ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

The Protective Effect of Taxifolin Against Amikacin-induced Ototoxicity in Rats: a Biochemical and Histopathological Evaluation

Taxifolinin Sıçanlarda Amikasin ile İndüklenen Ototoksisiteye Karşı Koruyucu Etkisi: Biyokimyasal ve Histopatolojik Değerlendirme

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ABSTRACT

Aim: *Amikacin is a semi-synthetic aminoglycoside derivative antibacterial drug. Amikacin's cochleotoxic effects causing permanent hearing loss have been reported. Over-production of reactive oxygen species (ROS) are responsible for the ototoxicity of amikacin. The study investigates the protective effect of Taxifolin (3.5.7.3.4-pentahydroxy flavanone), an antioxidant flavonoid, against amikacin-induced ototoxicity. The objective is to biochemically and histopathologically investigate the protective effect of taxifolin against amikacin-induced ototoxicity in male albino Wistar rats.*

Material and Method: *TAK group animals were administered taxifolin 50 mg/kg (n-6) by oral gavage. AMK (n-6) and HG (n-6) groups were administered distilled water as solvent using the same method. One hour after taxifolin and distilled water administration, TAK and AMK groups were given 200 mg/kg amikacin by intramuscular route. This procedure was performed once a day for 14 days. Cochlear tissues extracted from the animals killed after this period with high doses of ketamine (120 mg/kg) were investigated biochemically and histopathologically.*

Results: *Histopathological damage such as edema and deconstruction was observed in cochlear tissues of the AMK group where malondialdehyde (MDA) and total oxidant status (TOS) were high and total glutathione (tGSH) and total antioxidant status (TAS) were low. However, no histopathological finding except for mild edema was observed in the TAK group where MDA, TOS, tGSH, and TAS levels were close to those of the HG group.*

Conclusion: *Taxifolin may be beneficial in terms of preventing amikacin-induced cochlear damage.*

Key words: *amikacin; taxifolin; ototoxicity; rat*

ÖZET

Amaç: *Amikasin, yarı sentetik aminoglikozid türevi antibakteriyel bir ilaçtır. Amikasinin kalıcı işitme kaybına yol açan kokleotoksik etkileri rapor edilmiştir. Amikasin ototoksisitesinden, reaktif oksijen türlerinin (ROS) aşırı üretimi sorumlu tutulmaktadır. Çalışmamızda amikasin ile indüklenen ototoksisiteye karşı koruyucu etkisini araştıracağımız Taxifolin (3.5.7,3,4-pentahydroxy flavanone) antioksidan bir flavonoiddir. Taxifolinin albino Wistar türü erkek sıçanlarda amikasin ile indüklenen ototoksisiteye karşı koruyucu etkisini biyokimyasal ve histopatolojik olarak araştırmak amaçlanmaktadır.*

Materyal ve Metot: *Hayvanların TAK grubuna taxifolin 50 mg/kg (n-6) oral yoldan gavajla verildi. AMK (n-6) ve HG (n-6) gruplarına ise çözücü olarak distile su aynı hacimde aynı yöntemle uygulandı. Taxifolin ve distile su verildikten bir saat sonra TAK ve AMK gruplarına amikasin 200 mg/kg intramusküler yoldan enjekte edildi. Bu prosedür günde bir defa 14 gün boyunca uygulandı. Bu süre sonunda yüksek doz ketamile (120 mg/kg) öldürülen hayvanlardan çıkarılan koklea dokuları biyokimyasal ve histopatolojik olarak incelendi.*

Bulgular: *Malondialdehid (MDA) ve total oksidan statusun (TOS) yüksek, total glutatyon (tGSH) ve total antioksidan statüsun (TAS) düşük bulunduğu AMK grubunun koklea dokusunda ödem ve destrüksiyon gibi histopatolojik hasar görülmüştür. Ancak, MDA, TOS, tGSH ve TAS düzeyleri HG grubuna yakın olan TAK grubunda hafif ödem dışında herhangi bir histopatolojik bulgulara rastlanmadı.*

Sonuç: *Taksifolin, amikasinle ilişkili koklea hasarının önlenmesinde yararlı olabileceğini işaret etmektedir.*

Anahtar kelimeler: *amikasin; taksifolin; ototoksitite; rat*

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Introduction

Amikacin is a semi-synthetic aminoglycoside derivative antibacterial drug obtained by acetylation of kanamycin[1]. Amikacin is resistant to bacterial enzymes that can inactivate other natural aminoglycosides[2]. Aminoglycoside antibiotics (AGAs) are known for their rapid activity, low bacterial resistance, synergistic activity with beta lactams and their low cost[3]. Although AGAs include a wide range and have numerous areas of application, their adverse effects limit their use[4]. According to various studies, the ototoxicity incidence of AGAs range between 10% and 80% [5]. Similar to other AGAs, amikacin also has cochleotoxic effects causing permanent hearing loss[4]. Ototoxicity is a clinical condition caused usually by the harmful effects of some chemical agents on hearing and balancing functions of the ear[6]. Over-production of reactive oxygen species (ROS) is held responsible for the pathogenesis of amikacin ototoxicity[7,8]. Aksoy F. et al.[9] reported that there were many experimental studies demonstrating that antioxidant agents can prevent AGA ototoxicity.

The present study investigate the protective effect of Taxifolin (3.5.7.3.4-pentahydroxy flavanone or dihydroquercetine), against amikacin-induced ototoxicity. Taxifolin is an antioxidant flavonoid that can be found in citrus and onion in high quantities[10]. In literature, flavonoids have been reported to inhibit lipid peroxidation and enzymatic reactions that produce ROS[11]. Taxifolin has been reported to protect tissues from oxidative damage by inhibiting malondialdehyde (MDA) increase in various organ tissues and glutathione (GSH) decrease, which is an endogenous antioxidant[12–14]. Taxifolin use is also recommended to minimize lipid oxidation in food and pharmaceutical products[15]. All this information demonstrate that ROS is a major component in the pathogenesis of amikacin-induced ototoxicity. It is believed that taxifolin can be beneficial in treating amikacin-induced ototoxicity damage. There was no information in the literature about the protective effect of taxifolin against amikacin-induced ototoxicity. For this reason, the objective of our study is to biochemically and histopathologically investigate the protective effect of taxifolin against amikacin-induced ototoxicity in male albino Wistar rats.

Material and Methods

Animals

Male albino Wistar rats to be used in the study were obtained from the Medical Experimental Research and Application Center of Ataturk University. A total of 18 male albino Wistar rats weighing between 290 and 305 grams were used for the experiment. Prior to the experiment, animals were housed and fed in groups at normal room temperature (22°C).

Chemicals

Ketamine used in the experiment was supplied from Pfizer Ilaçlari Ltd. Şti. (Turkey), amikacin from Sanofi Ilaç San. (Turkey) and taxifolin from Evalar-Russia.

Animal Groups

Experimental animals were divided into three groups: amikacin (AMK) administered, taxifolin+amikacin administered and the control group (HG).

Experimental Procedure

Pharmacological procedure: TAK group animals were administered taxifolin 50 mg/kg (n-6) by oral gavage. AMK (n-6) and HG (n-6) groups were administered distilled water as solvent using the same method. One hour after taxifolin and distilled water administration, TAK and AMK groups were given 200 mg/kg amikacin by intramuscular route. This procedure was performed once a day for 14 days. Cochlear tissues extracted from the animals killed after this period with high doses of ketamine (120 mg/kg) were investigated biochemically and histopathologically. Biochemical and histopathological examinations were performed on removed cochlear tissues. Biochemical and histopathological results obtained from TAK and HG animal groups were compared with those of the AMK group.

Biochemical analyses: Homogenates of cochlear tissues were prepared for biochemical analysis. Total glutathione (tGSH) and malondialdehyde (MDA) levels of supernatants, which were obtained from the homogenates, were determined using appropriate methods based on the literature.

Preparation of samples: At this stage of the study, 0.2-gram from each removed tissue was weighed. They were rounded up to 2 ml in 1.15% potassium chloride solution for determining the MDA and in phosphate buffer with a pH of 7.5 for determining the tGSH and homogenized in an ice-cold medium. It was then centrifuged at 10000 rpm at +4°C for 15 minutes. The supernatant was used as an analysis sample.

Determination of MDA: It is based on spectrophotometric measurement at 532 nm wavelength of the

absorbance of the pink colored complex formed by thiobarbituric acid (TBA) and MDA at a high temperature (at 95°C) _ENREF_100[16]. Homogenates were centrifuged at 5000 g for 20 minutes and these supernatants were used to identify the MDA amount. 250 µl homogenate, 100 µl 8% sodium dodecyl sulfate (SDS), 750 µl 20% acetic acid, 750 µl 0.08% TBA and 150 µl distilled water were put into capped test tubes via pipetting and vortexed. The mixture was incubated at 100 °C for 60 minutes, and then, 2.5 ml of n-butanol was added and measured spectrophotometrically. The red color intensity was read using 3 ml cuvettes at 532 nm and MDA amount of samples was determined by a standard graph developed using an MDA stock solution prepared by considering the dilution coefficients.

Determination of tGSH: DTNB [5.5'-Dithiobis (2-nitrobenzoic acid)] in the measuring medium is a disulfide chromogen easily reduced by sulfhydryl compounds. Yellow color intensity was measured spectrophotometrically at 412 nm[17]. Homogenates were centrifuged for 10 minutes at 12000 g and supernatants were used to determine the GSH amount. 1500 µl measuring buffer (200 mM Tris-HCl containing 0.2 mM EDTA, pH=8.2), 500 µl supernatant, 100 µl 5.5'-Dithio-bis (2-nitrobenzoic acid) (DTNB) and 7900 µl of methanol were put into capped test tubes via pipetting and then vortexed. The mixture was incubated at 37 °C for 30 minutes and measured spectrophotometrically. The amount of yellow form was read using 3 ml quartz cuvettes at 412 nm and GSH amount of samples was determined by a standard graph developed using an GSH stock solution prepared by taking into consideration dilution coefficients.

Measurements of TOS and TAS: TOS and TAS levels of cochlear tissue homogenates were determined using a novel automated measurement method and commercially available kits (Rel Assay Diagnostics, Turkey), both developed by Erel[18,19]. The TAS method is based on the bleaching of characteristic color of a more stable ABTS (2.2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants and, measurements is performed 660 nm. The results are expressed as nmol hydrogen peroxide (H_2O_2) equivalent/L. In TOS method, the oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules, abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured at 530 nm spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The results were expressed as µmol Trolox equivalent/L. The percentage ratio of TOS to TAS was used as the oxidative stress index (OSI). OSI was calculated as TOS divided by 100xTAS.

Histopathological examination: Following a routine tissue monitoring, sections of 5 µm were obtained for histopathological examination. These sections were stained using Hematoxylin-Eosin (H&E) and cochlear tissues were evaluated using light microscopy (Olympus BX 51, Japan) by a pathologist uninformed of the treatment protocol, and the photographs were taken using a digital camera (Olympus DP71).

Statistical Analyses

Results obtained from the experiments were expressed as" mean value ± standard error " (x ± SEM). Level of significance of the difference among groups were determined using the one-way ANOVA test. After that, Fisher's post-hoc LSD (least significant differences) was conducted. All statistical procedures were carried out on "IBM SPSS Statistics Version 20" and $p<0.05$ was considered significant.

Results

Biochemical Findings

MDA and tGSH Analysis Results: As can be seen in Figure 1, the amount of MDA in cochlear tissues of animals administered amikacin increased significantly compared to healthy and taxifolin groups. However, the difference in MDA amount in healthy and taxifolin groups was statistically insignificant. The tGSH amount in amikacin group was found to be less compared to that of healthy and taxifolin groups. The difference in the amount of tGSH in the healthy group and the taxifolin group was found insignificant.

TOS and TAS analysis results: It was found that amikacin significantly increased the level of TOS in the cochlear tissue of animals compared to healthy and taxifolin groups. TOS levels were similar in healthy and taxifolin groups. Amikacin led to a decrease in TAS level in the cochlear tissue of animals. However, taxifolin significantly prevented the decrease of TAS level associated with amikacin and the difference in TAS level between the healthy and the taxifolin groups was found to be insignificant (Figure 2).

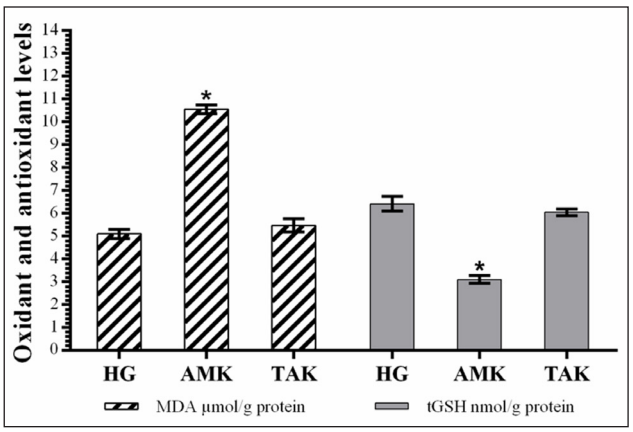

Figure 1. *MDA and tGSH levels in the cochlea tissue of study groups. According to HG and TAK groups *=p<0.0001, (n=6).*

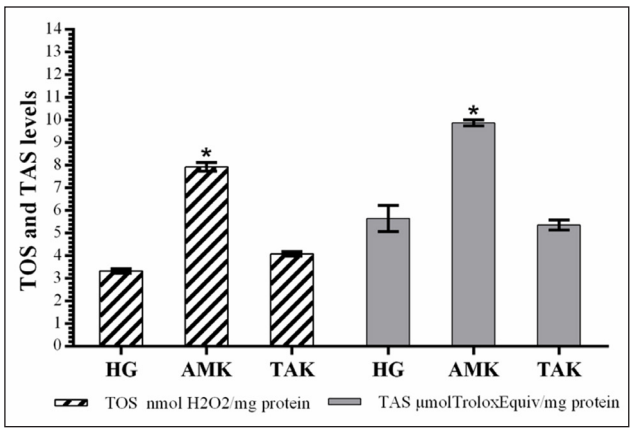

Figure 2. *TOS and TAS levels in the cochlea tissue of study groups. According to HG and TAK groups *=p<0.0001, (n=6).*

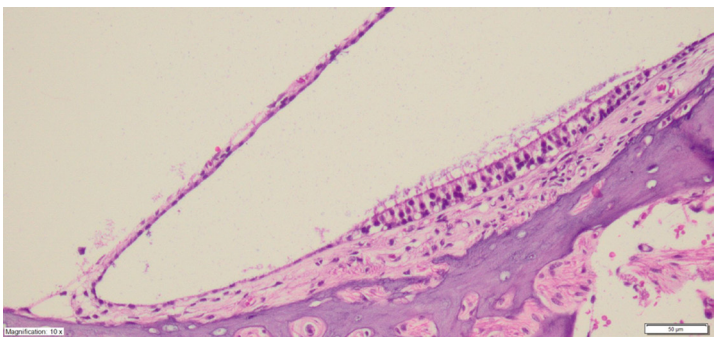

Figure 3. *Cochlear tissue of the healthy animal group (HE×200).*

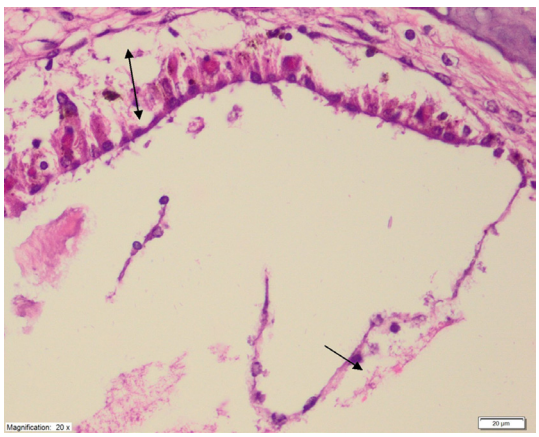

Figure 4. *Animal group administered only amikacin: cochlear edema and deconstruction (double-headed arrow), vestibular membrane degeneration (single-headed arrow, HE×400).*

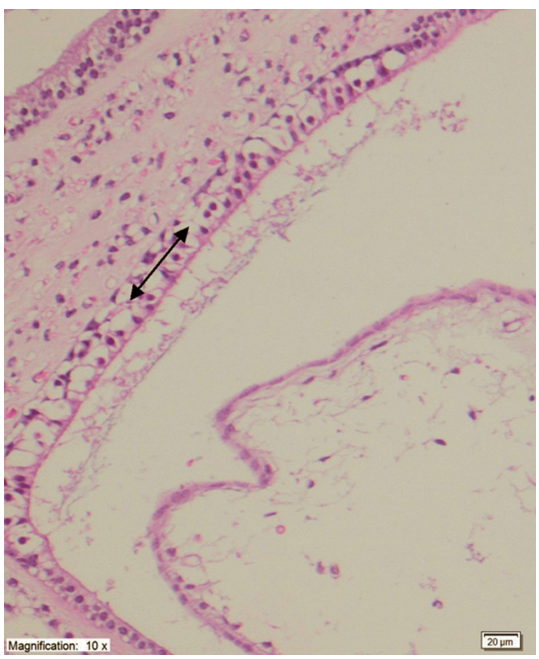

Figure 5. *Animal group treated with taxifolin: Edema in cochlea (double-headed arrow, HE×200).*

Histopathological Findings

Figure 3 shows normal cochlear tissue of the healthy group. Severe edema and deconstruction can be seen in cochlea of the group only administered amikacin. Vestibular membrane degeneration was also detected in the amikacin group (Figure 4). In addition to edema in the cochlea, no other histopathological finding was observed in the animal group treated with taxifolin (Figure 5).

Discussion

Since any other alternatives have not been found for amikacin and other AGAs, ototoxicity caused by these drugs is still open to discussion. Even though there have been various experimental studies to prevent amikacin-induced ototoxicity, clinical studies are still conducted for new strategies on its prevention[1]. The present study investigated the protective effect of taxifolin on amikacin-induced ototoxicity in rats biochemically and histopathologically. Literature suggests that AGAs are both vestibulotoxic and cochleotoxic[8]. In this group, streptomycin and gentamycin

were reported to be more vestibulotoxic, while other AGAs were reported to be cochelotoxic[1]. As mentioned above, over-production of ROS is responsible for the pathogenesis of amikacin ototoxicity [7,8]. The results of the biochemical experiments performed under this study show that MDA and TOS levels in the cochlear tissues of animals administered amikacin significantly increased, while tGSH and TAS levels decreased compared to healthy and taxifolin groups. A correlation was reported between increased MDA levels associated with amikacin in the cochlea and auditory dysfunction[20]. Aksoy F et al. reported that the increase in total oxidant level (TOS) due to amikacin led to sensorineural hearing loss in rats [9]. In our study, tGSH level in the amikacin group was found to be lower compared to healthy and taxifolin groups. In addition to low tGSH, TAS level was also low in the amikacin group. These results suggest that the physiological balance between the oxidant and the antioxidant is disrupted in the amikacin group. Literature reveals that the disruption of this balance leads to tissue damage, which is called oxidative stress[21]. It has been reported that a decrease in the amount of antioxidants in the cochlear tissue may result in auditory dysfunction[20]. It has also been reported that low TAS caused hearing loss in rats [9].

In our study, we used high MDA and low tGSH levels as a base for evaluating the oxidative damage to cochlear tissue. MDA is a reliable end product of lipid peroxidation (LPO) [22]. This is the reason why MDA is widely used as an indicator of oxidative status [23]. Increased MDA level indicates increased ROS. The MDA formed as a result of LPO is toxic and may cause further destruction[24]. However, in healthy tissues, the harmful effects of these oxidants are prevented by GSH and other enzymatic and non-enzymatic antioxidants. GSH is an antioxidant tripeptide composed of L-glutamate, L-cysteine, and glycine found in cells. GSH reacts with hydrogen peroxide (H_2O_2) and organic peroxides to detoxify and protect cells from SOR damage)[25].

The cochlear structure (architecture) was examined histopathologically to prove the accuracy of the results of our biochemical experiment. While edema and destruction were observed in the cochlear tissue of the group only administered amikacin, degeneration was identified in the vestibular membrane. In a study, which was conducted long ago by Lenoir M et al. amikacin was shown to result in morphological changes in the cochlea[26]. Cazals et al. reported that amikacin caused destruction of the cochlea[27]. No destruction was observed on the cochlear tissue of the taxifolin group. In addition, degeneration was found in the vestibular membrane. Our histopathological findings are consistent with biochemical findings. There is no information in the literature about the protective effect of taxifolin on the cochlea. However, there are studies showing that taxifolin has a cytoprotective effect and protects tissues from oxidative damage[13,28,29]. Taxifolin has been reported to prevent bone loss due to osteoporosis by inhibiting ROS and proinflammatory cytokine overproduction[30]. In conclusion: Amikacin caused oxidative cochlear damage in animals. The cochleotoxic effect of amikacin has been demonstrated by biochemical and histopathological findings. It has been understood that taxifolin prevents amikacin-associated oxidative cochlear damage. This suggests that taxifolin may be beneficial in treating amikacin-associated ototoxicity.

Conflict of Interest

No potential conflict of interest was reported by the author (s).

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