The GADD45 and Wild p53 Expressions Resulting from Moderate Swimming Exercise on Mus musculus Injected by Benzopyrene

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Abstract

Cancer prevalence is increasing nowadays. In Indonesia, cancer prevalence reaches1.4% out of 1,000 people. Cancer is mainly caused by two factors; genetic and environment, including a carcinogenic agent known as benzopyrene. Physical exercises can decrease the levels of blood glucose and fatty acid within the blood; can increase expression of Bax and caspase-3; but its effect on GADD45 and wild p53 expression, is still unknown. This study aimed to determine the expressions of GADD45 and wild p53 resulting from moderate swimming exercise on Mus musculus injected by benzopyrene. 18 mice were divided into three study groups; control group 1 (K1) which was not treated with any physical exercise and benzopyrene; control group 2 (K2) which was not treated with any physical exercise but induced with 0.08 mg of benzopyrene; and treated group (K3) which wastreated with physical exercise in moderate intensity and induced with 0.08 mg of benzopyrene. The buccal mucosa tissue samples were taken and stained for immunohistochemistry to be further examined under a light microscope at 400x magnification with 10 different angles. **Result**: There was no difference inGADD45 expression between the K1, K2, and K3 groups (p = 0.611), with a significant difference of wild p53 expression (p = 0.000). Moderate swimming exercise has no effect on GADD45 expression, but could increase wild p53 expression on Mus musculus injected by benzopyrene.

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Introduction

Cancer is a condition where new tissues grow due to a continuous abnormal cell proliferation, which has the capability to attack and harm other tissues. Nowadays, the head and neck cancer ranks sixth on the most cancer cases in humans,and holds the highest mortality rate among all types of malignancy.[1,2] Approximately 95% of oral cavity cancer is squamous cell carcinoma.[3]

The factors that increase the risk of oral cancer, including squamous cell carcinoma,are cigarettes, alcohol, inflammation, infection, preneoplasia, marijuana, malnutrition, denture irritation, mouthwash, dental plaque, candidiasis, diabetes mellitus, and viruses.[1,2] Cigarettes are known to be the most common factor for the emergence of squamous cell carcinoma.[4] At present, Indonesia was in the fourth place of the countries with the highest cigarette consumption worldwide after China, Russia, and USA.[5]

The substances contained in cigarettes include: nicotine, tar, carbon monoxide (CO), and other chemicals such as benzopyrene, isoprene, toluene, nickel, arsenic, cadmium, methane, ammonia, nitrogen oxide, hydrogen sulfide.[6] Benzopyrene is a chemical compound within the hydrocarbon group derived from incomplete combustion of organic materials, which can be directly absorbed by the body through inhalation, food ingestion, and exposure to the skin. Benzopyrene metabolites have been proven to cause DNA mutation which changes the behavior of the cells to be abnormal.[7]

Our body has a regulation system to every cellular change. The presence of DNA

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mutation is responded by the body in two ways; reparation and excision. Reparation is carried out by repair genes such as the Growth Arrest and DNA Damage-inducible 45 (GADD45).[8]When the damage of DNA is irreparable, the body will excise the mutated cells through apoptosis. Apoptosis is a programmed cell death which can occur both physiologically and pathologically.[9] The process is also controlled by the pro-apoptotic gene and the anti-apoptotic gene. The main gene of the apoptosis is referred as the guardian of the genome (p53 or wild p53) which is a tumor suppressor gene.[10]

Many studies that have been conducted in relation to cancer mainly discuss the use of drugs, both chemicals and herbs, intended for medical treatment. Research conducted by Irmawati, et al. shows that moderate intensity physical exercise can increase the expression of caspase-3.[11] However, the effect of moderate intensity swimming training on the expression of GADD45 and wild p53 is unknown.

Therefore, this study aimed to determine the expression of GADD45 and wild p53 resulting from sports activity, especially swimming activity in medium intensity.

Materials and methods

This study had been approved with ethical clearance from Committee of Ethical Clearance of Health Research, Faculty of Dental Medicine, Universitas Airlangga, Indonesia (No. 08 /KKEPK.FKG/II/2014).

This research was an experimental research with randomized block design. The samples were 18 male mice (Mus musculus) of Swiss Webster strain (Balb/c), agedapproximately 2 months and weighed 25-35 grams.

The mice were divided into 3 groups: control 1 (K1), control 2 (K2), and treatment (K3). In K1, the mice were exposed to water with 70% of the maximum swimming capacity (MSC),3 times a week for 12 weeks. In week 5, they were injected with oleum olivarumas much as 1.13 mL per Kg of their body weight on their buccal mucosa of the upper right oral cavity for3 times a week for 4 weeks. In K2, the mice were exposedto water with 70% of MSC,3 times a week for 12 weeks. In week 5, they were induced with benzopyrene (Merck, Sigma-Aldrich Pte. Ltd., Singapore) as much as 2.67 mg benzopyrene/1.33 mL oleum olivarum per kg of their body weight on their buccal mucosa of the upper oral cavity for 3 times a week for 4 weeks. In K3, the mice were given swimming exercise with loads on their body weighing 3% of their body weight with 70% MSC, 3 times a week for 12 weeks. At week 5, they were induced with 2.67 mg benzopyrene/1.33 mL oleum olivarum per Kg of their body weight on their buccal mucosa of the upper right oral cavity for3 times a week for 4 weeks.[12]

At the beginning of week 13, the mice on the entire group were anesthetized with ether. Tumor was identified as abnormal enlargement of the buccal mucosa. Tumor tissues in the buccal mucosa on the right side of the oral cavity were extracted. The mice were then sacrificed the tumor tissueswere processed into paraffin blocks. The paraffin blocks were cut by 4µ thick and placed on a microscope slide to undergo immunohistochemistry imaging using GADD45 and wild p53 monoclonal antibodies. The examination of GADD45 and wild p53 expressions was carried out using an optical microscope (Olympus CX31; Olympus Singapore Pte. Ltd., Singapore) at 400x magnification on 10 different fields of view. Finally, the research data were collected in the form of primary datato be further analyzed using Statistical Package for the Social Sciences (SPSS) 20.0 software for Windows 8 (IBM SPSS Inc, Chicago, United States). Since the data of GADD45 expression was normally distributed, One-way ANOVA test was used as parametric analysis. However, Brown-Forsythe test used to analyze wild p53 expression because the data was not normally distributed.

Results

Figure 1 showed the immunohisto-chemistry imaging of GADD45 expression. Positive results markedas brown-stained squamous cells. However, One-way ANOVA analysis revealed that moderate swimming exercise had no significant differences between K1, K2, and K3 (Table 1). Figure 2 showed the immunohistochemistry imaging of wild p53 expression, which positive results also marked as brown-stained squamous cells. Based on Table 2, the wild p53 expression in K3 was lower than that in K1 and the differences between the groups were found to be statistically significant ($p < 0.05$).

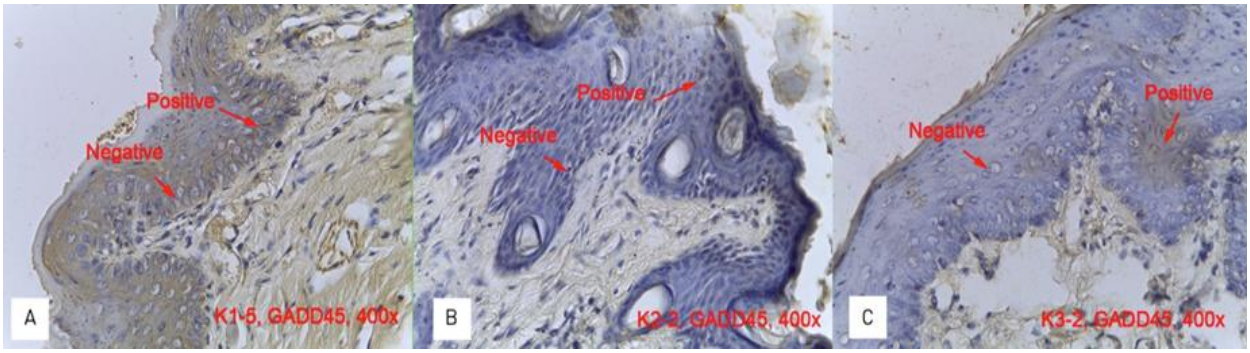

Figure 1. The Expression of GADD45 on the Oral Squamous Epithelial Cells in K1 (A), K2 (B), and K3 (C).

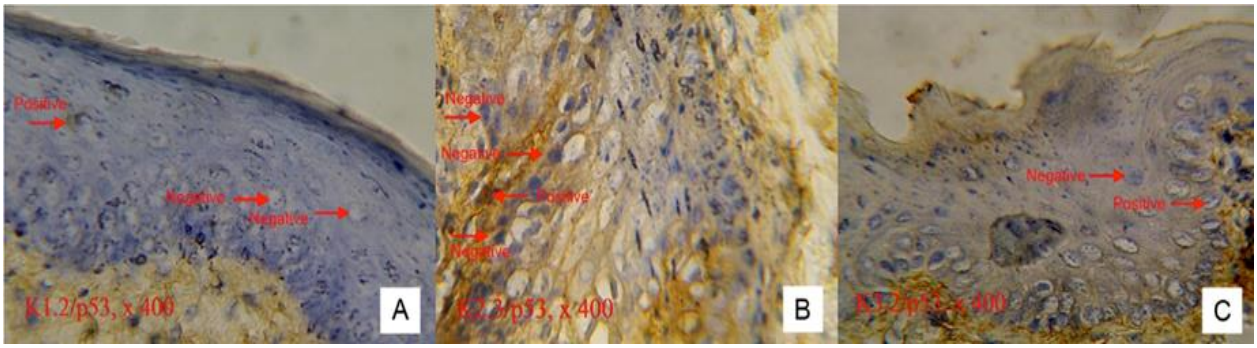

Figure 2. The Expressions of Wild P53 on the Squamous Oral Epithelial Cells in K1 (A), K2 (B) and K3 (C).

Group	n	GADD45	One-way
		Mean ± SD	ANOVA
K1	6	0.32 ± 0.27	
K2	6	0.47 ± 0.38	p=0.611
K3	6	0.45 ± 0.15	

Note: *significant at α = 0,05

Table 1. The Test Results of GADD45 Expression with One-Way ANOVA Test.

Group	n	wild p53	Brown-Forsythe
		Mean ± SD	
K1	6	2.80 ± 0.77	
K2	6	0.52 ± 0.13	p=0.000*
K3	6	1.13 ± 0.73	

Note: *significant at α = 0,05

Table 2. The Test Results of Wild P53 Expression with Brown Forsythe Test.

Discussion

This research has revealed that moderate intensity of exercise by swimming indicated no significant differences among experimental groups on GADD45 expression. This condition could be caused by the induction of benzopyrene into the body of the animal subjects that might cause the formation of Benzo [a] pyrene-7,8-diol-9,10-epoxide (BPDE) metabolites which were capable of binding to the DNA to form bulky DNA adduct.[11] Physiologically, the body has a system to keep cells in a state of homeostasis. When chemicals pass in the body, the body will protect itself by detoxifying the chemicals and removing them to prevent the formation of defective DNA in the target tissues. Whenever a defective DNA (mutation) occurs, the body would attempt to repair the DNA so that it does not pose any risky potentials of causing cellular behavior change into malicious cells (transformed cells), whichare the precursors of cancer cells. The results of this study did not agree with the research conducted by Marfe, *et al*.[13] (2010) which showed that a marathon run by a healthy amateur runner could increase the GADD45 expression.

GADD45 is a gene or protein that has implications for the regulation of a number of cellular functions such as DNA repair, cell cycle

control, the aging process, and genotoxic stress. There are 3 GADD45 families, GAD45 α, β, and γ. The GADD45 α is the first family identified through the screening of cDNA library that enhances transcription after ultraviolet radiation in Chinese Hamster Ovary cells (CHO cells). The GADD45 β, also known as MyD 118, is identified as the primary response of transient gene induced by interleukin 6 (IL-6) in myeloid leukemia cell lines. The GADD45 γ is an IL-2 inducible gene (gene that exists when there is IL-2 induction), also known as CR6 (Oncostatin-M inducible gene IOG37), and GADD-related protein 17 kDa (GRP17).[7]

When DNA damage occurs, the entire GADD45 families (α, β, and γ) are immediately induced, resulting in cell cycle arrest and/or apoptosis, or an active role-taking in DNA repair.[15]

The role of GADD45 in DNA repair is through the NER pathway, which is the interactions with Proliferating Cell Nuclear Antigen (PCNA) to reach the site of DNA experiencing lesions or, otherwise, to facilitate the access of defective DNA to reach the repair protein. The N-terminal region of GADD45 α (composed of the 1-94amino acid residues) is identified as the determinant region to interact with PCNA, or GADD45 α and β to interact with PCNA mediated by the C-terminal region (amino acids 137-165 and 114 -156). In addition, the central region and C-terminal of GADD45 γ (76-159residues) are also determined to be important to interact with PCNA.[16] The interaction between GADD45 and PCNA will stimulate the NER process by transferring the nuclear localization from the DNA replication site to the damaged DNA site.[17,18]

The result showed (Table 1) that GADD45 expression in K2 was higher than that in K3. This result was probably due to benzopyrene induction which was not followed by any intervention in the animal subjects, causing more number of DNA to be mutated, causing the body to work harder to repair the defective DNA by increasing the synthesis of GADD45 repair genes.

In Figure 1, the increase in GADD45 resulting from moderate swimming exercise had no significant difference between K1 and K2. In addition, the reason why the GADD45 expression did not increase in the treatment group, the authors suspect because the removal of tumor tissue was carried out 8 weeks after the first benzopyrene induction. In the early weeks of benzopyrene induction, a gene mutation was immediately responded by the body by repairing mutated genes, one of which was through GADD45 activity. Therefore, that it is suspected that GADD45 expression increases during the initial week of exposure to benzopyrene. However, due to continuous exposure to benzopyrene carcinogenic substances (3 times a week for 4 weeks), the process of gene repair can no longer occur. With the increasing number of genes that mutate but repair genes cannot be carried out by the body, the body must eliminate these mutant genes through the apoptotic pathway. Therefore, further research is needed to ascertain the role of GADD45 in the repair of genes that are mutations due to benzopyrene.[19]

The role of GADD45 in DNA repair was considered important as it was proven on the GADD45 null mice which experienced sensitivity enhancement towards radiation-induced carcinogenesis, genomic instability, abnormal chromosomes (marked with aneuploidy), centrosomic amplification, mitotic and cytokinesis disorder.[20] Furthermore, the absence of GADD45 indicated a decrease in NER activity and an increase in mutations.[7]

When DNA damage occurs, the transcription process of p53 mRNA will be triggered. The role of the normal p53 gene (wild p53) in Figure 2 did not only inhibit the cell cycle in the G1 phase but also held an important function in the apoptosis. Due to the cessation of the cell cycle in phase G1 caused by the p53 activity, the regulatory region of the p53 binding site would directly activate the transcription of the Bax gene located within the mitochondria.[21]The Bax played a role in opening permeability transition pore (Pt-pore) so that cytochrome-c came out from the mitochondria. Then, the Cytochrome-c activated apoptosis protease activating factor 1 (Apaf-1) and, together with Apaf-1, they formed apoptosome complex which would activate the caspase 9 as a caspase initiator.Then, caspase 9 would stimulate caspase 3 which was the executor of the caspase 3 and caused apoptosis.[22,23]

Based on Table 2, the wild p53 expression in K3 was lower than that in K1. This result might occur because, in the K3, the direct induction of carcinogenic benzopyrene would lead to partial mutation of the p53 gene (mutated p53 formation). This means that if a part of p53

underwent a mutation, the number of normal p53 (wild p53) would deplete. Meanwhile, in K1, the benzopyrene exposure only occurred indirectly (passive) so that only a little amount of the p53 were transformed (mutated). Thus, the wild p53 expression was more dominant than the mutated p53. However, the wild p53 expression in the K2 was the lowest among the K1 and K3 groups. This phenomenon might occur because of the continuous induction of benzopyrene that caused the transformation of wild p53 into mutated p53 and also the lack of the mice's physical ability in protecting and keeping the p53 in normal condition.

In this study showed that the moderate swimming exercise can increase the expression of wild p53. This might occur because the moderate swimming exercises can stimulate the p53 mRNA transcription through MAPK activation. This is in line with a study conducted by Thompson *et al*.[24] which found that the untrained subjects that were treated with downhill run at 10º for 30 minutes were proven to increase the MAPK response in 48 hours after the exercise. The MAPK is activated through Src and Ras-GAP signal transduction (GTP-ase activating protein).[25] This study is also consistent with the research conducted by Irmawati3 that moderate exercise can increase the expression of Vascular Endothelial Growth Factor (VEGF).

Conclusion

Moderate swimming exercise has no effect on GADD45 expression, but could increase wild p53 expression on Mus musculus injected by benzopyrene.

Conflict of interests

Conflict of interests is declared none.

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