

COMBINATIONAL ADMINISTRATION OF AMINOGLYCOSIDES AND LOOP DIURETICS AS AN EFFICIENT STRATEGY TO ESTABLISH DEAFNESS MODELS IN RATS

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Abstract

It is known that aminoglycoside antibiotics can damage the vestibular and auditory sensory epithelia, and the loop diuretics can enhance the ototoxic effect of aminoglycosides. Previous studies on the synergistic effect of these two types of drugs have used mice, guinea pigs and cats, but not rats. The aim of this study was to determine this synergistic effects in rat cochleae. Rats received intravenous injections of different doses of furosemide and/or intramuscular injections of kanamycin sulfate. Auditory brainstem response (ABR), scanning electron microscopy (SEM) and immunocytochemistry were used to determine the effects of drug administration. In the group receiving combined administration of furosemide and kanamycin, the ABR threshold showed significant elevation 3 days after drug administration, greater than single drug administration. The hair cells showed various degrees of injury from the apical turn to the basal turn of the cochlea and from the outer hair cells to the inner hair cells. Neuron fibers of the hair cells showed significant loss 7 days after the drug administration, but the number of spiral ganglia did not decrease and supporting cells showed no signs of injury. Our study suggest that combined administration of furosemide and kanamycin has a synergistic ototoxic effect, and can result in hair cell loss and hearing loss in rats.

Keywords: Kanamycin; Furosemide; Deafness model; Hair cell injury, rat

Introduction

The animal model of deafness is an important tool in studying the pathogenesis of deafness and hearing regeneration. It can be created through noise exposure, ototoxic drug administration and gene manipulation. Drug administration is more frequently used than the other two due to its rather complete elimination of auditory hair cells. To date, numerous methods have been developed using ototoxic drugs to induce deafness, including local application of carboplatin and cisplatin, neomycin infiltration through the round window membrane^[1,2], and intramuscular injection of kanamycin, gentamicin and amikacin^[3]. Nevertheless, these

methods may bring uncontrollable side effects other than deafness, e.g., infection and mechanical damage of the inner ear from local administration of ototoxic drugs; and severe kidney dysfunction with high mortality from systemic administration of aminoglycosides. Therefore, safer and more effective drug administration methods need to be explored. Fortunately, combined application of aminoglycosides and loop diuretics, such as kanamycin and furosemide, has been demonstrated to be able to cause hair cell injury in a faster and safer manner^[4-9]. This inspired us to explore a novel method to construct deafness models. In this study, we aimed to testifying the synergistic effect of kanamycin and furosemide in rat cochleae and establishing a preliminary

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criterion in drug dosages for deafness induction. The reason of choosing rats was that they are more advantageous than other rodent species for deafness model construction. First, rats costs lower in breeding than guinea pigs. Second, rats have a larger body than mice, more suitable for cochlear surgeries. The auditory brainstem response (ABR) was measured to determine the hearing threshold after drug application. Immunocytochemistry and confocal microscopy were performed to evaluate the inner ear morphology. To our knowledge, this is the first report of establishing rat deafness model through combinational application of aminoglycosides and loop diuretics.

Materials and Methods

Drug Administration

Ninety healthy 4-week-old SD rats (100-110 g) were randomly assigned into 6 groups, with 15 animals in each group (Table 1). The animals were anesthetized with intraperitoneal injection of 10 % chloral hydrate (0.45 ml/100 g). The left jugular vein was exposed and injected with furosemide, whereas kanamycin was injected intramuscularly in the thigh as described previously [4]. Group A was the control without any treatments. Groups B and C were injected with either furosemide or kanamycin alone in the doses of 200 mg/kg or 1000 mg/kg, respectively. Groups C, D, E and F received both drugs in different dosage combinations. All the animals exhibited normal behavior and were on normal diet after the drug administration, and there were no signs of abnormal vestibular function. Care and use of animals were approved by the Institutional Animal Care and Use Committee of Chinese PLA General Hospital.

ABR Measurements

Details of ABR measurements were provided elsewhere [8]. Briefly, the animals were anaesthetized with xylazine and ketamine. The recording electrodes were inserted at the vertex and pinna. ABRs were evoked with clicks and/or 5 ms tone pips (0.5 ms rise/fall, at 30/sec) at frequencies of 4, 8, 16, and 32 kHz. The signal was amplified, filtered, and averaged using the Intelligent Hearing System (USA). The sound level was raised in 20- and/or 5-dB steps. At each level, 1024 responses were averaged. Both ears were measured.

Immunocytochemistry and Scanning Electron Microscopy

The cochleae were perfused with 4.0 % formalde-

hyde and were treated with 0.2 % Triton X-100/PBS. Goat serum (10 %) was used to block nonspecific binding. The tissue was then incubated with 200 kD Neurofilament Heavy antibody (Abcam, diluted 1:200). The samples were washed with PBS, followed by incubation with the secondary antibodies. To stain nuclei, the DNA-specific label propidium iodide (PI) was used as described previously [10]. The samples were mounted on the glass slides with antifade solution (Prolong Antifade Kit, Molecular Probes) and examined using confocal scanning system (LSM 510 META, USA) with three lasers mounted on a Zeiss AxioPlan 2IE MOT motorized upright microscope.

For SEM, the cochleae were fixed with 2.5 % glutaraldehyde in the 0.1 M sodium cacodylate buffer (pH 7.4) containing 2 mM CaCl_2 , then was washed in PBS and post-fixed for 15 minutes with 1 % OSO_4 in the same buffer. The tissues were dehydrated in an ethanol series, critical point dried from CO_2 and sputter-coated with gold then examined using a Hitachi S-3700N scanning electron microscope.

Statistical Analysis

All the data were analyzed using the statistical software STATA7.0 (STATA Corp, L.P., College Station, TX, USA). The student's *t* test was applied with *p* value 0.05 being significant. The data were presented as mean $\pm$ standard deviation (SD).

Results

ABR Results

We performed ABR measurements at three time points after the drug administration (3 days, 1 week and 2 months, respectively). The purpose of doing so was to test whether the combined administration of furosemide and kanamycin would be able to induce stable hearing loss over a long-term period. Fig. 1 shows the average ABR thresholds of each group measured at four testing frequencies (4, 8, 16 and 32 kHz). Compared with the control (group A) at any time point, the ABR thresholds of the groups receiving kanamycin or furosemide alone (Groups B and C) did not show significant changes at all four frequencies tested (Fig. 1; $p > 0.05$). In the groups receiving both drugs (Groups D, E and F), however, the ABR thresholds were elevated significantly at all tested frequencies ($p < 0.01$). In Groups D and E, we applied the same dose of furosemide (100 mg/kg), but used different doses of kanamycin (500 mg/kg for Group D and 1000 mg/kg for Group E). We noticed that the higher dose of kanamy-

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