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HSP70 Gene Expression in Serum and Tissue of Rat Cochlear (*Rattus norvegicus*) Due to Noise Exposure and Heat

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Abstract : This study evaluated the activity of HSP70 gene in serum and tissues of the cochlea as a result of noise and heat in rats (*Rattus norvegicus*). The study was purely experimental studies in vivo with the design following the research design Completely Randomized Design (CRD) using a completely randomized design (CRD). Samples used 30 male rats *Rattus norvegicus* strain. The treatment group comprised 3 groups. Each group consisted of 10 rats. P0 group (control) = do not provide treatment noisy and hot. P1 = treatment noisy groups of 80-110 dB, and the group P2 = heat treatment 27-40^{o}C. The results showed that there was significant effect giving sound and heat ($p < 0.05$) against HSP70 gene expression both in serum and in cochlear tissue.
Keywords : Noise, rats, serum HSP70, Cochlear.

Introduction

Noisy according to the Occupational Safety & Health Administration (OSHA) with a limit of no more secure noisy 85 dB. Noise exposure depend duration of exposure, frequency, intensity noise and sensitivity of the individual ear and some of the factors preventing of cochlea.[1] In Indonesia, the intensity of noise in the workplace that allowed the plant is 85 dB for 8 hours of working time per day.[2] If the threshold value is exceeded continuously for a long time, it will cause disruption of hearing.[3] Another influential factor is the intensity of the sound is too high, the age of the employee, hearing impairment are present before work, noisy frequency, length of employment, the distance from the source sound, style of life of workers outside the job.[2]

According to the Occupational Safety & Health Orginazation (WHO), people with hearing loss ranging from 360 million (5.3%), of which 328 million (91%) most adults and 32 million (9%) suffered by children. Increased hearing loss is proportional to the rate of increase usia.[4] Exposure to noise and excessive heat from the safe threshold trigger oxidative stress on the organ of hearing. The outer hair cells of the cochlea into a spiral ganglional lose its integrity against neurons. Changes experienced by the anatomy of auditory neuron dendrites of pyramidal where encountered elongation and decreased spine density in apical and basal layer pyramidal neurons II-III and V-VI in the cortical regions. Cochlear redox status assessment showed increased production of superoxide and lipid peroxide in hair cells and spiral ganglional neuron.[5]

This noise exposure would cause permanent sensorineural deafness and related factors such as autoimmune antibodies, chronic infectious diseases, hypertension and atherosclerosis. HSP70 is formed as a result of exposure to noise, so that antibodies formed HSP70 allegedly stronger than HSP60.[6] Formation of antibodies in the plasma such as HSP27, HSP60, HSP70, HSP72 and HSP90 in the automobile industry workers in China are getting exposure to noise, dust, heat, and carbon dioxide, benzene occurs in the work

environment will increase HSP70 antibody in the body of this worker. This antibody has potential as biomaker to assess damage to cells in the body of industrial workers experience a stress due to the heat and exposure bising.[7] Antibodies against HSP70 could be expected to damage the tissue structure is similar to the HSP70 protein as well, an increase in other cell proteins may disrupt the function of cells in the body's stress pathways intracelluler and cause stiffness in the outer hair cells of cochlea.[3] Serum HSP70 is a protein formed by HSP70 gene that expressed for their stressors, especially coming from the increase in ambient temperature. All living organisms react to an increase in ambient temperature. This situation will induce the formation of serum HSP70 will be expressed in the body as autortegulasi. Hot response is the main mechanism for protecting cells against various stressors on the organism. Heat shock protein response is regulated by a shock gene transcription of heat.[8,9]

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Material and Methods

Research design

This research method is a purely experimental studies in vivo. The study design followed the Direct Random Design (RAL) consists of 3 groups of mice treated with a type of adult male and 10 replications conformity with the formula Federer (1963), namely $(t-1)(n-1)\geq 15$. Then obtained 3 treatments and 10 replications. The treatment group, Group I (P0) = mice without exposure to noise and heat. Group II (P1) = rats given the treatment noisy 80-110 dB. Group III (P3) = rats given heat treatment 27-40^{o}C. Giving noise and heat do 8 hours/day for 8 days.

Animal experiments

The study was conducted in facilities and equipment maintenance in laboratory test animals, namely Biochemistry - Universiatas Biomolecular Medicine Faculty of Brawijaya. The study used 30 male rats aged 8-11 weeks of Wistar strain and body weight of 150-200 grams, during the observation of the 7 days before treatment healthy condition with normal activity and behavior. Mice kept in a cage the size of 15x30x10 cm3 plastic, covered with iron wire and covered with 2 cm thick rice husks and replaced every 3 days. Each cage was placed 5 mice. CP551 standard feed from PT. Charoen Phokphan and giving drink ad libitum.[10] Prior research conducted maintenance Ethical Clearance from Health Research Ethics Committee so getting permission to use animal studies with letter No.170/KEPH-Science/2016.

Examination of HSP70 by ELISA

Rat blood sampling was done by aseptically from rat heart. Mice are anesthetized with ether in a sealed bottle until a dead rat, and the rat is placed on a surgical board. After iu, blood was collected using a syringe 1 cc of rat hearts and placed in a centrifuge tube and centrifuged for 10 minutes to obtain serum at a speed of 6000 rpm. Serum is stored at -20°C. Do ELISA (Enzyme-linked immunosorbent assay) HSP70 in all samples, including the control and taken the blood of rats as much as 1 cc after treatment.

Treatment and Decision Network Cochlear Mice

Adapted mice for two weeks in a laboratory environment, white mice can be treated in accordance with the planned group. Previous surgery, rats given inhaled ether. Furthermore, the temporal bone tissue necropsy. Once the tissue sample is taken, the immediate fixation with 10% buffered formalin solution. Then, tissue samples examined in the laboratory integrated.

Immunohistochemistry staining of HSP70

Cochlear tissue samples were fixed in 10% buffered neutral formalin and embedded (embedding) in paraffin. The thickness of the tissue cutting is 6 mL and this piece is affixed to the glass object with neofren. The elimination of endogenous peroxidase with 0.3% H2O2 in methanol (0.5 ml H2O2 plus 50 ml of methanol) for 15 minutes. Blocking nonspecific using normal rabbit serum 10% (Nichire Corporation, Histofine) and incubated at 37 ° C for 60 minutes. The opening of epitopes on the network retrieval is done by using the enzyme trypsin. HSP-70 primary antibody diluted 1: 100 and incubated at room temperature for two hours. Secondary antibody (Biotinylated anti-rabbit immunoglobulin Dako Nichirei Corporation, Histofine Japan) were incubated at 37 ° C for 30 minutes and the addition of avidin-biotin-peroxidase complexes (ABC produced Nichirei Corporation, Histofine Japan) were incubated at 37 ° C for 30 minutes. Visualization is done by using 3,3'-diamino-benzidine (DAB) was dissolved in tris-buffer and H2O2 (1 mg DAB + 5 ml tris buffer + 5 mL H2O2) was incubated at 37 ° C for 45 minutes. Counterstain is done with hematoxylin and closing preparations (mounting) with entelan.[11,12,13]

The calculation of the number of cells

The calculation of the number of cells that form a positive color Hsp-70 (forming the color brown) are calculated on a comprehensive view of 60x45 μm^2 using a microscope (Olympus) at a magnification objective 100 times with the aid of a video microscope (Video measuring gauge IV-560, for Company Limited) in 5 per visual field preparations. How cell counting cell histology can be seen in Table 1. This reading is called by IOA (Interobesrver agreement).Criteria readings can follow the way proposed by Leakeet al.[14] At least 100 cells were counted for each case. For scoring the calculation results can be seen in Table 1.

Table 1. Suggestions scoring system has been a lot of reference to international journals.

Scores proportion staining	**intensity staining score**	**Score Extra**
0 = no stained nucleus	0 = no staining	0 = no treatment response
1 = <1% core Coloring	1 = weak staining	2-3 = small treatment response (20%)
2 = 1-10% core stained	2 = moderate staining	4-6 = moderate treatment response (50%)
3 = 11-33% core stained	3 = strong staining	7-8 = good response to treatment (75%)
4 = 34-66% core stained	0 = no staining	
5 = 67-100% core stained		

Note: The maximum score is the result of the addition of eight.[14]

Data analysis

Data is collected and carried Shapiro-Wilk test for normality test data and Levene test for homogeneity test data. Data were analyzed using ANOVA test alpha level of 5%. Then proceed with the Post-hoc Bonferroni test to see significant differences between the treatment groups (P0-P3).

Results

Immunohistochemical examination of HSP70 in the group P0 (control) looks epithelial Organ of Corti (blue) is still normal, the group P1 (noisy> 80-110 dB) looks epithelial Organ of Corti (yellow) is reduced, group P2 (heat 27-40 C) looks epithelial organ of Corti slightly reduced.

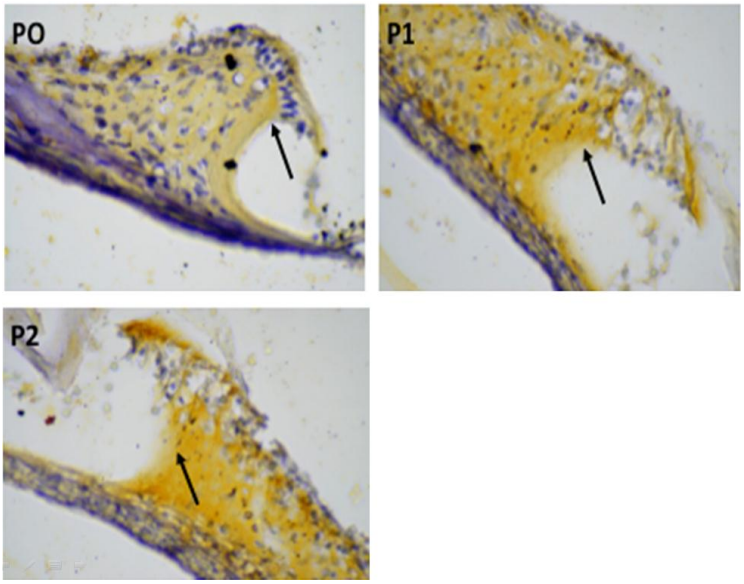

Figure 1. Immunohistochemistry HSP70 cochlear

Immunohistochemical examination showed HSP70 expression of HSP70 antibody with the intensity of 1-9 on a group of PO to P2. PO group (control) no HSP70 expression in the Organ of Corti of the cochlea. Group P1 (noisy 80 s/d 110 dB) no HSP70 expression looks brown with a score of 4-7 (moderate intensity). Group P2 (heat 40-70^{o}C looks brown with a 2-4 (weak intensity). The group P0 (control) compared with the group P1 (noisy 80-1110 dB) obtained a significant difference ($p < 0.01$), group P0 (control) compared with P2 heat 27-40^{o}C) significant difference ($p < 0.01$). Elisa examination HSP70 expression each treatment group compared to the group P0 (control) can be seen in Table 1 below;

Tabel.1. Based Treatment Group with ELISA examination CPI HSP 70 and HSP 70

Groups	HSP70 ELISA		
	OD	Concentration	HSP70 IHC
P0	0,09±0,01	2,79±0,43	3,80±1,30
P1	0,40±0,07	17,08±3,22	21,00±1,58
P2	0,21±0,03	8,47±1,20	12,40±2,70

Description: The group P0 (control) = do not provide treatment noisy and hot. P1 = treatment noisy groups of 80-110 dB, and the group P2 = heat treatment 27-40^{o}C.

The increase in the expression of the group P0 value of the concentration of the lowest value of 2.117gdL^{-1} and the highest score 3.103gdL^{-1}, the increase in the expression of the group P1 lowest concentration value of 13.552 gdL^{-1} and the highest value 20.502 gdL^{-1}, an increase in expression in the group P2 the lowest value of the concentration of 7.453gdL^{-1} and the highest 10.368gdL^{-1}.

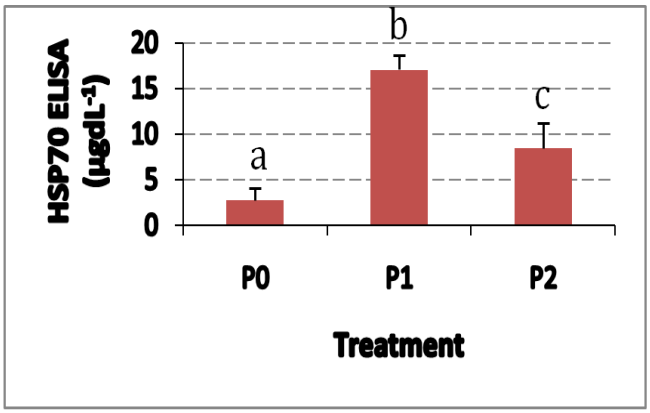

Figure 2. The difference in concentration of Elisa HSP 70 each treatment group

In Figure 2, we can see Elisa examination involves the HSP70 group P1 (noisy 80-110 dB) higher than P0 group (control) and group P2 (heat 27-40^{o}C). HSP70 expression in the P2 (heat 27-40^{o}C) was also higher ($p<0.01$) compared with controls.

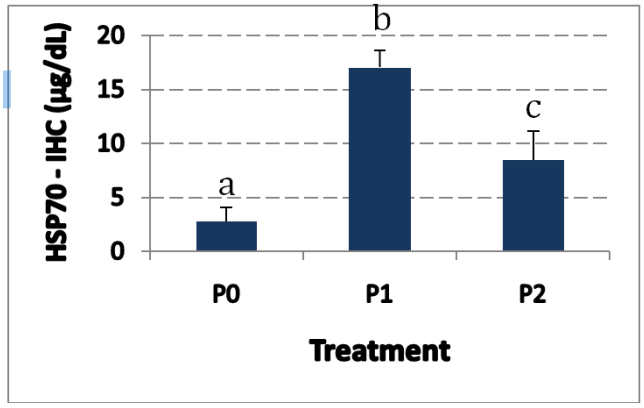

Figure 3.Variation IHC HSP 70 each treatment group

In Figure 2, it can be seen immunohistochemical examination involves the HSP 70 group P1 (noisy 80-110 dB) higher than P0 group (control) and group P2 (heat 27-40^{o}C). HSP70 gene expression in the group P2 (at 27-40^{o}C) also significantly different ($p <0.01$) with the controls.

Discussion

HSP70 gene expression in rat cochlea organ of corti tissues were significantly higher after administration noisy (80-110 dB) (Figure 1 and Figure 3). This is caused by the effect of mechanical stress and metabolic mechanisms in auditory sensory organ. Sound waves that exceed the physiological limits of cells in blood vessels and cells organ of corti cause these cells. Vibration noise source is disturbing the balance of the molecules of the surrounding air so that the air molecules vibrating and delivered to the cells-the cells around the organ of corti. The molecular vibrations may cause changes in the structure of the cell and activates protein HSP70 gene to form protein HSP70 improve the structure of the protein changes. Such statements of Sharma and Masison[15], HSP70 (*heat shock proteins*) protect cells from various conditions of stress. Hsp70, the most ubiquitous and highly conserved HSP, helps proteins adopt native conformation or regain function after misfolding. Various co-chaperones specify Hsp70 function and broaden its substrate range.

The vibration caused by noisy ongoing basis (8 days over 8 hours) may also affect the levels of protein in the blood or serum. So that the protein in serum may interfere with the active lead to deter damage HSP70 protein / enzyme further other in the blood. Similar situation also occurs in the heat of the perotein disruption in serum and in the organ of corti cells of the cochlea. It causes the active genes to produce proteins HSP70 chaperone HSP70 is useful to repair damaged proteins in serum and in the organ of corti cells of the cochlea. According to a statement Shiber and Ravid[11], the 70 kilodalton heat shock proteins (HSP70s) are a family of conserved ubiquitously expressed heat shock proteins. Proteins with similar structure exist in virtually all living organisms. The HSP70s are an important part of the cell's machinery for protein folding, and help to protect cells from stress.[16]

Conclusion

1. Gene expression HSP70 or HSP70 protein in serum was highly significant ($p<0.01$) in the provision of noisy 80-110 dB, 8 hours/day for 8 days.
2. Gene expression HSP70 or HSP70 protein in tissues organ of corti of the cochlea is very significant ($p<0.01$) in heat exposure 27-40^{o}C, 8 hours/day for 8 days.
3. HSP70 gene expression in serum was higher ($p<0.01$) in both the 80-110 dB noise and heat exposure 27-40^{o}C.

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