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Commentary

Entry of aminoglycosides into renal tubular epithelial cells via endocytosis-dependent and endocytosis-independent pathways

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ABSTRACT

Aminoglycoside antibiotics such as gentamicin and amikacin are well recognized as a clinically important antibiotic class because of their reliable efficacy and low cost. However, the clinical use of aminoglycosides is limited by their nephrotoxicity and ototoxicity. Nephrotoxicity is induced mainly due to high accumulation of the antibiotics in renal proximal tubular cells. Therefore, a lot of studies on characterization of the renal transport system for aminoglycosides so far reported involved various in-vivo and in-vitro techniques. Early studies revealed that aminoglycosides are taken up through adsorptive endocytosis in renal epithelial cells. Subsequently, it was found that megalin, a multiligand endocytic receptor abundantly expressed on the apical side of renal proximal tubular cells, can bind aminoglycosides and that megalin-mediated endocytosis plays a crucial role in renal accumulation of aminoglycosides. Therefore, megalin has been suggested to be a promising molecular target for the prevention of aminoglycoside-induced nephrotoxicity. On the other hand, recently, some reports have indicated that aminoglycosides are transported via a pathway that does not require endocytosis, such as non-selective cation channel-mediated entry, in cultured renal tubular cells as well as cochlear outer hair cells. In this commentary article, we review the cellular transport of aminoglycosides in renal epithelial cells, focusing on endocytosis-dependent and -independent pathways.

1. Introduction

In spite of the introduction of newer classes of antibiotics, aminoglycoside antibiotics such as gentamicin, amikacin and tobramycin are still continuously used worldwide for the treatment of various infections by Gram-negative bacteria and bacterial endocarditis [1–3]. Among them, gentamicin is the most commonly used because of its relatively low resistance levels and low cost. Streptomycin, the first aminoglycoside, is still an important component of anti-tuberculosis therapy regimens. In addition, aminoglycosides are administered to cystic fibrosis patients for chronic endobronchial infection complicated by frequent exacerbation [4]. However, the side effects of nephrotoxicity and ototoxicity are serious problems, and are the dose-limiting factors for the use of aminoglycosides [5–7].

The rate of nephrotoxicity induced by aminoglycoside treatment in clinical situations reportedly ranges from 1.7% to 58% [8]. The nephrotoxicity, which usually occurs after at least a week of treatment, is nonoliguric, and it is characterized by a slow increase in serum creatinine and a hypoosmolar urinary output. It is likely that aminoglycoside-induced nephrotoxicity is directly related to

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; CR, complement type repeat; EDTA, ethylenediaminetetraacetic acid; FITC, fluorescein isothiocyanate; GTTR, gentamicin conjugated to Texas red; K_d, dissociation constant; OAT, organic anion transporter; OCT, organic cation transporter; PAI-1, urokinase-plasminogen activator inhibitor type-1; RAP, receptor-associated protein; TRP, transient receptor potential.

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high accumulation of the drug in renal proximal tubular cells after glomerular filtration. Therefore, the mechanisms underlying not only the renal transport but also the tubular cytotoxic effect of aminoglycosides, especially gentamicin, have been investigated by means of a variety of in-vivo and in-vitro techniques [9–13].

Most aminoglycosides injected into the body are excreted into the urine without being metabolized, the rest being selectively accumulated in renal proximal tubular cells. Aminoglycoside taken up into the cells stays there for a long time, leading to renal damage including structural changes and functional impairment of the plasma membrane, mitochondria and lysosomes in the renal proximal tubular cells [5,14–16]. Thus, there has been a great interest in the molecular mechanism responsible for aminoglycoside transport across the plasma membrane of renal proximal tubular cells. Since aminoglycosides are hydrophilic polycationic compounds with three to five amino groups (Fig. 1) and do not readily penetrate cell membranes, the involvement of a specific membrane transport system is essential for the entry of aminoglycosides into the epithelial cells in the kidneys. The purpose of this commentary is to provide an overview of the cellular transport of aminoglycosides in renal epithelial cells, focusing on endocytosis-dependent and endocytosis-independent pathways. As far as we know, this is the first commentary article that summarizes the two pathways involved in cellular entry of aminoglycosides in the kidneys.

2. Aminoglycoside transport via the endocytosis-dependent pathway

2.1. Renal distribution of aminoglycosides after systemic administration

Early studies involving autoradiography and immunohistochemistry revealed that gentamicin is localized to the proximal tubules after a systemic injection [17–20]. Gentamicin is almost exclusively located over the brush-border membranes and the apical endocytic vesicles of proximal tubular cells at 10 min after a systemic injection. No or little gentamicin is seen on the basolateral membranes of proximal tubular cells or the distal parts of nephrons from the beginning of the loop of Henle to the end of the medullary collecting duct. After 1 h, gentamicin is observed in small lysosomes in the apical part of the cells. At 24 h, gentamicin is distributed throughout the matrix of large lysosomes. Gentamicin is distributed in the lysosomes of proximal tubular cells even 10 days after an injection. The apparent elimination half-life of gentamicin in the kidneys after a single injection was estimated to be 98–166 h [21,22]. These observations indicated that gentamicin is incorporated into proximal tubular cells through endocytosis and stays there for a long time.

Furthermore, in-vivo studies showed that gentamicin uptake in the renal cortex is saturable, and competitively inhibited on coinfusion of other aminoglycosides such as tobramycin, netilmicin, neomycin and amikacin [23,24]. The cortical levels of aminoglycosides are much higher than the serum levels, indicating the involvement of a concentrative uptake system for aminoglycosides [21,22]. These results substantiate the involvement of a specific molecule in the uptake pathway responsible for aminoglycoside accumulation in the renal proximal tubular cells, most likely via receptor-mediated endocytosis.

2.2. Involvement of megalin and cubilin, multiligand endocytic receptors, in aminoglycoside uptake

Identification of the aminoglycoside binding receptor responsible for the renal accumulation of aminoglycosides would be essential to understand the mechanisms underlying their tissue-specific distribution and toxicity. Acidic phospholipids such as phosphatidylinositol 4,5-bisphosphate and phosphatidylserine, components of the cell membrane, bind aminoglycosides in a saturable manner [25,26]. Since aminoglycosides are polycationic at physiological pH due to their amino residues, they could interact with acidic phospholipids in the brush-border membrane of renal proximal tubular cells, serving as the initial binding site for renal uptake of aminoglycosides. However, considering the broad distribution of acidic phospholipids in the plasma membranes of various tissues, other factors should be prerequisite for concentrated and specific accumulation of aminoglycosides in the renal proximal tubular cells.

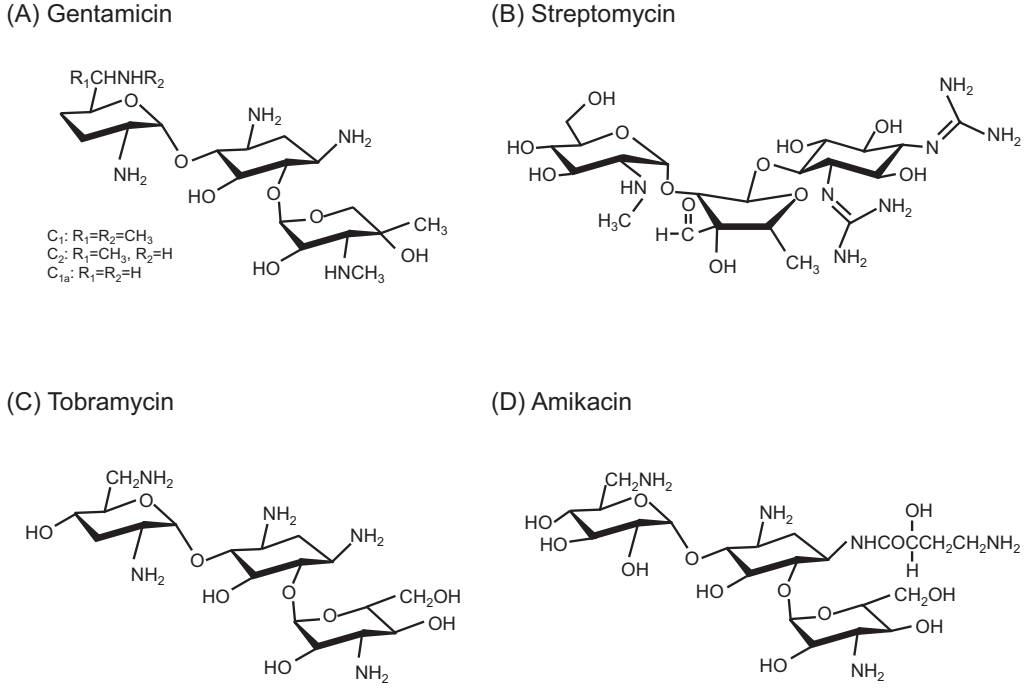

Fig. 1. Chemical structures of aminoglycoside antibiotics. (A) Gentamicin, (B) Streptomycin, (C) Tobramycin, (D) Amikacin.