Distribution of Gentamicin by Immunofluorescence in the Guinea Pig Inner Ear*

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Summary. We studied the distribution of gentamicin in the inner ear, brain and kidney of the guinea pig following intraperitoneal administration or perfusion of gentamicin through the perilymphatic space. The resulting histopathological changes were examined by immunofluorescence using antiantibiotics serum. After perfusion of gentamicin through the perilymphatic space, specific fluorescence was found in the cochlea, and was especially prominent in the outer hair cells, basilar membrane and basilar crest. Although no fluorescence was observed in the cochlea following intraperitoneal administration of high doses of gentamicin, type I hair cells in the vestibule were seen to be selectively stained with the antibody. Furthermore, some of the vestibular ganglion cells, Purkinje cells and unidentified nuclei in the brain stem were also stained. In particular, fine granules showing relatively intense fluorescence were recognized in the cytoplasm of the stained cells. In the cortex of kidney, only proximal tubular cells were stained with intense fluorescence. Our results suggest that the aminoglycoside antibiotics have two sites of action: one is the cell membrane of the sensory hair cells and the other is the cytoplasm.

Key words: Gentamicin distribution – Inner ear – Brain – Immunofluorescence – Kidney

Introduction

Since the introduction of the aminoglycoside antibiotics into clinical use for the treatment of various infectious diseases, their toxicities to various organs have become a significant problem. The major target organs for these drugs are

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known to be the inner ear and the kidney. Each of the aminoglycoside antibiotics has a different effect on the inner ear. Kanamycin and neomycin affect the cochlea most intensively, whereas gentamicin and streptomycin sulfate show greater toxicity in the vestibular system. Furthermore, vestibular symptoms suggest that streptomycin also damages the central nervous system [5]. These facts led us to investigate the mechanisms by which these antibiotics affect the sensory cells of the inner ear. In their recent research, Orsulakova et al. [10] showed the strong affinity of the aminoglycoside antibiotics to inositol phospholipid, an important compound for transmembrane signalling [3]. However, a possible role of inositol phospholipid as an aminoglycoside receptor is not thoroughly understood.

During the past three decades, many investigators have studied the toxicities of the aminoglycoside antibiotics, but their findings have been restricted to the destructive or degenerative changes in the tissue affected. Since the initial reaction of the antibiotics involves a cellular binding, investigation of the distribution of these drugs and inositol phospholipid is important in elucidating any subsequent mechanisms of tissue reactions. Consequently, we used immunofluorescent studies to examine the distribution of gentamicin in the inner ear, brain and kidney following its intraperitoneal injection.

Materials and Methods

**Animals.** The Hartley strain of guinea pigs (average body weight, 200–250 g) with normal Preyer reflexes was used in this series of experiments.

**Administration of Gentamicin.** In one group of guinea pigs, animals were treated with a perilymphatic perfusion of 1 ml phosphate-buffered solution containing gentamicin (5 mg/ml). This solution was perfused from the scala tympani to the oval window for 15 min and the animals were immediately decapitated. Another group of guinea pigs received various doses of gentamicin (1, 2, 10, 30, 50, 100, 200 mg/kg) by daily intraperitoneal injection for 7 days; these animals were then decapitated 24 h after the final injection of the drug. The temporal bones, brains and kidneys were fixed with 10% buffered formalin for 2 days. Following fixation, the temporal bones were decalcified with 10% EDTA in phosphate-buffered saline (PBS) for 1 week. They were embedded in paraffin, sectioned 5–6 μm in thickness, and mounted on glass slides previously coated with 0.1% neoprene (polychloroprene) in toluene. In addition, sections were stained with hematoxylin and eosin for routine histopathological study.

**Immunofluorescence.** Type VII protease was obtained from Sigma Co. (St. Louis, Mo) and was used for treatment of formalin-fixed paraffin-embedded specimens to recover the antigenicity present [6]. Deparaffinized tissue sections were first treated with 0.1% protease in PBS containing 0.02% CaCl₂ at 37°C for 15 min. Specificity of anti-gentamicin antiserum was determined by the Ouchterlony double-immunodiffusion method, using various aminoglycoside-conjugated ovalbumins as the antigen; the antiserum was found to make a precipitin line only with gentamicin-conjugated ovalbumin. The titer of the antiserum was 1:1600, as determined by enzyme-linked immunosorbent assay. After three washings with PBS, tissue sections were reacted overnight with anti-gentamicin antiserum (Miles Lab., Elkhart, In.) at 1:80 dilution with PBS at 4°C. Thereafter, the sections were treated for 1 h with goat FITC-anti-rabbit IgG antiserum (Cappel Lab., Cochranville, Pa.) at 1:20 dilution at 37°C. In each reaction, the sections were incubated in the humid chamber, and were washed three times with PBS. After mounting with buffered glycerol (pH 9.4), all sections were observed under a fluorescence microscope (Olympus, BH, Tokyo). In this experiment, the kidney specimen