Antioxidant Effect of Curcumin on the Prevention of Oxidative Damage to the Cochlea in an Ototoxic Rat Model Based on Malondialdehyde Expression

Tengku Siti Hajar Haryuna[1] Dyah Fauziah[2] Sari Anggraini[1]
M. Pahala Hanafi Harahap[1] Juliandi Harahap[3]

[1] Department of Otorhinolaryngology, Head and Neck Surgery, Faculty of Medicine, Universitas Sumatera Utara, Medan, North Sumatera, Indonesia
[2] Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia
[3] Department of Community Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan, North Sumatera, Indonesia

Address for correspondence Tengku Siti Hajar Haryuna, Department of Otorhinolaryngology - Head and Neck Surgery, Universitas Sumatera Utara, Jl. Dr. Mansyur Padang Bulan No. 5, Medan, North Sumatra 20155, Indonesia (e-mail: tengkusitihajarharyuna2@gmail.com).

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Abstract

Introduction Aminoglycoside, as an antimicrobial medication, also has side-effects on the inner ears, bringing about hearing disorders. Curcumin has been proven to be a strong scavenger against various reactive oxygen species (ROS), and the increase in ROS production is considered to play an important role in the process of hearing disorder.

Objective To prove that curcumin is an effective antioxidant to prevent cochlear damage based on malondialdehyde (MDA) expression.

Methods The present research used 32 *Rattus norvegicus*, of the Wistar lineage, randomly divided into 8 groups: negative control, ototoxic control (a single dose of 40 mg/ml of gentamicin via intratympanic injection), 2 groups submitted to ototoxic control + curcumin treatment (100 mg/kg, 200 mg/kg), 2 groups who iunderwent ototoxic control + curcumin treatment for 7 days, and two groups submitted to curcumin treatment as prevention for 3 days + ototoxic induction.

Results The results showed that the lowest dosage of curcumin (100 mg/kg) could decrease MDA expression on the cochlear fibroblastic wall of the ototoxic model; however using greater doses of curcumin (200 mg/kg) for 7 days would provide a better effect. Curcumin could also significantly decrease MDA expression when it was administered during the preototoxic exposure.

Conclusion Curcumin can be used as a therapy for ototoxic prevention based on the decrease in MDA expression.

Keywords
- curcumin
- malondialdehyde
- cochlear fibroblast
- reactive oxygen species
- ototoxicity
- gentamicin

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Introduction

Various kinds of medicines and topical agents can cause ototoxicity.[1] Toxic reactions induced by medicines often trigger the development of hearing disorders in some people. They can occur in one or both ears with different levels of damage, and their effects can be identified in the short or long terms.[2] The effect of this ototoxicity can last for a day or a few weeks after the medicine enters body circulation.[3]

Numerous medicines can be categorized as ototoxic because of their side effects on the inner ears. Ototoxicity can be divided into cochleotoxicity (when it affects hearing) or vestibulotoxicity (when it affects balance).[4] Cochleotoxicity occurs in 2% to 25% of patients, while vestibulotoxicity occurs in 15% of patients who consume aminoglycosides.[3] Some medicines which contain ototoxic effects are diuretic loop, cytostatic, tuberculostatic, quinine, and aminoglycoside drugs. Ototoxic agents damage the structure of the inner ear and change the mechanoelectrical transduction, which causes functional problems.[5]

As wide-spectrum antibiotics, aminoglycosides have been reported to have strong bactericidal activity against Gram-negative bacteria. Their potency to kill bacteria is preferred over that of other medicines which only possess bacteriostatic capabilities.[6] The popularity and universal use of aminoglycosides have made its ototoxicity side effect as the main cause of hearing disorder. Even though aminoglycoside ototoxicity is usually associated with accumulated dosage and the administration process, cochlear dysfunction could still occur in some patients within or below the indicated therapeutic dosages.[7]

The molecular mechanism of ototoxicity can cause an elevated production of reactive oxygen species (ROS), depletion of the natural antioxidant glutathione and its enzymes, and the average increase in fat oxidation, oxidative modification of proteins, nucleic acid damage through caspase activation, and modification of important cochlear proteins.[8] Reactive oxygem species can also cause cell dysfunction, necrosis or apoptosis in the tissues, as well as induce posttranslational changes that can affect the function of protein cells and signal pathways.[9] Increasing ROS can damage deoxyribonucleic acid (DNA), disintegrate endothelial and generate apoptosis.[10] Increasing the levels of ROS in the testicles, which are linked to membrane lipids, proteins and DNA, can affect sperm production and quality. This peroxidation damage to the tissues indicates a biochemical basis for reperfusion injury.[11]

Oxidative stress markers have been the focus of various studies as important tools to assess the biological redox status, the condition and course of the disease, as well as the effects of antioxidants on human health[12] Soltani et al.[13] (2018) conducted a study on oxidative stress and malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels. It is known that the last product of lipid oxidation is MDA, which has been widely regarded as a reliable biomarker of oxidative stress.[14]

Curcumin, a well-known bioactive phytochemical extracted from turmeric (*Curcuma longa*), has been used for centuries in Chinese and Indian traditional medicines.[15] Curcumin is also used throughout Asia as a spice and seasoning to give food a certain taste and yellowish color.[16] Numerous studies have reported that curcumin has many healing properties, such as an antioxidant, anti-inflammatory agent, it acts in the prevention and treatment of cancer,[17,18] and is an anti-angiogenic,[17] anti-thrombotic, and hepatoprotective agent.[18] Curcumin has long been known as a safe ingredient. However, there are some side effects that have been reported in several studies, which are diarrhea, headache, rash, yellow stool, nausea, and an increase in the serum levels of alkaline phosphatase and lactate dehydrogenase.[19]

The present research aims to assess whether curcumin can work as an effective and safe antioxidant to prevent and slow the development of damage to the cochlea fibroblasts based on the decrease in MDA expression in an ototoxic model of rats.

Methods

The present experimental study employed a randomized post-test only control group design. All procedures were approved by the Health Research Ethical Committee, Faculty of Medicine of Universitas Sumatera Utara/H. Adam Malik General Hospital, under number 777/TGL/KEPK FK USU-RSUP HAM/2016, in accordance with the Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes. In total, 32 adult male white rats (*Rattus norvegicus* of the Wistar lineage) were obtained from the Animal Experimental Unit of the Biochemistry Laboratory, Faculty of Medicine, Universitas Airlangga, Indonesia. Before observation, the rats were subjected to a 12-day clinical evaluation in a preconditioned environment (20°C to 25°C, 12 hours of light) and ad libitum feeding to control body weight between 150 g and 250 g.

The curcumin used in the research was extracted directly from *C. longa* (The Testing Service Unit, Faculty of Pharmacy, Universitas Airlangga, Surabaya, number 0632/SA/V/2016) with levels of $16.62 \pm 0.14\%$ w/w compared with Standard using Thin Layer Chromatography and Densitometry, provided by professor doctor Suprapto Ma'at, MS (Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia). Curcumin was administered to the rats at dosages of 100 mg/kg and 200 mg/kg, and it was suspended in 0.5% carboxymethyl cellulose (CMC) and delivered through a nasogastric tube (NGT).

The 32 rats were randomly divided into /8 groups (with 4 rats in each group), as seen on ►**Table 1**.

To induce ototoxicity on the test subjects, 0.1 ml of gentamicin (40 mg/ml) was injected (intratympanic) at the anterosuperior part within 60 to 80 minutes of the anesthesia (7.5 mg/kg of xylazine and 100 mg/kg of ketamine intraperitonially).[20] The functional and morphological changes in the cochlea were observed in terminated test subjects 18 hours after the administration of gentamicin.[21]

It is known that the administration of 100 mg/kg of curcumin once a day for 7 days to posthepatectomy rats

Table 1 Setup of the ototoxic model group

Group 1	Group 2	Group 3	roup 4	Group 5	Group 6	Group 7	Group 8
Control	gentamycin 40 mg/ml	gentamycin 40 mg/ml ↓ *curcumin* 100 mg/kg after 18 hours	gentamycin 40 mg/ml ↓ *curcumin* 200 mg/kg after 18 hours	gentamycin 40 mg/ml ↓ *curcumin* 100 mg/kg for 7 days	gentamycin 40 mg/ml ↓ *curcumin* 200 mg/kg for 7 days	Days 1–3: *curcumin* 100 mg/kg per day ↓ gentamycin 40 mg/ml	Days 1–3: *curcumin* 200 mg/kg per day ↓ gentamycin 40 mg/ml
↓	↓	↓	↓	↓	↓	↓	↓
Termination in 18 hours	Termination in 18 hours			Termination in day 9		Termination in 18 hours	

could decrease MDA significantly.[22] Another research[23] showed that curcumin was able to induce the proliferation of Langerhans cells and protect against oxidative stress in damaged cell tissues related to diabetic cognitive disorder. In the present research, we also tested a double dose to see if we could achieve a better result.

We used the MDA NB 100–62737 antibody (Novusbio, Novus Biologicals, Centennial, CO, US), the biotinylated secondary antibody (anti-rabbit), and peroxidase-labeled streptavidin. After the administration, the test subjects were euthanized by cervical dislocation, and a necropsy of temporal bone tissues was conducted to obtain tissue samples, which were fixed with 10% formalin buffer and subjected to ethylenediaminetetraacetic acid (EDTA) decalcification for 4 weeks. The laboratory examination was performed through fixation by deparaffinized tissue blocks dyed with haematoxylin eosin (HE). The MDA expression was assessed using a immunohistochemical (IHC) grading technique,[24] by multiplying 0% of the cells labeled in the IHC (it was said 0 when it was 0%, 1 when it was $< 30\%$, 2 when it was between 30% and 60%, and 3 when it was $> 60\%$) with the intensity of the IHC reaction (it was called 0 when there was no reaction, 1 when the reaction was week, 2 when it was moderate, and 3 when it was strong), so that the final value was between 0 and 9.

The results were submitted to analysis of variance (ANOVA; $p \leq 0.05$) using the Statistical Package for the Social Sciences (IBM Corp., Armonk, NY, US) software, version 22.0. Data comparison was performed using the Mann-Whitney test followed by the Kruskal-Wallis test to determine the statistical differences between each group.

Results

MDA as the Marker of Oxidative Stress

After the desired cochlea areas were identified through HE pigmentation, we performed the IHC grading to measure MDA expression in the fibroblast of the lateral walls of the cochlea (►**Fig. 1**).

The administration of gentamicin triggered the incidence of the oxidative process indicated by the increase in MDA expression in the cochlear fibroblast, marked by the browning of cytoplasm. This was also clearly observed with the increase in the brownish intensity of the cells in the fibroblast area within the group without curcumin administration (group 2). Curcumin could decrease MDA expression in the cochlear fibroblast of ototoxic model of rats (groups 3 to 8) compared with those in group 2. Group 8 had the lowest MDA expression. The change in MDA expression could be seen in the IHC description, as it was shown in ►**Fig. 2**.

Analysis of the Data

Based on the statistical assessment of the measurement result, group 2 had the highest mean value of MDA expression, while the lowest mean value was found in group 8, as shown in ►**Fig. 3**.

In ►**Table 2**, a significant difference ($p \leq 0.05$) in fibroblast MDA expression is observed between the induced-ototoxicity group (group 2) and the control group (group 1), but there was no significant difference between the induced-ototoxicity group and the groups who only received one dose of curcumin (groups 3 and 4). There was a significant difference ($p \leq 0.05$) in the ototoxic model groups who received only 1 dose of curcumin (groups 3 and 4) compared with the ototoxic model groups who underwent 7 days of curcumin treatment (groups 5 and 6). No significant differences were observed between groups 7 and 8; but, overall, there was a

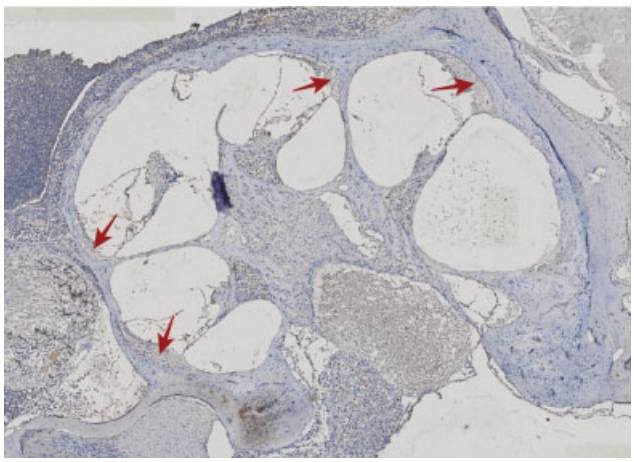

Fig. 1 Supporting tissues and cochlea lateral walls with immunohistochemical (IHC) pigmentation (magnification: 10x). The directional arrow indicates the lateral walls.

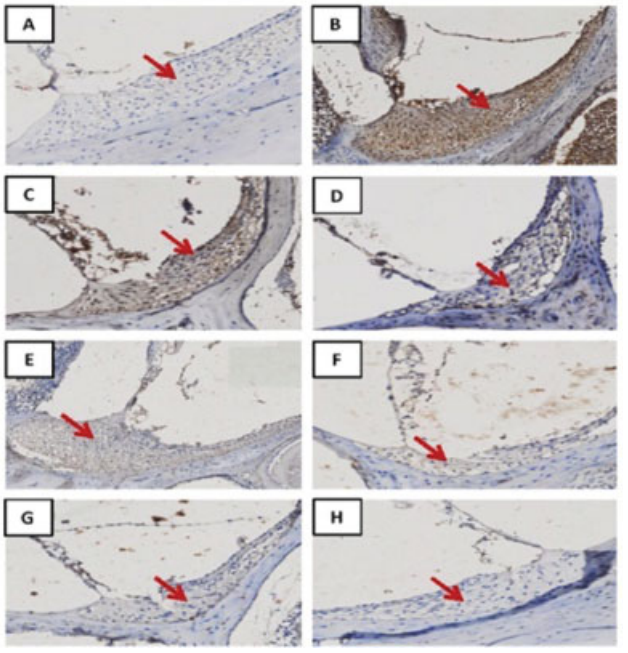

Fig. 2 Malondialdehyde (MDA) expression in each group (magnification: 40x): (**A**) group 1; (**B**) group 2; (**C**) group 3; (**D**) group 4; (**E**) group 5; (**F**) group 6; (**G**) group 7; and (**H**) group 8. The directional arrow indicates the brownish color that characterizes MDA expression in the cochlear fibroblast.

significant difference between the groups who underwent the curcumin treatment and those who did not.

Discussion

The present research assessed MDA expression in the cochlear fibroblast of white rats, *R. norvegicus* of the Wistar lineage. Rodents (especially *Mus musculus* and *R. norvegicus*) have been widely used as guinea pigs in biomedical research for years.[25] Fluorescent imaging shows that gentamicin attaches to the stria vascularis, especially to its marginal cells. Huth et al.[3] point out that, after aminoglycoside enters the systemic current, it enters cochlea in only a few minutes through capillary blood vessels. Aminoglycosides can also be seen in the basal and fibrotic cells. Schacht et al.[4] point out that the outer hairs of corti cells are easily damaged, which gradually affects the lower apex regions. The damage usually occurs from the first up to the third layers, and inner hair cells gradually disappear. Aminoglycoside is also found in the stria vascularis, where degradation of tissue and marginal cells occur. Damage to the ganglion spiral cells usually follows the loss of hair cells, which can persist long after the therapy has finished. It is also pointed out that anomalies in the stria vascularis can occur without damage to the hair cells.

The significant MDA expression among the gentamycin-induced ototoxic model groups compared with the control group supports the statement of Ayala et al.,[14] which point out that MDA constitutes a reliable marker to pinpoint oxidative stress in clinical studies; therefore, MDA reactivity and toxicity are often correlated in biomedical research.

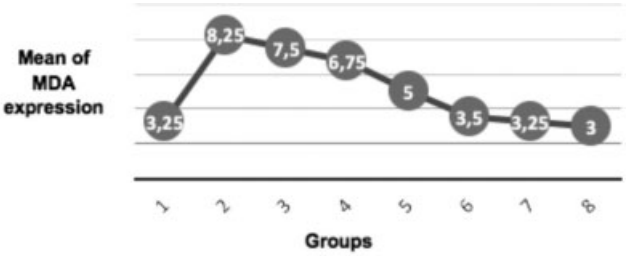

Fig. 3 The mean value of MDA expression in the fibroblast of cochlear lateral walls in an ototoxic rat model during the IHC examination.

Table 2 Analysis of variance for malondialdehyde expression

Group	Group	Standard deviation	*p-value*
Group 1	2	5.000* ± 1.070	.000*
	3	4.250* ± 1.070	.001*
	4	3.500* ± 1.070	.003*
	5	1.750 ± 1.070	.115
	6	.250 ± 1.070	.817
	7	.000 ± 1.070	1.000
	8	.25 ± 1.070	.817
Group 2	3	.750 ± 1.070	.490
	4	1.500 ± 1.070	.174
	5	3.250* ± 1.070	.006*
	6	4.750* ± 1.070	.000*
	7	5.000* ± 1.070	.000*
	8	5.250* ± 1.070	.000*
Group 3	4	.750 ± 1.070	.490
	5	1.750 ± 1.070	.028*
	6	3.250* ± 1.070	.001*
	7	4.250* ± 1.070	.001*
	8	4.500* ± 1.070	.000*
Group 4	5	1.750 ± 1.070	.115
	6	3.250* ± 1.070	.006*
	7	3.500* ± 1.070	.003*
	8	5.750* ± 1.070	.002*
Group 5	6	1.500 ± 1.070	.174
	7	1.750 ± 1.070	.115
	8	2.000 ± 1.070	.074
Group 6	7	.250 ± 1.070	.817
	8	.500 ± 1.070	.645
Group 7	8	.250 ± 1.070	.817

Note: * $p \leq 0.05$: statistically significant difference.

Dierckx et al.[26] found high levels of MDA in the lipid autoxidation pathway in diabetic patients compared with healthy groups.

This is also in accordance with the theory that states that aminoglycoside induces the establishment of ROS, which are central in the molecular path to ototoxicity. Aminoglycosides are known to directly modulate the enzymatic activity related to ROS metabolism by interfering with other antioxidants.[27] Reactive oxygen species can be produced in several sources, including the mitochondria, xanthine oxidase, and unoccupied nitric oxide synthase.[9] The excessive production of ROS promotes oxidative stress that causes many molecular changes such as nucleic acid mutations and protein misfolding that leads to diseases.[15] An increase in ROS levels can produce oxidative stress, activated apoptosis, and cause damage to DNA in the tissues.[28] A study reported that excess ROS decrease sperm motility and morphology, resulting in DNA damage and apoptosis.[29] These factors play their role in initiating cell death as the result of the oxidative response.[30] Numerous pathways are believed to trigger the apoptosis of cochlear cells through ROS activity, such as the caspase-dependent pathway, caspase-independent pathway, lipid autoxidation, and induction of Ca^{2+} influx.[31] Lipid peroxidation (autoxidation) is a chain reaction which continuously provides the supply of peroxide-free radicals and initiates the next peroxidation in the lipoprotein membrane by ROS; among which MDA belongs.[32]

Oxidative damage can occur as the result of overproduction of ROS and/or the lack of antioxidant scavenging ability to neutralize free radicals. There are at least three strategies to avoid the development of oxidative stress within the inner ears: ROS detoxification with antioxidants, ROS inhibition by oxidant scavengers, or cutting off the associated downstream ROS signal pathway.[27] Curcumin shows a strong scavenging activity regarding various ROS, including anionic superoxide, hydroxyl radicals, and nitrogen dioxide radicals.[33]

A significant difference in MDA expression in both ototoxic models was observed between the groups in whom the duration of the curcumin treatment varied (18 hours and 7 days). The results could mean that the longer curcumin treatment with either dose (100 mg/kg or 200 mg/kg) might yield better results.

The decrease in MDA expression caused by curcumin activity within the cochlea fibroblast could be inferred from previous medical observations. Correa et al.[34] pointed out that oxidative stress constituted the basis of the risk factor for mortality in senior hemodialysis patients. It was also found that curcumin could decrease cardiac complications in some patients.

A significant difference was observed between model groups with curcumin administration as therapy (curcumin was administered after gentamycin) and the groups with curcumin supplement as prevention measures (curcumin was administered before gentamycin). Kuhad et al.[35] point out that curcumin has protective effects in cases of cisplatin-induced nephrotoxicity, hinting its function as a strong anti-inflammatory and antioxidant. It is believed that curcumin would also be suitable to prevent the hearing impairment associated with oxidative stress.

Considering that aminoglycosides cannot be metabolized and persist in hair cells for several months, it is necessary to find a potential compound which can suppress the development of ototoxicity during the long-term therapy.[3] Alrawaiq and Abdullah[36] noted that curcumin was considered a strong phytochemical to prevent and alleviate various diseases, and could be used as potential therapeutic agent. The importance of MDA as an important marker of oxidative stress and its role in causing health disorders should be further explored, including the development of better and more reliable testing methods to be applied in nutritional and medical studies.[37]

From the results obtained, it is expected that curcumin can become an effective and safe plant-based medication to prevent and treat hearing loss due to ototoxicity in a biomolecular level. Despite this, animals are not representive of human beings. Therefore, it is necessary to do further research on human beings.The present research is expected to be the basis for further research to assess cochlear function using tools such as otoacoustic emissions (OAEs).

Conclusion

The results show that curcumin could effectively prevent and decrease gentamycin-induced oxidative stress by limiting MDA expression in the cochlea fibroblast. It is implied that the antioxidative properties of curcumin could slow and prevent the development of ototoxicity. We encourage further exploration of this research and turning it into proper clinical research.

Conflict of Interests

The authors have no conflict of interests to declare.

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