Modulation of antioxidant status by curcumin prevents cochlear damage after noise exposure

**Tengku Siti Hajar Haryuna[a*], Wibi Riawan[b], Muhammad Reza[a]
and Abdul Rachman Saragih[a]**

[a]Department of Otorhinolaryngology-Head and Neck Surgery, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
[b]Department of Biochemistry, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

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ABSTRACT

Several studies have discovered that curcumin possesses broad biological functions, especially antioxidant and antiinflammatory. Noise exposure consequently leads to increased levels of radical oxygen species (ROS) production in the cochlea, causing cochlear damage and noise-induced hearing loss (NIHL). Treatments that enhance antioxidant defences might protect susceptible individuals from NIHL. This study was conducted to demonstrate curcumin as the safe and effective therapeutic agent in the prevention and treatment of oxidative damage in fibroblasts within the cochlear supporting tissues and lateral wall following noise exposure by modulating endogenous antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT). Twenty-four Rattus norvegicus were randomly divided into 4 groups (n = 6). Group 1: The control group; group 2: noise (+); group 3: noise (+), 50 mg/day curcumin (+); group 4: noise (+), 100 mg/day curcumin (+). All groups (except group 1) were subjected to 100 dB SPL for 2 hours per day for 14 days. Curcumin was administered orally for 14 days. All samples were immunohistochemistrically examined for the expressions of SOD in cochlear fibroblasts and colorimetrically examined for CAT levels in cochlear tissues using colorimetric reader. All statistical analyses were performed by Statistical Analysis System (SAS). The results showed that curcumin is potentially effective in the prevention and treatment of oxidative damage in fibroblasts within the cochlear supporting tissues and lateral wall following noise exposure by modulating the expressions of SOD and CAT levels.

Keywords: Curcumin, SOD, CAT, Antioxidant, Cochlea
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INTRODUCTION

In developing and developed countries, noise overexposure is the greatest compensatable occupational jeopardy and the growing problem which causes hearing loss worldwide [1, 2]. World Health Organization estimated that globally 360 million people suffered from disabling hearing loss (more than 5% of the world's population) in 2012 and 16% of the disabling hearing loss in the adult population worldwide is contributed by occupational noise exposure [3].

Following noise overexposure, the cochlea endures dramatic cellular injury as well as various pathological consequences or structural changes to its various cells, and all of these cumulative phenomena play a critical role in permanent Noise-Induced Hearing Loss (NIHL) development, which is the second most common form of acquired sensorineural hearing loss following age-related hearing loss (presbyacusis). The classical features of NIHL at the cellular level include loss of sensory hair cells, damage to hair cell stereocilia, swelling of afferent dendrites and spiral ganglion neurons in Rosenthal's canal, damage to inner hair cell - auditory nerve synapse, acute swelling of stria vascularis, reduced cochlear blood flow and loss of fibrocytes in spiral limbus and ligament [3, 4].

Cochlear fibrocytes in the lateral wall, albeit non-sensory cells, play a crucial role in the regulation of inner ear ion and fluid homeostasis, inflammatory responses, predicted by their protein expression profiles [5, 6]. Cochlear fibrocytes of the spiral ligament contain Na^+/K^+-ATPase and $Na^+/K^+/Cl^-$ co-transporters which are necessary for the endocochlear potential maintenance and ionic homeostasis. According to previous study that analyzed cochlear fibrocytes using a rat model of mitochondrial toxin-induced acute cochlear energy failure, hearing loss was prompted by apoptosis of fibrocytes in the cochlear lateral wall, not by damage to sensory cells. Other previous studies also reported that degeneration of the lateral wall fibrocytes leads to hearing loss due to the endocochlear potential reduction, and the principal cause of hearing loss is apparently attributed to the loss of fibrocytes in the cochlear lateral wall, especially in the spiral ligament since cochlear sensory cells (hair cells or spiral ganglion cells) still survived even after severe hearing loss taken place [6].

At present, according to animal models studies, the mechanisms that lead to NIHL can be classified into two major categories: direct mechanical trauma and metabolic damage to the organ of Corti via various biochemical pathways which converge and cumulatively prompt hair cell death [4, 7]. Noise can trigger intense metabolic activity, leading to intense mitochondrial oxidative phosphorylation and reactive oxygen species (ROS) overproduction, overwhelming endogenous antioxidant defenses, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [8, 9]. Accordingly, many researchers have made efforts to develop therapeutic agents against NIHL. In particular, pharmacological intervention-targeted oxidative stress using a great variety of antioxidants, such as *N*-acetyl cysteine, glutathione, ebselen, resveratrol, D-methionine, acetyl-L-carnitine and many others, have shown certain degrees of protection against NIHL in animal model systems. Moreover, clinical trials of these antioxidants are in progress, but their efficacy may be limited and some studies failed to see protection effects, an issue that yet needs to be resolved [7, 9, 10].

Several natural products derived from plants have been studied as potential therapeutic agents for various types of human illnesses [11]. Curcumin, a yellow pigment extracted from the rhizomes of *Curcuma longa* L., is a major component of turmeric originated from Asia and commonly used as a spice and food-coloring agent [12]. Several studies have discovered that curcumin possesses broad biological functions, especially antioxidant and antiinflammatory. It has been reported that curcumin is a bifunctional antioxidant (exerting antioxidant activity in a direct and an indirect way by scavenging ROS and inducing an up-regulation of various endogenous antioxidant, such as SOD, CAT and GPx) [11]. Its potent antioxidant properties are attributed to the presence of phenollic OH and CH_2 groups in β-diketone moiety of this compound [12].

The role of curcumin in the prevention and treatment of noise-induced cochlear damage through its antioxidant effect against oxidative stress and the mechanisms involved in fibroblasts within the cochlear supporting tissues and lateral wall has still never been observed and arouses our interest to explore the possibility of whether curcumin can prevent noise-induced cochlear injury as well as the molecular mechanisms of how curcumin protects fibroblasts within the cochlear supporting tissues and lateral wall using animal model system based on its antioxidant effect. We also conducted this study to demonstrate that higher dose of curcumin (100 mg/day) exerts more beneficial effect on antioxidant status in cochlear fibroblasts within the supporting tissues and lateral wall following noise exposure than low dose of curcumin (50 mg/day).

EXPERIMENTAL SECTION

This experimental study was conducted on *Wistar* strain white rats (*Rattus norvegicus*) with randomized posttest-only control group design and approved by Health Research Ethical Committee of North Sumatera c/o Medical School, Universitas Sumatera Utara.. Noise-exposed groups were subjected to 1-10 kHz noise at 100 dB SPL for 2 hours per day for 14 days. Curcumin administered in this present study was derived from *Curcuma longa* L. (turmeric) with $(28.1 \pm 1.0)\%$ curcumin content compared to Standard. Twenty-four rats were randomly divided into 4 groups, where n = 6 for each group. Group 1 rats served as the control group. Group 2 rats were subjected to experimentally encountered noise-induced oxidative stress by exposing them to noise of 100 dB for 2 hours per day for 14 days. Group 3 rats were exposed to noise of 100 dB for 2 hours per day followed by the administration of 50 mg curcumin for 14 days. Group 4 rats were simultaneously exposed to noise of 100 dB for 2 hours per day followed by the administration of 100 mg curcumin for 14 days.

All samples were immunohistochemistrically examined for the expressions of SOD in cochlear fibroblasts and colorimetrically examined for CAT levels in cochlear tissues using colorimetric reader. The data collected were statisically analyzed by Analysis of Variance (ANOVA) using SAS for Windows. The significance was taken as P = 0.05.

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RESULTS AND DISCUSSION

The expression of SOD and CAT level were found to be decreased in group exposed to noise of 100 dB for 2 hours per day for 14 days (group 2) compared to control group (group 1) Curcumin increased the expression of SOD and CAT level following noise-exposure of 100 dB for 2 hours per day for 14 days and they were found to be higher when treated with curcumin at higher dose (100 mg) than lower dose (50 mg) (Table 1).

Table 1: The mean values of the expressions of SOD and CAT levels in all groups

Group (n = 6)	Mean	
	SOD (relative expression/field)	CAT (pmol/ml)
Group 1	17.17	10.19
Group 2	2.33	7.46
Group 3	8.17	6.74
Group 4	18.50	11.83

SOD: superoxide dismutase; CAT: catalase

Superoxide Dismutase (SOD)

After being evaluated immunohistochemistrically, group 2 [Fig. 1(B)] showed lower density and less SOD-expressed fibroblasts than other groups ([Fig. 1(A), (C) and (D)]). Curcumin-treated groups ([Fig. 1(C) and (D)]) showed higher density and more SOD-expressed fibroblasts than other groups ([Fig. 1(A) and (B)]). This result showed differences in the number of SOD-expressed cells among all groups.
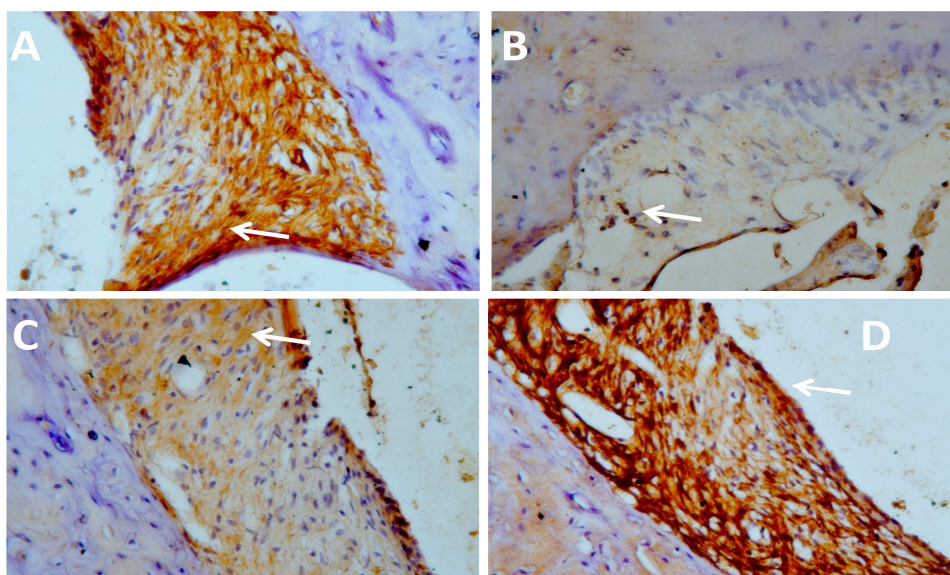
Fig. 1: The expression of SOD in each group (1000x zoom): (A) group 1; (B) group 2; (C) group 3; (D) group 4
The white arrow indicates the expression of SOD in cochlear fibroblasts marked by the brown color.

Table 2: The results of ANOVA test for the expressions of SOD in all groups

Group (n = 6)	Mean* ± standard deviation
Group 1	$17.17^a \pm 1.47$
Group 2	$2.33^c \pm 1.03$
Group 3	$8.17^b \pm 1.84$
Group 4	$18.50^a \pm 2.17$

*Means followed by the same letter are not significantly different at the α=0.05 level (comparisonwise), using Fisher's LSD [13]

Data in Table 2 showed significant differences for the expressions of SOD (P<0.05) in all groups, except in group 1 compared to group 4. Group 4 (treated with 100 mg curcumin per day for 14 days) showed statistically significant increase in the expression of SOD compared to group 3 (treated with 50 mg curcumin per day for 14 days) (P<0.05).

Noise exposure consequently leads to increased levels of ROS production, such as superoxide radicals ($O_2\bullet-$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH•), in the cochlea, causing cochlear damage and NIHL in

susceptible individuals. Many factors are likely to influence individual susceptibility to NIHL and treatments that enhance antioxidant defences might protect susceptible individuals from NIHL [14].

The expression of SOD was found to be decreased significantly in the cochlear fibroblasts of the noise-exposed group (group 2) compared to control group (group 1) ($P<0.05$). SOD is one of endogenous antioxidant enzymes that catalyzes the dismutation of more toxic superoxide anion radicals ($O_2\bullet-$) into less toxic hydrogen peroxide (H_2O_2) and oxygen (O_2) [15, 16]. Significant increases in the superoxide anion radicals ($O_2\bullet-$) have been observed in the cochlea after noise exposure [17]. The excessive generations of those highly reactive radicals consequently accelerate the rate of SOD depletion, tipping the balance from sufficiency to deficiency. Accordingly, SOD is not able to completely catalyze the dismutation of excessive amounts of superoxide anion radicals ($O_2\bullet-$). Similarly, Rewerska et al. (2013) found the significant decrease in SOD activity after exposure to 4 kHz octave band noise at the intensity of 110 dB SPL for 8 hours in C57BL/6 mice [8]. Ersoy et al. (2014) discovered that SOD values were significantly decreased in *Wistar* albino rats after noise exposure of 100 dB (A) noise for 4 hours/day for 20 days [18].

Curcumin proved to be able to significantly increase the expression of SOD in cochlear fibroblasts ($P<0.05$); and higher dose of curcumin (100 mg/day) (group 4) statistically exerted more significant SOD expression-increasing effect ($P<0.05$) than low dose of curcumin (50 mg/day) (group 3). Curcumin is able to enhance the activity and gene expression of SOD to convert more superoxide anion radicals ($O_2\bullet-$) into less damaging species: hydrogen peroxide (H_2O_2) and oxygen (O_2), thereby the overproduction of superoxide anion radicals ($O_2\bullet-$) induced by noise can be immediately neutralized.

Catalase (CAT)

Table 3 - The results of ANOVA test for CAT levels in all groups

Group (n = 6)	Mean* ± standard deviation
Group 1	$10.19^a \pm 1.43$
Group 2	$7.46^b \pm 2.52$
Group 3	$6.74^b \pm 0.49$
Group 4	$11.83^a \pm 0.60$

Means followed by the same letter are not significantly different at the α=0.05 level (comparisonwise), using Fisher's LSD [13]

Data in Table 3 showed significant differences for CAT levels ($P<0.05$) in all groups, except in group 1 compared to group 4 and group 2 compared to group 3. Group 4 (treated with 100 mg curcumin per day for 14 days) showed statistically significant increase in CAT level compared to group 3 (treated with 50 mg curcumin per day for 14 days) ($P<0.05$).

In this study, CAT level was found to be decreased significantly in the cochlear tissue of the noise-exposed group (group 2) compared to control group (group 1) ($P < 0.05$). CAT is also one of endogenous antioxidant enzymes that catalyzes the conversion of hydrogen peroxide (H_2O_2) produced by the dismutation of superoxide anion radical ($O_2\bullet-$) to water (H_2O) and oxygen (O_2). Hydrogen peroxide (H_2O_2) is a harmful by-product of many normal metabolic processes. In order to prevent damage, it must be immediately converted into other harmless substances. CAT and GPx play a significant role in cellular antioxidant defense mechanisms by limiting the accumulation of hydrogen peroxide (H_2O_2). CAT rapidly catalyzes the decomposition of hydrogen peroxide (H_2O_2) into less reactive gaseous oxygen (O_2) and water molecules (H_2O), while GPx converts hydrogen peroxide (H_2O_2) into two molecules of water (H_2O) using glutathione as a substrate, thereby decreasing the participation of hydrogen peroxide (H_2O_2) in Fenton and Haber-Weiss reactions, which can lead to the formation of the highly reactive hydroxyl radicals ($OH\bullet$) [19, 20, 21, 22]. As a result of noise-mediated oxidative stress, the production of superoxide anion radicals ($O_2\bullet-$) as well as hydrogen peroxide (H_2O_2) would be overwhelming, due to the immediate conversion from superoxide anion radicals ($O_2\bullet-$) into hydrogen peroxide (H_2O_2) by SOD, resulting in the accumulation of hydrogen peroxide (H_2O_2). Consequently, CAT and GPx enzymes could not effectively compete with the accumulation of hydrogen peroxide (H_2O_2) and thereby unable to convert them into water (H_2O) and oxygen (O_2) completely. In agreement with our results, Derekoy et al. (2004) also discovered that CAT enzyme levels were significantly low in the group of rabbits after noise exposure (100 dB SPL, 100 kHz) for 1 hour [23]. Yildirim et al. (2007) found that CAT levels in textile workers exposed to 105 dB noise were lower than CAT levels in control subjects. They suggested that decreased CAT levels after noise exposure have been interpreted as reflecting the consumption of CAT as a consequence of free radical neutralization [24].

In this study, curcumin also proved to be able to significantly increase CAT level in cochlear tissue (P<0.05); and higher dose of curcumin (100 mg/day) (group 4) statistically exerted more significant CAT level-increasing effect (P<0.05) than low dose of curcumin (50 mg/day) (group 3). Curcumin can enhance the activity and gene expression of CAT in catalyzing the conversion of hydrogen peroxide (H_2O_2) produced by the dismutation of superoxide anion radical ($O_2\bullet$-) to water (H_2O) and oxygen (O_2). The overwhelming production of hydrogen peroxide (H_2O_2) due to the immediate conversion from superoxide anion radicals ($O_2\bullet$-) to hydrogen peroxide (H_2O_2) by SOD can be attenuated by curcumin, thereby limiting the accumulation of hydrogen peroxide (H_2O_2) and furthermore preventing the generation of the highly reactive hydroxyl radical (OH•). SOD constitutes the first-line of antioxidant defense against the hazardous effects of reactive oxygen intermediates, whereas CAT acts as the second-line defense [25, 26, 27]. In this present study, curcumin at lower dose (50 mg) presumably only induced the expression of SOD as the first-line of antioxidant defense instead.

CONCLUSION

This present study indicates that curcumin is safe and effective therapeutic agent in the prevention and treatment of oxidative damage in fibroblasts within the cochlear supporting tissues and lateral wall following noise exposure by modulating the expressions of SOD and CAT levels. Moreover, it provides more insight into the mechanism of curcumin as an exogenous antioxidant against noise-induced oxidative stress and may serve as a scientific basis in the traditional systems of medicine for the management of NIHL in the future.

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