Effect of Curcuma longa L. extract on the AP1 expression in rat cochlear fibroblasts under noise conditions

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Abstract: Noise-induced cellular stress can cause damage to fibroblasts within the cochlear supporting tissues and lateral wall. In the present study, we aimed to evaluate the role of curcumin as the safe and effective therapeutic agent in the prevention and treatment of this condition according to the expression of activator protein-1 (AP1). A total of 24 Rattus norvegicus were randomly divided into four groups (n = 6). Group 1: control; group 2: noise (+); group 3: noise (+), 50 mg/day curcumin (+); group 4: noise (+), 100 mg/day curcumin (+). All groups (except for group 1) were subjected to a sound pressure level (SPL) of 100 dB for 2 h/day during 2 weeks. Curcumin used in this study was derived from Curcuma longa L. (Turmeric), and it was orally administered for 2 weeks. All samples were immunohistochemically examined for the expression of AP1 in cochlear fibroblasts. The results showed that there were significant differences for the AP1 expression (P<0.05) among all groups, except for between groups 1 and 3, or between groups 1 and 4. Our data proved that curcumin was potentially effective in the prevention and treatment of damage of fibroblasts within the cochlear supporting tissues and lateral wall due to the decreased AP1 expression following noise exposure.

Keywords: Noise, Curcumin, AP1, Fibroblast, Cochlea

1. Introduction

Immoderate noise is increasingly dealt with in many aspects of everyday life and predominantly found in the developing and industrial countries with poor hearing conservation1,2. According to recent global estimates released by the World Health Organization (WHO, 2012), there are 360 million people worldwide (more than 5% of the world’s population) with disabling hearing loss3. Excessive noise exposure, both long-term and a single exposure to an extremely intense sound, can damage the auditory system, leading to noise-induced hearing loss (NIHL)2. Noise exposure is able to negatively affect all three areas of the cochlea, the organ of Corti, the lateral wall and the spiral ganglion neurons (SGN). Most studies on NIHL have concentrated on the sensory hair cells in the organ of Corti where auditory transduction takes place. However, there is increasing awareness that the SGN and lateral wall of the cochlea are also negatively affected by noise4. Within the cochlear lateral wall, the spiral ligament is situated between the otic capsule and the stria vascularis (medially), which are predominantly composed of connective tissue elements, including extracellular material and cells from mesenchymal origin. Fibroblasts with stress fibers (tension fibroblasts) containing contractile proteins are discovered in the tissue that anchors the spiral ligament to the lateral aspect of the basilar membrane (BM), suggesting that the spiral ligament is capable of producing and/or regulating BM tension5.

The molecular mechanisms that adjust the balance of cell death and cell survival in the inner ear are not well-understood. However, there is increasing consciousness...
that mitogen-activated protein kinase (MAPK), a stress-activated member of the MAPK family, may play a crucial role. It serves as an importing necessary signaling protein that links activity at the cell membrane to downstream signaling in the nucleus. The MAPK family consists of three subfamilies: the extracellular signal-regulated kinases (ERKs), the c-Jun N-terminal kinases (JNKs), and the p38. Activator protein-1 (AP1) is phosphorylated by MAPKs. AP1 is a potent stress response transcription factor and able to trigger the de novo expression of many transcription factors or adaptor proteins, aiding the matrix metalloproteinase-9 (MMP-9) promoter activity. MMPs represent a large family of calcium-dependent zinc-containing endopeptidases that play a role in the tissue remodeling and degradation of the extracellular matrix, including collagens, elastins, gelatin, matrix glycoproteins and proteoglycan.

For years, many researchers have made efforts to use natural plant-derived compounds as potential therapeutic agents for a variety of diseases in humans. Curcumin, a yellow pigment extracted from the rhizomes of Curcuma longa Linnaeus, is a major component of turmeric originated from Asia and commonly used as a spice and food-coloring agent. Curcuminoids refer to a group of phenolic compounds present in turmeric, which are chemically related to its principal ingredient curcumin. The composition of curcuminoids is approximately 70% curcumin (curcumin I), 17% demethoxycurcumin (curcumin II), 3% bis-demethoxycurcumin (curcumin III) and 10% cyclocurcumin (curcumin IV).

The role of curcumin in the prevention and treatment of noise-induced fibroblast damage within the cochlear supporting tissues and lateral wall mechanism has never been investigated. In the present study, we aimed to assess whether higher dose of curcumin (100 mg/day) exerts more beneficial effects on inhibition of AP1 pathway compared with low dose of curcumin (50 mg/day).

2. Material and methods

Male Wistar strain white rats Rattus norvegicus (150–250 g, 8–12 weeks of age) were used in this study. The study was conducted in the standardized laboratory, which has complete equipment and sufficient experience in the maintenance of experimental animals, in the Laboratory of Biochemistry Faculty of Medicine, University of Airlangga (Surabaya, Indonesia). This experimental study was also approved by Health Research Ethical Committee of North Sumatera c/o Medical School, Universitas Sumatera Utara (Medan, Indonesia).

Curcumin was derived from Curcuma longa L. (Turmeric), and it was identified by Dr. Rer. Nat. M. Yuwono, MS., Apt. (sheet number No. 2036/SA/XII/2012) at Assessment Service Unit Faculty of Pharmacy Airlangga University. The curcumin content level was 28.1±1.0% w/w compared with standard, and it was suspended in 0.5% carboxymethyl cellulose. Afterwards, the suspension was directly administered to the stomach of rat via nasogastric tube, once a day for 2 weeks. The 24 Rattus norvegicus samples were divided into four groups. Group 1: the control group; group 2: noise (+); group 3: noise (+), 50 mg/day curcumin (+); group 4: noise (+), 100 mg/day curcumin (+). The given dose of noise exposure was 100 dB SPL for 2 h during 2 weeks.

After 2 weeks, the rats were sacrificed by ether inhalation, and necropsy procedure on temporal bone of rats was performed. All samples underwent standard tissue processing with fixation in buffered formaldehyde, followed by dehydration in graded alcohol solutions. Subsequently, they were embedded in paraffin blocks, serially cut into 4 µm thick sections, and placed on glass slides. Representative sections were stained with hematoxylin and eosin (H&E). Immunohistochemical staining was carried out to examine the expression of AP1.

Immunohistochemistry procedures were described as follows. Briefly, the slide was cleared in xylene and rehydrated through graded alcohol solutions. Endogenous
peroxidase activity was blocked with 3% hydrogen peroxide in absolute methanol. Non-specific binding of the secondary antibody was prevented by incubation with 10% non-immune serum (0.25% Triton X-100 in PBS). C-Fos antibody (SANTA CRUZ, sc-271243) served as the primary antibody, and it was separately applied to each specimen and incubated in a humidified chamber. After rinsing with PBS, sections were incubated with biotinylated secondary antibody, and then the sections were washed once more and incubated with a horseradish streptavidin-peroxidase conjugate. Next, a substrate of chromogen solution (3,3’-diaminobenzidine tetrahydrochloride) was added. This reaction involved peroxidase catalysis of the substrate and conversion of the chromogen to a brown deposit that marked the antigen. The final steps included counterstaining with H&E and application of coverslips.

The samples in each slide were examined by three investigators, and the fibroblasts within the cochlear supporting tissues and lateral wall which expressed AP1 in all fields were manually calculated with hand counter. The expression of AP1 was quantitatively calculated for the average distribution of fibroblasts with single nucleus expressing AP1 (showing brown-colored cytoplasm).

The data were processed using the Statistical Package for the Social Sciences (SPSS) one-way analysis of variance (ANOVA), and a \( P \) value of less than 0.05 was considered as statistically significant.

3. Results

Immunohistochemistry analysis showed that the AP1 expression was increased in the noise-exposed group (Fig. 1B) compared with other groups. The curcumin-treated groups showed lower density of the brown color, and less AP1-expressing fibroblasts than the noise-exposed group (Fig. 1C–1D).

Table 2 reveals that there were significant differences in the AP1 expression (\( P < 0.05 \)) among all groups, except for between control group and 50 mg curcumin-treated group, or between control group and 100 mg curcumin-treated group. A dose of 100 mg curcumin per day significantly decreased the AP1 expression compared with a dose of 50 mg curcumin per day.

The AP1 expression was increased in group 2 (noise-exposed group), and it was decreased in curcumin-treated groups (groups 3 and 4). In general, a dose of 100 mg curcumin per day reduced the AP1 expression compared with a dose of 50 mg curcumin per day (Fig. 2).

![Figure 1](image-url)

**Figure 1.** The expression of AP1 in each group (1000× zoom): (A) Group 1; (B) Group 2; (C) Group 3; (D) Group 4. The white arrow indicates the expression of AP1 in cochlear fibroblasts marked by the brown color.

### Table 1. Inhibitory effect of curcumin on the AP1 expression in each group (primary data).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Group</th>
<th>Mean difference</th>
<th>Std. error</th>
<th>Sig.</th>
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<tbody>
<tr>
<td>Curcumin</td>
<td>I (Control)</td>
<td>II (Noise)</td>
<td>III (Noise+C50)</td>
<td>IV (Noise+C100)</td>
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<tr>
<td>1</td>
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*Denotes statistically significant.
4. Discussion

Noise-induced fibroblast damage within cochlear supporting tissues and lateral wall can be caused by many pathways, and the underlying mechanisms remain unclear. In 2013, Haryuna et al. have proved on rat model that noise inflicts fibroblast damage within cochlear supporting tissues and lateral wall viewed due to the increased expression of Hsp-70, and curcumin can significantly prevent such a damage[11]. However, noise influence on AP1 expression and role of curcumin in the prevention and treatment of noise-induced fibroblast damage have never been conducted.

Both physiological and pathological stimuli (growth factors, stress signal, infections and oncogenic stimuli) can regulate AP1 activity[12]. In this study, we found that the AP1 expression was significantly increased in cochlear fibroblasts in noise-exposed group (group 2) compared with the control. MAPKs, consisting of three subfamilies (ERK, JNK and p38), are crucial signal transducers, and they regulate distinct cellular functions and can be activated by phosphorylation in cytoplasm and translocated into nucleus, where they stimulate phosphorylation of AP1[6].

AP1 is confirmed as fundamental transcriptional factor for MMP-9 expression[13]. MMPs contribute to tissue remodeling and degradation of extracellular matrix, including collagens, elastins, gelatin, matrix glycoproteins, proteoglycan[8]. Collagen is an important structural component of cochlear lateral wall, and its most plentiful type (type II) is an extracellular matrix material and sub-epithelial connective tissue of inner ear which can be also detected in BM[14].

Wang et al. reported that AP1 acts as an important modulator in regulating inflammation prompted by pathogens (Group B streptococcus, Herpes simplex virus 1, Helicobacter pylori)[15]. Moghadamtousi found that AP1 is critical for transcriptional regulation of high-risk human papillomaviruses (HPVs)[16]. Mishra et al. reported a fundamental role of AP1 transactivation in severe lesions during oral carcinogenesis[17].

JNK activation inflicted by cellular stress results in c-Jun activation, and its translocation from cytoplasm to nucleus is vital for AP1 activity[18,19]. Curcumin may interfere with multiple cell signaling pathways, and it apparently inhibits AP1 binding via its action on mitogen-activated protein kinase kinase-1 (MEK1)-JNK pathway, glutamate-induced JNK phosphorylation, c-Jun phosphorylation, AP1 binding activation and excitotoxicity in a concentration-dependent manner[20,21].

In the present study, we proved that curcumin was able to suppress AP1 expression in cochlear fibroblasts. Aggarwal et al. (2006) stated that curcumin inhibits AP1 and NF-κβ activation induced by tumor promoters[22]. The c-Jun domain of AP1 is a positive regulator of cyclin D1 expression, contributing to tumorigenic phenotype stimulation[23]. Jurenka (2009) reported curcumin’s great potential as a therapeutic agent for various inflammatory diseases[24]. Farhangkhoei (2006) found that curcumin treatment of diabetic rats reduces the damage of oxidative DNA and protein, which is mediated by decreased activation of redox-sensitive transcription factors, NF-κβ and AP1[25].

In this study, we found that a dose of 100 mg/day curcumin could significantly decrease the expression of AP1 compared with its half-dose (50 mg/day). The expression of AP1 in groups 3 and 4 was not significantly different from the control group, indicating that higher dose of curcumin was able to prevent AP1 activation, and thereby its expression was found to be nearly similar from control.
5. Conclusions

Curcumin is considered to be a safe and effective therapeutic agent in the prevention and treatment for the noise-exposed damage of fibroblasts within the cochlear supporting tissues and lateral wall. Moreover, the study thus provided more insights into the mechanism of curcumin against AP1, and we showed that curcumin could inhibit AP1 signaling pathways. Moreover, our study also served as a scientific basis for its usage in the traditional systems of medicine for the management of NIHL in the future.

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