Braz J Otorhinolaryngol.
2012;78(6):47-50.

DOI: 10.5935/1808-8694.20120032

ORIGINAL ARTICLE

BJORL.org

# Persistence of the otoprotective effect. How long does otoprotection against amikacin lasts?

**Andreia Ardevino de Oliveira[1], Matheus de Souza Campos[2], Adriana de Andrade Batista Murashima[3], Maria Rossato[4], Miguel Angelo Hyppolito[5], José Antônio Apparecido de Oliveira[6]**

**Keywords:**
amikacin,
aminoglycosides,
drug toxicity,
hair cells, auditory, inner,
hair cells, auditory, outer.

## Abstract

There is evidence that a "resistance phenomenon" occurs when a none-damaging dose of amikacin protects the hair cells from ototoxicity. Our goal is to prove that this resistance is persistent.

**Method:** Experimental study - 14 albino guinea pigs (*Cavia porcellus*) divided into three groups. The auditory function was assessed by distortion product otoacoustic emissions (DPOAE): before exposure to amikacin, on the 15th day after the non-damaging dose was injected, at the end of the damage dose injection and prior to decapitation.

**Results:** Group A (control) presented normal hearing and histological pattern. Group B (amikacin 20 mg/kg/day (IM) for 30 days and affecting dose (400 mg/kg/day) for 12 days and Group C (same protocol of Group B, but kept for 60 days and slaughtered), the DPOAE confirmed normal auditory function in the pre-exposure and maintenance of the standard-dose; however, significant loss of auditory function after the end of the damaging dose injection.

**Conclusion:** The protection phenomenon did not extended for a period of 30 to 60 days after the application of damaging doses of amykacin.

[1] PhD in Otorhinolaryngology - School of Medicine of Ribeirão Preto - University of São Paulo (USP) - Department of Ophthalmology, Otorhinolaryngology and Head and Neck Surgery. Assistant Physician - University Hospital - Medical School of Ribeirão Preto - USP).
[2] MD. ENT - School of Medicine of Ribeirão Preto - University of São Paulo (USP) - Department of Ophthalmology, Otorhinolaryngology and Head and Neck Surgery.
[3] MSc student in Medical Sciences - School of Medicine of Ribeirão Preto - University of São Paulo (USP) - Department of Ophthalmology, Otorhinolaryngology and Head and Neck Surgery - Lab Technician.
[4] Lab Technician.
[5] PhD in Medical Sciences; Professor at the School of Medicine of Ribeirão Preto - University of São Paulo (USP) - Department of Ophthalmology, Otorhinolaryngology and Head and Neck Surgery.
[6] Full Professor - retired - School of Medicine of Ribeirão Preto - University of São Paulo (USP) - Department of Ophthalmology, Otorhinolaryngology and Head and Neck Surgery.
Send correspondence to: Andréia Ardevino de Oliveira. Departamento de Oftalmologia, Otorrinolaringologia e Cirurgia de Cabeça e Pescoço. Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto - USP - SP. Avenida Bandeirantes, nº 3.900, 12º andar. Monte Alegre. Ribeirão Preto - SP. CEP: 14049-900.
E-mail: andreiaoliveira@hcrp.fmrp.usp.br
Paper submitted to the BJORL-SGP (Publishing Management System - Brazilian Journal of Otorhinolaryngology) on May 30, 2012;
and accepted on October 2, 2012. cod. 9233.

## INTRODUCTION

Aminoglycosides are among the most used antibiotic agents in the world because of their high efficacy and low cost. Nonetheless, they do have important side effects, such as kidney toxicity and ototoxicity.

The ototoxic action of these antibiotics happen directly on the polyphosphoinositides receptors, located on the membrane of hair cells of the organ of Corti, of the saccular and utricular macula and the ampullary crests of the vestibular system. These receptors are lipidic components of the cell membrane which form complexes with aminoglycosides, bringing about changes to the membrane permeability, which may cause cellular failure with consequent hearing loss[1].

The ototoxicity mechanism against the hair cells involves the capture of these aminoglycosides by these cells, through receptor-mediated endocytosis[2], individual genetic predisposition to cell damage involving mitochondrial DNA alterations[3,4], the damage action of Reactive Oxygen Species (ROS) - formed as a consequence of aminoglycosides scavenging iron[5,6] - all the way to programmed cell death by apoptosis[7].

Ototoxicity may appear during acute or late exposure to aminoglycosides, even months after the exposure. It may evolve to a more severe degree or even recovery of normal auditory thresholds (prior to the exposure). When cochlear lesion ensues with destruction of the organ of Corti hair cells, hearing loss is irreversible[8].

Amikacin, the first described aminoglycoside, is a derivative of kanamycin, active against most microbial species resistant to gentamicin and to kanamycin itself[9].

The pattern of injury to the Organ of Corti involves the initial damage to the external hair cells on the basal turns of the cochlea, later progressing with a lesser degree of cochlear lesion, towards the cochlear apex[10]. The lesions affect preferably the outer hair cells, initially reaching the first row of cells, then following to the second and third rows[11].

A dose of 400 mg/kg/day of intramuscular amikacin for 12 days causes the complete destruction of outer hair cells and a partial lesion to the internal ones, on the first and second turns of the cochlea of guinea pigs, with lesser lesions on the third and fourth turns[10].

Non-damaging sound stimuli (low intensity ones), employed during a long period of time prior to the exposure to traumatic noise of the same type, protect the cochleas of lab animals, reducing the physiological alterations and the lesions to the sensorial cochlear cells[12], a phenomenon known as resistance. It is very likely that the conditioning stimuli would change the cell, making it more capable of withstanding damaging stimuli and the protection seems to be mediated by cochlear changes[13].

It has been proved that the resistance phenomenon is also manifested after the prior and longstanding administration of non-damaging doses of amikacin before employing the ototoxic doses, in other words, the non-damaging dose of amikacin significantly protects the hair cells against the ototoxicity of amikacin itself on the two most basal turns[14].

Our goal has been to study the otoprotection of the outer hair cells against the ototoxicity caused by amikacin is temporarily persistent.

## METHOD

We utilized fourteen albino lab animals (*Cavia porcellus*), weighing 250g and with the Preyer's reflex. The study was approved by the Ethics Committee in experimentation with animals of our institution, approval protocols # 075/2008.

The drug utilized in this study was intramuscular amikacin. The Preyer's reflex was tested daily, the same happened with the assessment of body weight until the maximum time before animal slaughtering.

The animals were distributed in three groups:

**Group A (control)** - Four guinea pigs injected with intramuscular distilled water for 30 days (two animals) and 60 days (two animals).

**Group B** - Five animals injected with intramuscular amikacin 20 mg/kg/day, for 30 days (protective dose) and, afterwards, a dose of 400 mg/kg/day of amikacin (damaging dose) for 12 days, the mean necessary time for the elimination of the Preyer's reflex. After that, the animals were kept for 30 days on a regular diet, and they were afterwards slaughtered.

**Group C** - Five animals in the same amikacin administration regimen from Group B were maintained for 60 days and then slaughtered.

The technique used to study the histopathology changes in this study was the scanning electron microscopy (SEM), with attention to the structural damages caused to the Organ of Corti on the different cochlear turns, especially to the outer hair cells which received different doses of amikacin.

The guinea pigs were anesthetized by inhaling ether and then they were beheaded, their temporal bones containing their bullae were removed. The apex and the round window were opened and, for fixation, we used glutaraldehyde at 2.5% in a 0.1% phosphate buffer (Sorensen) at 4°C. The microdissection carried out preserved the spiral lamina with the organ of Corti. This material was preserved for 12 hours in a 0.1M buffer solution, and it was reaffixed in an osmium tetroxide solution with 0.1M phosphate buffer for 1 hour at 4°C. The following stages were: dehydration in ethanol with drying using the critical point of liquid carbon dioxide in a BALTEC - CPD 030 - "CRITICAL POINT DRYER" device. The cochlear was then settled in a cylindrical specimen holder, fixed with carbon conductive paste and plated with gold (thin layer) through a vaporizer (BALTEC - SDC 050) for perfect visualization upon scanning electron microscopy.