

Apoptotic effect of gentamicin in cochlea ototoxic rat model (preliminary study)

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Apoptotic effect of gentamicin in cochlea ototoxic rat model (preliminary study)

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ABSTRACT: This study aimed to show the potency of gentamicin in increasing the apoptotic index in the cochlea lateral wall of ototoxic rat models. Eight *Rattus norvegicus* were divided into two groups: 1) control and 2) gentamicin administered. The rats were terminated to measure the apoptotic index using TUNEL assay of the fibroblasts of the cochlea lateral walls. Data were analysed by using ANOVA, with $p < 0.05$ used as the cut-off for statistical significance. Administration of gentamicin showed increased apoptotic index which was statistically significant ($p < 0.05$). This study demonstrates that gentamicin is a pro-apoptotic and demonstrates its potency.

Keywords: apoptotic, cochlea, gentamicin, ototoxic

INTRODUCTION

Gentamicin is one of the aminoglycoside antibiotics which is important in challenging Gram-negative bacteria (Mouedden et al., 2000), however, it is often criticised because of its nephrotoxicity and ototoxicity (Sardana et al., 2015; Huth et al., 2011; Petersen & Rogers, 2015).

Aminoglycoside usage induces reactive oxygen species (ROS) and caspase formation, and can lead to apoptosis (Glutz et al., 2015).

The patterns and mechanisms of hearing loss which are caused by aminoglycosides are not fully understood (Selimoglu, 2007). The aim of this research was to assess gentamicin as a pro-apoptotic in the cochlea lateral wall in ototoxic rat models.

MATERIALS AND METHODS

Eight male *Rattus norvegicus* of 150–250 grams in weight were used. They were separated into two equal-sized groups. The rats were anaesthetised using 10 mg/kg of xylazine and 90 mg/kg of ketamine intraperitoneally (Toydemir et al., 2015). Injections of gentamicin (40 mg/ml) 0.03–0.05 cc (Sagit et al., 2013), guided using a microscope, were placed anterosuperior to the tympanic membrane. Group 1 was the control group which was not treated, while group 2 was injected with gentamicin. Ad libitum food, and comfortable lightning, humidity and room temperatures of between 20°C to 26°C were provided. The rats were terminated 18 hours after being injected (Suzuki et al., 2008).

Temporal bone tissues were fixated with 10% formalin buffer solution and EDTA for four weeks and it was then possible to evaluate the apoptotic index of fibroblasts on the cochlea lateral wall.

The following procedural steps were carried out: the apoptotic index was evaluated using an Olympus XC 10 microscope with 40x magnification. Brown-stained TUNEL-positive markers were seen in the nuclei of cells, and counted in a masked manner. The researchers evaluated two fields which were randomly selected (Zhang et al., 2013).

The data were analysed using one-way ANOVA by using IBM SPSS statistical software with a significance level of 0.05.

RESULTS

Figure 1 shows the lateral wall of the cochlea and Figure 2 shows the role of gentamicin in the lateral wall of the cochlea of *Rattus norvegicus*, observed by using the TUNEL assay method. Using this method the researchers assessed the fragmentation of DNA in the cell nucleus as indicated by brown colouration. In group 2, in which the rats were given gentamicin, the cell nuclei are mostly brown, compared to group 1 where far fewer are seen.

Table 1 shows significant differences ($p < 0.05$) between the control group and group 2. Gentamicin administration increased the apoptotic index in group 2.

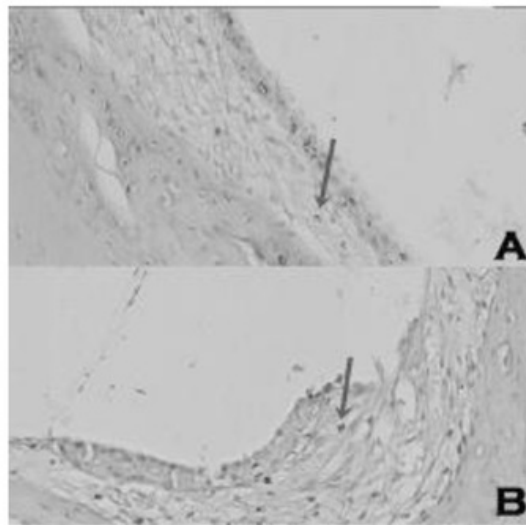


Figure 1. Apoptosis index in each group (40x magnification). The arrows indicate TUNEL (+) cells. A: Group 1; B: Group 2.

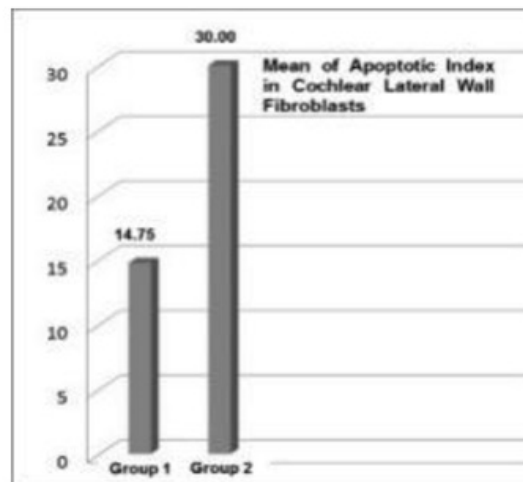


Figure 2. Average number of cells undergoing apoptosis in cochlea lateral wall fibroblasts for each treatment group.

Table 1. ANOVA test results for apoptotic index.

Group		Mean difference ± standard deviation	p-value
1	2	-15.250 ± 3.433	0.000*

*Denotes statistically significant.

DISCUSSION

The lateral wall of the cochlea is the first the part of the cochlea in which histological change occurs (Fujioka et al., 2014), and this led the researchers to investigate this site for this research.

In this study, the pro-apoptotic role of gentamicin as shown in the apoptotic index of the fibroblasts of ototoxic rat models was evaluated. Gentamicin at a dosage of 40 mg/ml was enough to increase the apoptotic index in lateral cochlear wall fibroblasts. Gentamicin toxicity enhances stress markers (caspase 12), pro-apoptotic BAX, released caspase 3 and blocked anti-apoptotic Bcl-2 (Jaikumkao et al., 2016). In chinchillas exposed to gentamicin it was found that cochlea cell death markers increased (Ding et al., 2010). In this present study the method of intratympanic gentamicin injection dose to a Wistar rat used in other research (Sagit et al., 2013) was followed. The chosen termination time of 18 hours after exposure was based on the results of a study of the round window membrane in guinea pigs, which gave the best TUNEL results (Suzuki et al., 2008).

After exposure, aminoglycoside binds to ferric iron (Fe^{III}) (Fetoni et al., 2012; Kurasawa and Steyger, 2011) leading to Fe^{II} -aminoglycoside complexes and forming ROS. ROS activate BAX which in turn triggers apoptosis (Kurasawa and Steyger, 2011).

CONCLUSION

This study showed that gentamicin is able to increase the index of apoptosis in rat models.

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