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INHIBITORY EFFECT OF TOBRAMYCIN ON SODIUM TRANSPORT ACROSS THE ISOLATED FROG SKIN

고신대학 의학부 의예과

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개구리 피부를 통한 Na 이동에 대한 tobramycin 의 억제효과

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= 국문요약 =

상피 조직의 Na 이동에 미치는 aminoglycoside의 작용 기작을 보기 위해 Short-Circuit Current(SCC)에 대한 tobramycin(TB)의 영향을 조사하였다. 0.1, 0.2, 그리고 0.5mg/mℓ의 TB농도를 개구리 피부 내측에 투여시 SCC가 각각 약물투여 전의 94, 77, 75%로 되었으며, TB 1.0mg/mℓ 농도에서는 TB의 SCC에 대한 억제효과가 오히려 0.5mg/mℓ농도보다 작았으며 약물투여 전의 78%였다. 피부외측에 TB를 위와 같은 농도로 (0.1~1.0mg/mℓ) 처리했을 때는 피부 내측에 처리시와 같이 SCC 억제효과가 나타났으며 약물처리 후 각각의 SCC는 투여 전의 96, 79, 75 및 84%로 나타났다. 피부 외측에 약물처리시 1.0mg/mℓ 농도TB의 SCC 억제효과는 크게 감소하여 0.2mg/mℓ 농도에서보다도 억제효과가 적게 나타났다. 피부 외측과 내측 용액 각각에 TB를 투여시 약물의 SCC 억제효과는 각각의 경우 모두 0.2mg/mℓ 농도에서 상대적으로 가장 컸으며, gentamicin의 약물효과는 0.1mg/mℓ에서 가장 컸다. 0.2mg/mℓ 농도의 TB를 피부 외측과 내측에 동시에 투여시에는 외측 또는 내측에 같은 농도의 TB 투여시보다도 더 큰 SCC 억제효과가 나타났으며, 이때의 개구리 피부의 산소 소모율은 대조군과 별다른 차이를 나타내지 아니했고, 산소 소모율은 70.18±3.38μl/gm/40min였다.

이상의 결과는 TB가 개구리 피부 외측막의 Na 투과도를 감소시키며, 피부 내측막에서는 능동적 Na 이동을 저하시키는 것으로 사료되며 또한 이와같은 TB에 의한 상피세포막 기능의 변화가 aminoglycoside에 의해 야기되는 신장기능 장애인 다뇨증과 근위세뇨관에서의 Na rejection의 한 원인이라고 간주되어진다.

Ⅰ. INTRODUCTION

The aminoglycosides are potent antibacterial agents in the management of severe Gram-negative bacterial infection. But serious nephrotoxicity is a major limitation to the usefulness of the aminoglycoside and the same spectrum of toxicity is shared by all members of aminoglycoside antibiotics.[7,19] Nephrotoxic potential of aminoglycoside appears to be increased according to the extent to which the drug is accumulated in the proximal tubular cells and the interaction between aminoglycoside and membranes of epithelial cells or intracellular organelles.[24]

One of the newest aminoglycoside agents is tobramycin(TB). TB was derived from nebramycin and demonstrated a great antibacterial activity, particularly with respect to *Pseudomonas*.[16] Like other aminoglycosides, TB are entirely excreted by the normal kidney and its accumulation in kidney cortex has been described previously.[3,18] Furthermore administration of TB to animals has produced histopathologic alterations in the proximal tubules which are similar to those produced by gentamicin.[1,2,27] Also the antimicrobial activity and pharmacokinetic properties of tobramycin are very similar to those of gentamicin[19] The similarities between tobramycin and gentamicin in drug-induced pathology suggest that information available about gentamicin may be relevant to tobramycin.

Nephrotoxicity from aminoglycoside is essentially a form of acute tubular necrosis and is initially manifested by the inability to concentrate the urine.[19] Recent studies by Lee and Park[8] concluded that the aminoglyciside (gentamicin or tobramycin)-induced polyuria and urine concentrating defect are due to osmotic diuresis induced by proximal Na rejection and the mechanism with which gentamicin and tobramycin impair renal functions is basically identical but nephrotoxic potential of gentamicin is relatively higher than that of tobramycin. Comparative toxicology studies in animals also showed that tobramycin exhibited a lesser nephrotoxic potential than did gentamicin.[2,5] In previous study using frog skin preparations[1] gentamicin increased the Na permeability of the mucosal border and inhibits the activity of the Na pump mechanism at the serosal border of the epithelium The above authors considered these alterations in membrane functions as the early events of aminoglycoside nephrotoxicity.

The objective of the present study was to further investigate the mechanism with which aminoglycosides impair transepithelial Na transport using frog skin preparations and to compare the effects of tobramycin on Na transport across the isolated frog skin with those of gentamicin previously reported from this laboratory.[1]

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 as described by Ussing and Zerahn.[21] The abdominal 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 Ringer-KCl-agar

bridges connected to the chamber 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, 1mM $CaCl_2$, 2.5mM $NaHCO_3$, and 3.5mM KCl and finally adjusted to pH 7.4. The solution was continuously stirred with a stream of air. The tobramycin sulfate stock solution (80mg/mℓ) was purchased from Daewoong Pharma Inc. Ltd., Korea. This solution was diluted with Ringer solution to the appropriate final concentration of tobramycin. In all cases pH of the tobramycin-Ringer solution was adjusted to 7.4 with NaOH.

When potential difference and SCC were stabilized which usually ocurred at 40~60 min after mounting the frog skin, tobramycin 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 skin was replaced by the tobramycin-Ringer solution, the other side was filled with fresh Ringer solution. In the same experiments oxygen consumption of the isolated frog skin was measured using a biological oxygen monitor (Yellow Spring Instrument, Model 53). After 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 20~50mgs of the wet tissue from each preparation were transferred to the polarography reaction vessel containing 2mℓ of the room-air saturated normal Ringer or tobramycin (0.2mg/mℓ) 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

In all experiments, tobramycin was applied to the appropriate medium when the SCC was stabilized (the basal SCC) after 40~60 min. The value of SCC after drug treament was expressed as the percentage of the basal SCC (control) of that preparation.

Fig. 1 illustrates changes in SCC across the frog skin after tobramycin was applied to the serosal bathing medium at a concentration of 0.1, 0.2, 0.5 or 1.0mg/mℓ. At 0.1, 0.2 or 0.5mg/mℓ of tobramycin, the relative SCC was increased

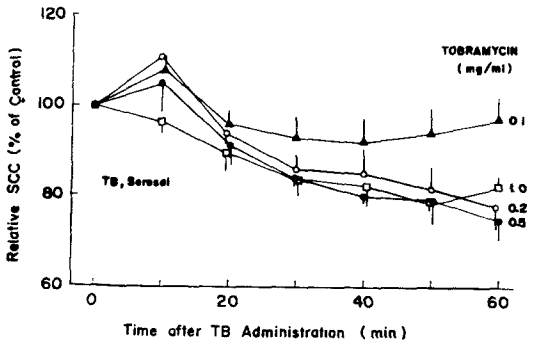

Fig. 1. Changes in short-circuit current (SCC) after tobramycin (TB) was applied to the serosal side of the frog skin. Each point represents the mean of 4~5 skins±SE. Absolute value of SCC at time zero was on the average of 225.3±12.8 μA/3.14cm^2 (n=36).

immediately during the initial 10 min period and followed by gradual reduction in SCC without stable value which usually occurred at 40~50 min after gentamicin administration (see Fig. 2 of Hong and Park,[1]). At 1.0mg/mℓ of tobramycin, initial increase in SCC disappeared and the magnitude of reduction in SCC was less than that of 0.5mg/mℓ of tobramycin after 40~60 min. The maximal magnitude of reduction in SCC was approximately 94, 77, 75, and 78% of the respective basal SCC at 0.1, 0.2, 0.5, and 1.0mg/mℓ of tobramycin respectively.

Changes in SCC after tobramycin was administered to the mucosal side of the frog skin at four different concentrations of tobramycin (0.1, 0.2, 0.5, and 1.0mg/mℓ) were shown in Fig. 2. At 0.1mg/mℓ of tobramycin, the initial increase in SCC was lasted 40 min and finally decreased to 96% of the control value at that concentration (of tobramycin). But at 0.2, 0.5, and 1.0mg/mℓ of tobramycin, the relative SCC at the point of maximal inhibition was approximately 79, 75, and 84% of the control value, respectively. Interestingly, on the mucosal border of the frog epithelium, this inhibitory effect of tobramycin on SCC was opposite to the stimulatory effect of gentamicin, while both drugs had the same inhibitory effect on the serosal side (see Fig. 3 of Hong and Park). Moreover, the inhibitory action of tobramycin on SCC was significantly decreased at 1.0mg/mℓ when compared with that observed at 0.5mg/mℓ. This pattern of tobramycin action on SCC was also shown when the tobramycin applied to the serosal side of the frog skin (see Fig. 1).

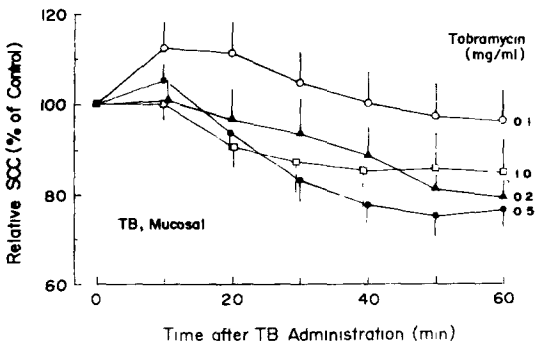

Fig. 2. Short-circuit current (SCC) change induced by tobramycin (TB) added to the mucosal side of frog skin. Each data point represents the mean of 4 frog skins±SE.

Fig. 3 summarizes the dose-response relationship for gentamicin and tobramycin applied to the serosal or mucosal side of the frog skin for one hour. It is seen that both the stimulation of SCC by the mucosal side gentamicin and the inhibition by the serosal side gentamicin were maximal at 0.1~0.2mg/mℓ. On the other hand the inhibition of SCC by the serosal or mucosal tobramycin was most effective at 0.2~0.5mg/mℓ. Thus inhibitory potential of gentamicin on SCC was relatively higher than that of tobramycin.

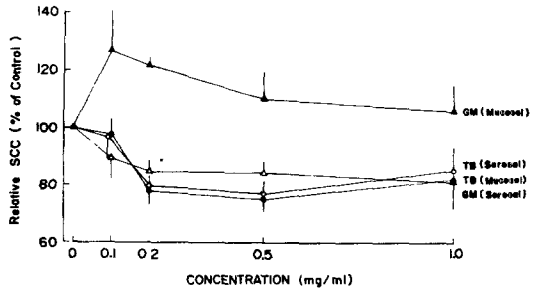

Fig. 3. Gentamicin and tobramycin dose-response curves in short-circuit current (SCC) across frog skin. Each data point represents the mean SCC±SE of 4~6 frog skins after 60 min exposure to gentamicin at the mucosal (M) and serosal (S) side.

Fig. 4 compares SCC changes observed in the skins treated with tobramycin (0.2mg/mℓ) at the serosal (S), mucosal (M), and both mucosal

and serosal (S+M) sides respectively. When tobramycin was added to both sides of the skin the SCC decreased further (71%) than those in which drug was applied to only one side of the frog skin.

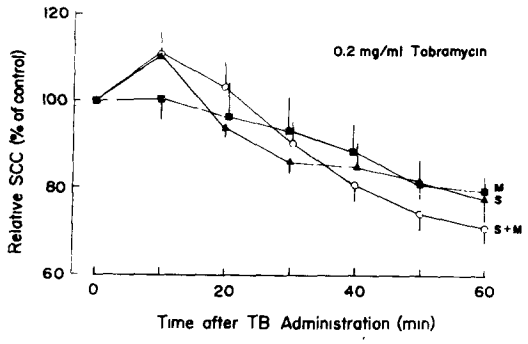

Fig. 4. Changes in short-circuit current (SCC) at 0.2mg/mℓ concentration of tobramycin (TB) administered to the serosal (S), mucosal (M), and both the serosal and mucosal (S+M) sides of frog skin. Each point represents the mean of 4 frog skins±SE

In order to test possibility that the reduction of SCC by tobramycin applied to the serosal and the mucosal side was secondary to the metabolic inhibition in epithelial cells, the study on the effect of tobramycin on oxygen consumption of epithelial cells was undertaken in the next series of experiments. The results, however, appear that oxygen consumption of the skin tissue was not significantly changed by 0.2mg/mℓ of tobramycin (Table 1).

Ⅳ. DISCUSSION

The objective of the present study was to further investigate the mechanism with which aminoglyciside impairs Na transport across the frog skin using tobramycin, one of the newest aminoglycoside agents.

In the present investigation, tobramycin (0.1 ~1.0mg/mℓ) administered to the serosal side of the frog skin resulted in 3~6% inhibition of net Na transepithelial Na transport (see Fig. 1). Since the Na pump site in frog skin was located on the basolateral (serosal) cell membrane[15] and the oxygen consupmtion of the skin tissue was not impaired by tobramycin (see Table 1), the inhibition of the Na transport was probably related to direct alterations in the activity of Na pump mechanism. In the basolateral side of the frog skin the mode of tobramycin is consistent with that of gentamicin.[4] These findings strongly suggest that aminoglycoside inhibition of Na pump mechanism is due to the interaction of aminoglycoside with the basolateral membrane of the frog skin.

Previous studies have provided various evidences that the cell membrane may be an important site of toxic action due to aminoglycosides. Williams et al.[28,29,30] demonstrated the binding of gentamicin to basolateral membranes in rat renal proximal tubule after *in vivo* administration of gentamicin and concluded that basolateral membrane change may be important

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

	QO_2 (μ1/gm/40 min)
Control	71.01 ± 2.35
Tobramycin (0.2 mg/ml)	70.18 ± 3.38

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

in the pathogenesis of gentamicin nephrotoxicity. They also reported that changes in basolateral membrane function involve the activity of Na-K-ATPase, an enzyme localized in the basolateral membrane of the renal cell. Furthermore, aminoglycosides may cause toxicity through involvement with plasma membrane is supported by additional work done in the kidney, the ear, and artificial membranes.[9,10,11,12,17,21] In recent studies, Lee and Park[8] concluded that tobramycin-induced polyuria and urine concentrating defect was primarily attributed to the osmotic diuresis associated with reduction in Na reabsorption in the proximal tubule. Therefore, tobramycin-induced inhibition of Na pump mechanism in frog skin and in the renal proximal tubule may be considered to be a result of the basolateral membrane change induced by interaction of aminoglycoside and the basolateral membrane. Sastrasinh at al.[20] identified the acidic phosphatidylinositol, one of major constituents of plasma membrane, as a prime target for a charge interaction with polycationic aminoglycosides due to amino side group at physiological pH.[19] In order to further access the role of cationic charge in nephotoxicity as a determinant of interaction between aminoglycoside and plasma membrane, Simmons et al.[22] examined the renal effect of polycationic diethylaminoethyl (DEAE) dextran, since DEAE possessed a molecular structure very similar to that of aminoglycoside antibiotics. They demonstrated that DEAE dextran had considerable mitochondrial and renal toxicity and these toxicities were not observed with neutral dextran. Therefore cationic charge seems to be a important molecular and pathogenic determinant of nephrotoxicity.

If this hypothesis is correct, the ability of aminoglycoside to interact with the anionic components of biological membranes is importantly influenced by the number of ionizable primary and secondary amino groups present within the molecules. The competitive binding studies with various aminoglycosides demonstrated that kanamycin and amikacin, each with four ionizable amino groups, had relatively similar affinities for the membrane binding site, whereas netilmicin, gentamicin, and tobramycin, each with five ionizable amino groups, were distinctly more attractive than kanamycin and amikacin, but did not differ markedly from one another. Neomycin, with six ionizable amino groups, was the most attractive aminoglycoside.[20] This appears to be close to the ranking for nephrotoxicity potential of aminoglycosides (neomycin>gentamicin>kanamycin>amikacin>tobramycin>streptomycin; gentamicin>tobramycin>netilmicin) observed in humans or in rats.[1,19]

On the other hand, although gentamicin and tobramycin have the same number of ionizable amino groups, the Na transport was most effectively inhibited at 0.2mg/mℓ of tobramycin and at 0.1mg/mℓ of gentamicin when each aminoglycoside was applied to the serosal bathing medium (see Fig. 3). Furthermore various studies showed that gentamicin was more nephrotoxic than tobramycin.[2,8] This different toxicity between gentamicin and tobramycin may be due to a lower affinity of tobramycin than that of gentamicin for the receptor of the plasma membrane as suggested by Soberon et al.[23] in their comparative studies of aminoglycoside nephrotoxicities.

When tobramycin was applied to the mucosal surface of the frog skin, tobramycin reduced the Na permeability of the mucosal border and subsequently decreased the net Na transport across the frog skin. In our previous studies gentami-

cin increased the Na permeability of mucosal border. The interpretation of these opposite effects of gentamicin and tobramycin is not yet provided. However, the hypothesis that aminoglycosides bind to phosphoinositides on plasma membrane suggest a possibility that these drugs may causes injury to frog ephithelia For example, the phosphoinositides have been implicated as part of the membrane receptor-complex for a number of agonists and are thought to regulate membrane permeability to cations.[6,13,14] Thus, it is possible that aminoglycosides, by interacting with the phosphoinositides, interfere a specific agonist-receptor interaction or promote a generalized dysfunction consequent to altered membrane permeability to cations. Furthermore, one of the aminoglycoside nephrotoxic actions is an interference with mitochondrial energy production. Weinberg and Humes[26] and Weinberg et al.[25] showed that gentamicin-induced dysfunction of renal cortical mitochondria was due to alterations in the Na and K permeability of inner mitochondrial membrane at Mg^{++}-sensitive sites They suggest that aminoglycoside may be specially acting to displace Mg^{++} from the same sites due to its cationic properties, and the ability to produce effects similar to those of gentamicin is a function of the extent to which they are cationic at physiological pH

The data presented in this paper and the previous paper[4] strongly suggest that both in the frog skin and in the renal proximal tubule tobramycin and gentamicin inhibit the activity of the Na pump mechanism at the serosal membrane and induce changes in the Na permeability of the mucosal membrane of the epithelium. These changes in membrane functions may be responsible for the early events of aminoglycoside nephrotoxicities characterized by proximal Na rejection and osmotic polyuria.

V. SUMMARY

The effect of tobramycin (TB) on sodium transport across the isolated frog skin was studied in order to further investigate the mode of aminoglycoside action. When TB was applied to the serosal side of frog skin short-circuit current (SCC) was increased immediately during the initial 10 min period and decreased gradually to 94% of control value at 0.1mg/mℓ, to 77% at 0.2mg/mℓ, and to 75% at 0.5mg/mℓ of TB. At 1.0mg/mℓ of TB, the initial increase in SCC disappeared and the maximal reduction in SCC was to 78% of the control value. The inhibitory effect of TB was greater at 0.5mg/mℓ than at 1.0mg/mℓ. When TB was added to the mucosal surface of frog skin, it decreased SCC to 96, 79, 75, and 84% of the respective control value at 0.1 0.2, 0.5 and 1.0mg/mℓ, respectively The inhibitory action of TB on SCC was significantly decreased at 1.0mg/mℓ when compared with that observed at 0.5mg/mℓ. When both sides of frog skin were treated with 0.2mg/mℓ of TB. the inhibition of SCC was greater than those treated with TB at the mucosal and serosal side alone The inhibitory action of tobramycin on transepithelial Na transport was most effective at 0.2mg/mℓ, whereas that of gentamicin was at 0.1mg/mℓ. The quantity of frog skin oxygen consumption at 0.2mg/mℓ of TB was 70.18±3.38μl/gm/40min which were not significantly different from the control value.

The present results suggest that 1) TB inhibits the activity of Na pump mechanism at the serosal membrane and decrease Na permeability of the mucosal membrane of epithelium, and 2) these alterations in membrane function are responsible for the early events of nephrotoxicity characterized by increased proximal Na rejection and polyuria.

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