na transport using frog skin preparations. Since the mechanism of Na transport in amphibian skins is similar to that in renal tubules, the isolated amphibian skin has been widely used as a model system to investigate the nature of Na transport process in the kidney tubule.[7,10,28,29,34] In the frog skin the transepithelial Na transport takes place in two consecutive steps. Na first diffuses into the cell across the apical border and then moves out of the cell by the active Na pump mechanism in the basolateral membrane.[4,14] This movement of the sodium ion generates an electrical current flow and in fact the magnitude of the short-circuit current across the skin has been proven to be equivalent to the active Na transport.[35] We therefore used in the present study the technique of short-circuit current in evaluating the gentamicin action on the Na transport process in frog skin epithelium.

Ⅱ. MATERIALS AND METHODS

The common frogs, ***Rana nigromaculata,*** were captured in the Kimhae field near Pusan, Korea, and brought to the laboratory where they were kept fasting in tap-water at the room temperature of approximately 25℃. The experiments were performed on these animals within 2~3 weeks of capture. The short-circuit current

(SCC) across the isolated skin was measured using the technique of Ussing and Zerahn.[35] The skin was removed from a frog and mounted as a flat sheet between two Lucite chambers having a cross-sectional area of 3.14㎠. The potential difference across the skin was measured with a pair of calomel electrodes connected to the chamber by Ringer-KCl-agar bridges and a DC digital voltmeter. Current was driven through the skin from an external source via Ag-AgCl electrodes connected to the chamber by another pair of Ringer-agar bridges. For the measurement of SCC a digital microammeter was used. The skin was bathed on both sides with a Ringer solution containing 115mM NaCl, 3.5mM KCl, 2.5mM $NaHCO_3$, and 1mM $CaCl_2$ and adjusted to pH 7.4. The solution was continuously stirred with a stream of air. The gentamicin sulfate stock solution (80㎎/㎖) was purchased from Kukjei Pharmacological Co., Korea. This solution was diluted with Ringer solution to the appropriate final concentration of gentamicin. In all cases, pH of the gentamicin-Ringer solution was adjusted to 7.4 with NaOH.

When potential difference and SCC were stabilized which usually occurred at 40~60 min after mounting the frog skin, gentamicin was applied to the mucosal, serosal, or both sides of the skin and followed the changes in SCC for the next 60 min When bathing solution of only one side of the skin was replaced by the gentamicin-Ringer solution, the other side was filled with fresh plain Ringer solution. In some experiment oxygen consumption of the isolated frog skin was measured using a biololgical oxygen monitor (Yellow Spring Instrument, Model 53). After the skin was removed from the animal it was divided sagitally into two identical pieces, which were incubated in normal Ringer solution with airation. After 60 min, approximately 20~50mg of the tissue from each preparation was transferred into the polarography reaction vessel containing 2ml of the room-air saturated normal Ringer or gentamicin (0.1㎎/㎖) containing Ringer solution, and the changes in PO_2 in the medium were measured using a Clark oxygen electrode for 40 min at 25℃. The amount of oxygen consumption by the tissue (QO_2) was calculated using the Henry's law :

$$\text{Change in oxygen content} = \alpha \times \frac{PO_2}{P_B} \times V$$

where α is the O_2 solubility in water at 25℃; PO_2, partial pressure of oxygen; P_B, barometric pressure; and V, volume of incubation medium in milliliters.

Ⅲ. RESULTS

Fig. 1. illustrates average time course of SCC change in 7 frog skin preparations bathed in normal Ringer solution. After the skin was mounted to the chamber the SCC was gradually reduced and stabilized at about 249.1㎂/3.14㎠ after 40~60 min. Therefore, in experiments on gentamicin effect, the drug was admi-

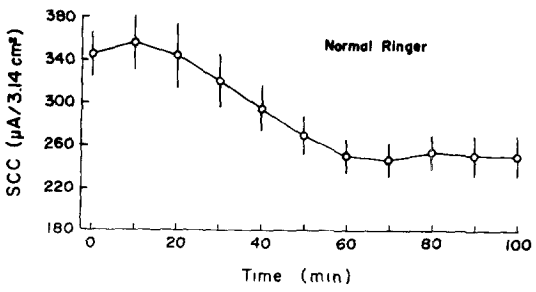

Fig. 1. Short-circuit current (SCC) change in 7 frog skins bathed in normal Ringer solution. Each point represents the mean±SE.

nistered to the chamber after the SCC was stabilized (this will be called as the basal SCC) and observed the SCC change during the following one hour Since, however, absolute value of the basal SCC was so variable between each skin preparation the value of SCC after gentamicin administration was expressed as the percentage of the basal SCC (control) of that preparation.

Fig. 2. shows changes in SCC across the frog skin after gentamicin was added to the serosal bathing medium at a concentration of 0.1, 0.2, 0.5, or 1.0mg/mℓ. At all concentrations tested, gentamicin slightly increased the SCC during the initial 10 min period and then gradually reduced to the stable value which was observed at 40~50 min after the drug administration. The degree of SCC inhibition by gentamicin was proportional to the concentration of the drug. The relative SCC at the point of maximal inhibition was approximately 88, 84, 82, and 75% of the respective control value at 0.1, 0.2, 0.5, and 1.0mg/mℓ of gentaicin, respectively.

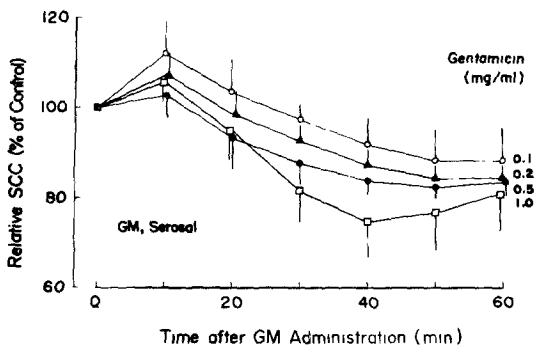

Fig. 2. Changes in short-circuit current (SCC) after gentamicin (GM) was added to the serosal side of the frog skin. Each point represents the mean of 4~6 skins±SE. Absolute value of SCC at time zero was on the average of 215.63±9.40μA/3.1㎠(n=40)

Surprisingly, when gentamicin was applied to the mucosal side of the frog skin the SCC was significantly increased, the effect being inversely proportional to the concentration of the drug in the medium. As depicted in Fig. 3, the SCC increased by about 51% at 0.1mg/mℓ, by 37% at 0.2mg/mℓ, and by 16~17% at 0.5~1.0mg/mℓ of gentamicin.

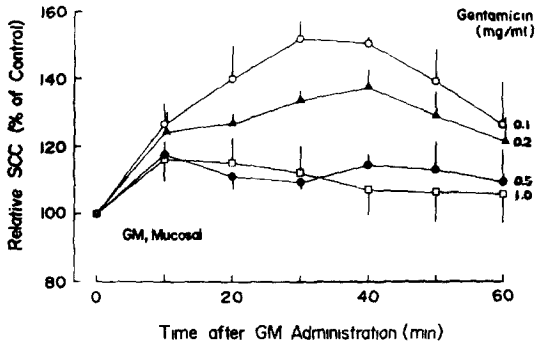

Fig. 3. Changes in short-circuit current (SCC) in gentamicin (GM) added to the mucosal side of frog skin. Each data point represents the mean of 4 to 6 frog skins±SE.

Since gentamicin appeared to have different effects on the Na transport at the two sides of the frog skin, we next tested the combined effect of gentamicin in the mucosal as well as serosal bathing media Fig. 4, compares SCC changes in the skins treated with gentamicin (0.1mg/kℓ) at the mucosal (M), serosal (S), and both mucosal and serosal (M+S) sides measured at the same day. As usual, gentamicin increased the SCC at the mucosal side and decreased at the serosal side. When gentamicin was applied to the both sides of the skin the SCC increased slightly (15%). Overall, the value of SCC in the later situation was somewhere between those observed with gentamicin at the mucosal or serosal side alone. However, inspection of the time courses of the SCC change

indicated that it was not a simple sum of the mucosal and serosal effects of gentamicin (see Fig. 4. dotted line). It thus seems that there is some interaction between gentamicins applied to mucosal and serosal borders of the epithelial cells in modulating overall Na transport.

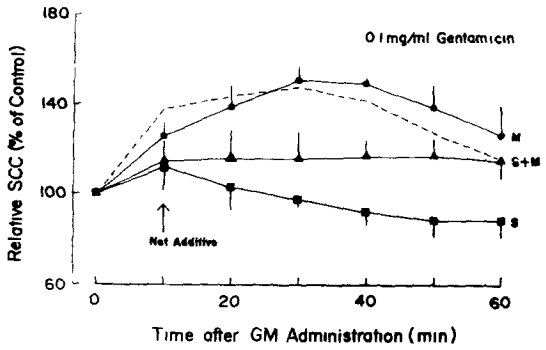

Fig. 4. Changes in short -circuit current (SCC) at 0.1mg/mℓ concentration of gentamicin (GM) added to the mucosal (M), serosal (S), and both the mucosal and serosal (S+M) sides of frog skin Each data point represents the mean from 3 to 5 frog skins± SE.

In order to test the possibility that the reduction of SCC by gentamicin applied to the serosal side was secondary to the metabolic alterations in epithelial cells, the effect of gentamicin on the oxygen consumption of the tissue was studied in the next series of experiments. The results, however, indicated that oxygen consumption of the skin tissue was not significantly changed by 0.1mg/mℓ of gentamicin (Table 1).

Ⅳ. DISCUSSION

The present study is primarily concerned with whether the gentamicin has acute effects on the transepithelial transport of Na ions in the frog skin.

In order to investigate the gentamicin action on the transepithelial sodium transport, it is necessary to understand the processes of sodium transport in the epithelial cell. According to a classical hypothesis[45] sodium transport across the frog skin involves at least two steps : sodium enters the epithelial cell across the mucosal border and then is actively extruded across the serosal border The first process appears to be a rate-limiting step for overall transport of sodium[2,41] and the second process is the one which involves the sodium pump.[25] Since under normal conditions the intracellular concentration of Na is maintained low, the sodium pump mechanism located in the serosal membrane is usually operating at a submaximal rate. In the present study, gentamicin (0.1~1.0mg/mℓ) applied to the serosal surface of the frog skin induced 12~25% inhibition of the SCC (i.e., net transepithelial Na transport. See Fig 2). This inhibition of Na transport was probably due to direct alterations in Na pump activity since the oxygen utilization of the tissue was not impaired by the drug (see Table 1). Interestingly, however, the same concentrations of gentamicin administered to the mucosal surface of the skin increased the net trans-

Table 1. Effect of Gentamicin on Oxygen Consumption of the Frog Skin.

	QO_2 (μl/gm/40 min)
Control	73. 18 ± 3. 25
Gentamicin (0. 1 mg/mℓ)	74. 12 ± 2. 67

Note. Each datum represents the mean ± SE for 5 preparations.

epithelial Na transport by about 15~50% Since the transport of Na across the cell is rate-limited by the mucosal entry step, the above finding suggests strongly that gentamicin increase the Na permeability of the mucosal membrane. This increase in Na permeability will lead to rise in cellular Na content and consequently activation of the Na pump mechanism at the serosal membrane. Whether gentamicin has similar effects on epithelial cells of the kidney tubules is not precisely known. However, several studies in mammalian kidneys suggest such a possibility. Williams et al.[19] reported that gentamicin inhibited the Na-K-ATPase activity in the isolated perfused rat kidney tubule. Since the enzyme plays a crucial role in the active transport of Na across the renal tubule[27] its inhibition by gentamicin will reduce extrusion of Na ions across the serosal (or basolateral) membrane and consequently the transepithelial Na transport will be decreased. The effect of gentamicin on the Na permeability of the mucosal membrane of the renal tubular cell has not been systematically explored However, the fact that gentamicin increases the permeability of the apical (mucosal) membrane to Ca^{++} and possibly other ions[23,24] suggests that the permeability to Na may also be increased by the drug. Whether gentamicin increases the ionic permeability at the tight junction area of the apical border is not known. If the Na permeability of the junctional area was also increased, then the gentamicin-induced increase in mucosal membrane Na permeability may not result in an increase of overall net Na transport. In the proximal tubule, a considerable fraction of Na transported into the basolateral side is normally leaked back into the lumen through the tight junction structure and the amount of the leakage is increased if the junctional permeability is increased[30] Thus, unlike in tight epithelia, as frog skin, modulations in proximal tubular Na reabsorption is due primarily to changes in the rate of backflux and not to alterations in active transport.[30]

In the present study the stimulation of net Na transport by gentamicin is inversely proportional to its concentration on the mucosal surface and directly proportional to its concentration on the serosal surface (see Figs 2 and 3). These suggest that some portions of drugs applied to the mucosal side move through the cell and alter Na pump mechanism at the serosal membrane. In fact, in the mammalian kidney, gentamicin is taken up by the proximal tubular cell through the mucosal membrane[3,17,32] possibly by the pinocytotic process and diffuses through the cytoplasm.[36] Williams et al.[40] observed that when isolated rat renal tubules were perfused with gentamicin the Na-K-ATPase activity was inhibited. They therefore suggested that this inhibition of the enzyme activity was due to interaction of gentamicin at the cytoplasmic face of the basolateral membrane.

The effect of gentamicin on the mucosal permeability may be more significant in affecting overall net Na transport in the intact kidney than in the isolated frog skin. Since under normal conditions approximately 70% of the glomerular filtrate is reabsorbed from the proximal tubule[8] the gentamicin concentration at the luminal side of the cell will become much higher than that at peritubular side.

On the basis of above considerations it is believed that both in the frog skin and in the renal proximal tubule gentamicin increases the Na permeability of the mucosal border and inhibits the activity of the Na pump mechanism at the serosal border of the epithelium These alterations in membrane functions may be re-

sponsible for the early events of gentamicin nephrotoxicity characterized by increased proximal Na rejection and polyuria.[16]

V. SUMMARY

In an attempt to evaluate the mechanism of gentamicin (GM) action in transepithelial Na transport, the effect of GM on the short-circuit current (SCC) across the isolated frog skin was studied. After the skin was mounted to the chamber the SCC was gradually reduced and stabilized at about 249.1 μA/3.14cm^2 after 40~60 min. When the GM (0.1~1.0mg/mℓ) was added to the serosal bathing medium the SCC increased slightly during the initial 10 min period and then declined gradually below the control level until it stabilized at 50~60 min. The relative SCC at the point of maximal inhibition was 88, 84, 82 and 75% of the respective control value at 0.1, 0.2, 0.5, and 1.0mg/mℓ of GM, respectively. When GM was applied to the mucosal side of the frog skin the SCC increased by 51% at 0.1mg/mℓ, by 37% at 0.2mg/mℓ, and by 16~17% at 0.5~1.0mg/mℓ. When 0.1mg/mℓ of GM was simultaneously administered to both the mucosal and serosal sides of the skin the SCC increased by about 15%. This increase in SCC was equivalent to the sum of the SCC changes induced by GM applied to mucosal or serosal side alone. The oxygen consumption of frog skin in the presence of 0.1mg/mℓ GM was 74.12±2.67 μl/gm/40min, and this value was not significantly different from the control value.

These results suggest that GM increases the Na permeability of the mucosal border and inhibits the activity of Na pump mechanism at the serosal border of the frog skin epithelium.

REFERENCES

1. Appel GB, Neu HC : The nephrotoxicity of antimicrobial agents (Second of three parts) N Engl J Med 269 : 722~728, 1977
2. Cereijido M, Herrera FC, Flanigan WJ, Curran PF : The influence of Na concentration on Na transport across frog skin. J Gen Physiol 47 : 879~893, 1964
3. Collier VU, Lietman PS, Mitch WE : Evidence for luminal uptake of gentamicin in the perfused rat kidney. J Pharmacol Exp Ther 210 : 241~251, 1979
4. Curren PF, MacIntosh JR : A model system of biological water transport Nature London 193 : 347, 1962
5. Feldman S, Wang MY, Kaloyanides GJ : Aminoglycosides induce a phospholipidosis in the renal cortex of the rat : An early manifestation of nephrotoxicity J Pharmacol Exp Ther 220 : 514~520, 1982
6. Feldman S, Wang M, Levine R, Pastoriza E, Kaloyanides GJ : Aminoglycosides induce a renal phospholipidosis in the rat. Clin Res 29 : 554A, 1981
7. Ferreira KTG : The effect of Cu^{2+} on isolated frog skin Biochem Biophys Acta 203 : 555~567, 1970
8. Harth O : Chapter 27. The function of the kidneys In *Human Physiology*, ed. by Schmidt RF and Thews G, Springer-Verlag, Berlin, 1983
9. Herrera FC, and Curran PF : The effect of Ca^{++} and antidiuretic hormone on Na^+ transport across frog skin I Examination of interrelationships between Ca^{2+} and hormone. J Gen Physiol 46 : 999~1010, 1963
10. Hong SK, Szekerczes J, Park YS, Kurata FK, Gardner KD Jr. : Effects of diphenylamine on sodium and water transport across the toad skin and bladder. Toxicol Appl Pharmacol 27 : 612

~620, 1974

11. Houghton DC, Hartnett M, Campbell-Boswell M, Porter G, Bennett W : A light and electron microscopic analysis of gentamicin nephrotoxicity in rats. Am J Pathol 82 : 589~612, 1976
12. Kacew S : Gentamicin or chlorphentermine induction of phospholipidosis in the developing organism : role of tissue and species in manifestation of toxicity. J Pharmacol Exp Ther 232 : 239~243, 1985
13. Kaloyanides GJ, Pastoriza-Munoz E : Aminoglycoside nephrotoxicity. Kidney Int 18 : 571~582, 1980
14. Koefoed-Johnsen V, Ussing HH : The nature of the frog skin potential. Acta Physiol Scand 42 : 298~308, 1958
15. Kosek JC, Mazze RI, Cousins MJ : Nephrotoxicity of gentamicin. Lab Invest 30 : 48~57, 1974
16. Lee HY, Park YS : Comparative nephrotoxicities of gentamicin and tobramycin in the rats. Korean J Internal Med 29 : 264~271, 1985
17. Lipsky JJ, Cheng L, Sacktor B, Lietman PS : Gentamicin uptake by renal tubule brush border membrane vesicles. J Pharmacol Exp Ther 215 : 390~393, 1980
18. Lipsky JJ, Lietman PS : Neomycin inhibition of adenosine triphosphatase : Evidence for a neomycin-phospholipid interaction. Antimicrob Agents Chemother 18 : 532~535, 1980
19. Lipsky JJ, Lietman PS : Aminoglycoside inhibition of a renal phosphatidylinositol phospholipase C. J Pharmacol Exp Ther 220 : 287~292, 1982
20. Luft FC, Kleit SA : Renal parenchymal accumulation of aminoglycoside antibiotics in rats. J Infect Dis 119 : 406~409, 1974
21. Luft FC, Patel V, Yum MN, Patel B, Kleit SA : Experimental aminoglycoside nephrotoxicity. J Lab Clin Med 86 : 213~220, 1975
22. Marche P, Koutouzov S, Girard A : Impairment of membrane phosphoinositide metabolism by aminoglycoside antibiotics : streptomycin, amikacin, kanamycin, dibekacin, gentamicin and neomycin. J Pharmacol Exp Ther 227 : 415~420, 1983
23. Michell RH : Inositol phospholipids in membrane function. Trends Biochem Sci 4 : 128~131, 1979
24. Michell RH, Jafferji SS, Jones IM : The possible involvement of phosphatidylinositol breakdown in the mechanism of stimulus-response coupling at receptors which control calcium gates. Adv Exp Biol 83 : 447~465, 1977
25. Millis JW, Ernst SA, Dibon DR : Localization of Na pump site in frog skin. J Cell Biol 23 : 88~110, 1977
26. Morin JP, Viotte G, Vandewalle A, Van Hoof F, Tulkens P, Fillastre JP : Gentamicin-induced nephrotoxicity : A cell biology approach Kidney Int 18 : 583~-590, 1980
27. Nechay BR, Nelson JA : Renal ouabain-sensitive adenosine triphosphatase activity and Na reabsorption J Pharmacol Exp Ther 175 : 717~726, 1970
28. Palmer LG, Edelman IS, Lindemann B : Current-voltage analysis of apical sodium transport in toad urinary bladder : Effects of inhibitors of transport and metabolism. J Membrane Biol 57 : 59~71, 1980
29. Park YS, Hong SK : Effects of insulin on short-circuit current and Na-K-ATPase activity of the toad skin and urinary bladder. Comp Biochem Physiol 58 : 155~158, 1977
30. Rose BD : Chapter 5 Proximal tubule. In *Clinical Physiology of Acid-Base and Electrolyte Disorders.* McGraw-Hill, Inc., 1977
31. Sastransinh M, Knauss TC, Weinberg JM, Humes HD : Identification of the aminoglycoside binding site in rat renal brush border mem-

branes J Pharmacol Exp Ther 222 : 350~358, 1982

32. Silverblatt FG, Kuehn C : Autoradiography of gentamicin uptake by the rat proximal tubule cell. Kidney Int 15 : 335~345, 1979
33. Simmons CF, Bogusky RT, Humes HD : Inhibitory effects of gentamicin on renal mitochondrial oxidative phosphorylation J Pharmacol Exp Ther 214 : 709~715, 1980
34. Takada M, Hayashi H : Interaction of cadmium, calcium, and amiloride in the kinetics of active sodium transport through frog skin. Jpn J Physiol 31 : 285~303, 1981
35. Ussing HH, Zerahn K : Active transport of sodium as the source of electric current in the short-circuited isolated frog skin Acta Physiol Scand 23 : 110~127, 1951
36. Wedeen RP, Batuman V, Cheeks C, Marquet E, Sobel H : Transport of gentamicin in rat proximal tubule Lab Invest 48 : 212~223, 1983
37. Weinberg JM, Humes HD : Mechanisms of gentamicin induced dysfunction of renal cortical mitochondria. I. Effects on mitochondrial respiration. Arch Biochem Biophys 205 : 222~231, 1980
38. Williams PD, Holophan PD, Ross CR : Gentamicin nephrotoxicity I Acute biochemical correlates in rats Toxicol Appl Pharmacol 61 : 243~251, 1981
39. Williams PD, Holohan PD Ross CR : Gentamicin nephrotoxicity. II Plasma membrane changes. Toxicol Appl Pharmacol 61 : 243~251, 1981
40. Williams PD, Trimble ME, Crespo L, Holohan PD, Freedman JC, Ross CR : Inhibition of renal Na, K-adenosine triphosphatase by gentamicin J Pharmacol Exp Ther 231 : 248~253, 1984