Inhibitory Activity of Dome Formation in LLC-PK$_1$ Cells Is a Selective Index of Aminoglycoside Nephrotoxicity

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(Received for publication March 4, 2002)

Aminoglycoside antibiotics are widely used clinically for a treatment of Gram-negative bacterial infection by the inhibition of bacterial protein synthesis. However, the aminoglycosides have clinical side effects such as nephrotoxicity and ototoxicity for a long-term treatment. Here we evaluated a simple and useful method for screening a new aminoglycoside without or less nephrotoxicity using LLC-PK$_1$ cells derived from a pig kidney proximal tubular epithelial cell.

Dome formation is a morphological trait of LLC-PK$_1$ cells through unidirectional water and salt transport in normal culture condition[1,2]. In the course of searching for a practical method for evaluating aminoglycosides nephrotoxicity, we reconfirmed usefulness of dome formation in LLC-PK$_1$ cells, which was originally reported by INUI *et al.*[1,2]. In this study, we modified and qualified the method for screening a new aminoglycosides, and determined whether it is applied to other antibiotics. As a result, an optimal condition was determined as follows. LLC-PK$_1$ cells obtained from American Type Culture Collection were maintained in a Dulbecco's modified Eagle's medium (DMEM; Nissui Pharmaceutical Co., Ltd.) supplemented with 10% fetal bovine serum (JRH Biosciences) at 37°C with 5% CO_2 condition without common additive antibiotics to exclude the background effects. The cells were inoculated in a 48-well plate (SUMILON; Sumitomo Bakelite Co., Ltd.) at 1×10^5 cells/ml (0.5 ml/well) with various concentrations of aminoglycosides and cultured for 4 days. All aminoglycosides were diluted in 10% DMSO before use. Then numbers of dome, which was formed more than 200 μm in diameter, in a well were counted microscopically.

As shown in Fig. 1, the dome formation was inhibited dose-dependently by almost all aminoglycosides except streptomycin. But its inhibitory activity of dome formation varied with each aminoglycoside antibiotic. Among them, neomycin and gentamicin C inhibited it most strongly, whereas streptomycin did not inhibit it and seemed to induce the formation. The order of inhibitory activity was similar to nephrotoxicity in a rat model[3]. Furthermore, our result was compatible with other reports, in which inhibitory activities of aminoglycosides were assessed by dome formation and membrane enzymes using LLC-PK$_1$ cells[1,2].

To ascertain whether this method is applied to other compounds in addition to aminoglycosides, we tested some compounds known to show nephrotoxicity. As shown in Fig. 2, polymyxin B (Sigma) and aspirin (Kanto Chemical Co.) did not inhibit the dome formation. The effect of aspirin was similar to streptomycin, but the dome formation was restricted in a peripheral area of a well and smaller than that of streptomycin. Although both of cephaloridine (Shionogi Co., Ltd.) and cephalothin completely inhibited the dome formation at 100 μg/ml, the effects were apparently cytotoxic. This result indicates that this method can not be appropriate for evaluation of nephrotoxicity by

Fig. 1. Effect of various aminoglycosides on dome formation in LLC-PK$_1$ cells.

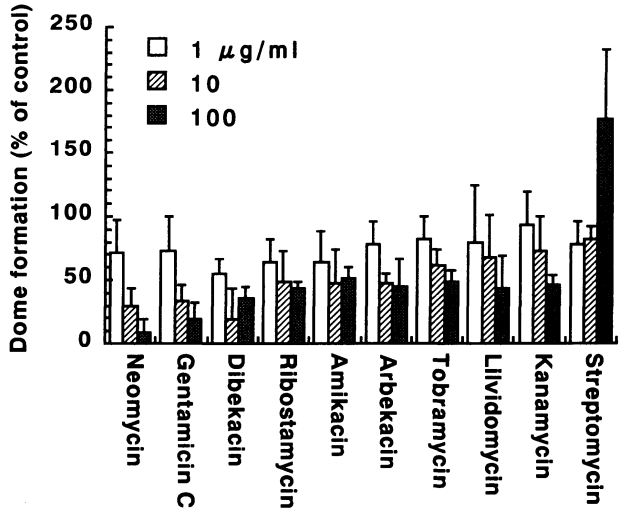

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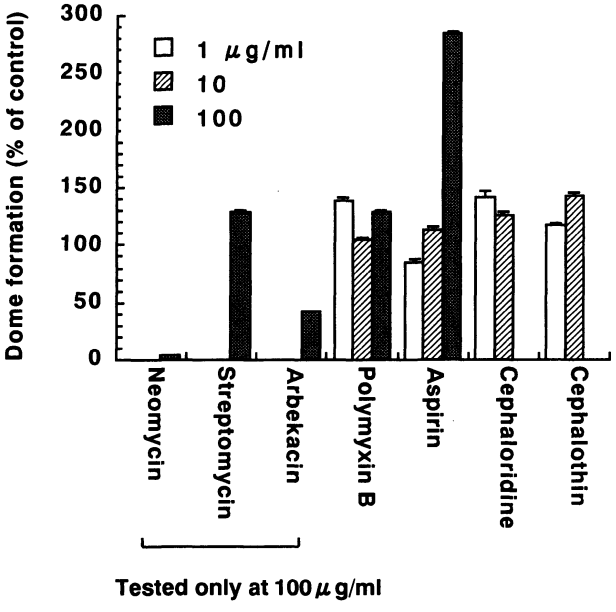
Fig. 2. Effect of other nephrotoxic compounds on dome formation.



other compounds except aminoglycosides. It is reported that aspirin, a non-steroidal anti-inflammatory drug, induces nephropathy for a long treatment[4], but its target causing nephrotoxicity is considered to be different from that of aminoglycosides. Cephaloridine and cephalothin are typical cephalosporin antibiotics and cephaloridine is reported to show nephrotoxicity more severely than cephalothin through accumulation in proximal tubular cells[5,6]. However, there was no clear difference in the order of inhibitory activity between these cephalosporins. While aminoglycosides are also reported to accumulate in proximal tubular cells[7], a mechanism of aminoglycosides nephrotoxicity is therefore different from that of cephalosporin. In conclusion, the inhibition of dome formation is selectively applied to aminoglycosides, and this method is useful for evaluation of aminoglycoside nephrotoxicity. Furthermore, a mechanism by which aminoglycoside inhibits dome formation will be a clue for elucidating its nephrotoxicity.

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