The Influence of Moderate Exercise on Caspase-3 Expression in Inhibiting Transformation of Oral Squamous Epithelial Cells

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Abstract

The prevalence of cancer is increasing nowadays. In Indonesia, the prevalency of cancer is 1,4% out of 1000 people. Cancer is mainly caused by two factors; genetic and environmental, one of them being is a carcinogenic agent benzopyrene. Physical exercise can decrease blood glucose and fatty acid levels within the blood but its effect on cancer prevention, especially on caspase-3 expression, is still unknown.

This study examines the expression of caspase-3 produced by moderate exercise in preventing the formation of transform cell epithelial of the oral squamous cell.

18 mice were divided into three study groups; control group 1 (K1) not given any physical exercise and benzopyrene, control group 2 (K2) not given any physical exercise but induced with 0,08 mg benzopyrene, treatment group (P) were given physical exercise in moderate intensity and induced with 0,08 mg benzopyrene. Buccal mucosa tissue samples were taken and stained for immunohistochemistry and examined under a light microscope at 400x at 10 different angles.

The mean of caspase-3 expression was found highest in group P (13.33), meanwhile in group K1 and K2 were 3.00 and 8.33, respectively. There was significant difference of caspase-3 expression between the groups (p = 0.000) Conclusion: Moderate exercise could increase caspase-3 expression in preventing the formation of the transform cell epithelial of the oral squamous cell.

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Introduction

The prevalence of cancer today is increasing. Exposure to cancer risk factors is not only present in industrialized countries, but also in the whole world.[1] Cancer is considered the second leading cause of death globally. According to data from Basic Health Research in Indonesia (RISKESDAS) 2013, the prevalence of cancer in Indonesia is considerably high at 1.4% for every 1,000 residents.[2]

Cancer can be caused by a multitude of factors; from a person's genetics, environment, infection and lifestyle-including smoking.[3] Smoking cigarettes causes the body to be exposed to carcinogenic substances, the risk of developing cancer are in direct correlation with death.[1,4] Since cigarette smokers inhale smoke into the lungs, the type of cancer that develop from smoking are lung cancers, cancer of the larynx and pharynx, and cancer of the oral cavity (oral cancer). Oral cancer is considered the cancer that occurs most often in cases of head and neck cancer worldwide with about 263,000 new cases each year. The most common case of oral cancer is squamous cell carcinoma that causes changes in the oral cavity and impacts basic human functions such as talking, swallowing, chewing, and saliva production. Toxic compounds contained in cigarette smoke consists of Reactive Oxygen Species (ROS), such as polycyclic aromatic hydrocarbons complex , one of which is benzo [a] pyrene (BaP).[1,5,6]

Benzopyrene is metabolized into Benzo [a] pyrene-7,8-diol-9,10-epoxide (BPDE) and binds to one of DNA purine base, guanine.[4] BPDE and DNA bond can cause DNA mutations

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that stimulate the process of apoptosis in cells.[7] Apoptosis is the mechanism of cell death, and can be divided into 2 types, one is programmed apoptosis which is physiological and the other is pathological apoptosis which is when there is damage to the cells due to disease or due to harmful agents. If there is interference in the apoptotic pathway function, it can cause diseases in humans such as cancer, autoimmune disorders, and other neurodegenerative diseases.[8] Complex proapoptosis pathways such as Bax, and caspase groups may be induced by physical exercise. Regular physical exercise with appropriate intensity can prevent chronic diseases such as cancer, the risk of recurrence of cancer, diabetes, and heart attacks.[9,10,11]

Physical exercise, is mostly effective if given moderate intensity. Physical exercise being undertaken tends to be physiological, which does not cause any interference with the balance at the cellular level to the organ level. Therefore, in this study, homeostasis at cellular and organ level will not be disturbed.[7,12]

Effect of moderate exercise on caspase-3 expression in prevention of the formation of transformation on oral squamous epithelial cells is still not clearly, therefore, the aim of this study is to analyses the effect of moderate exercise on the caspase-3 expression in prevention of the formation of transform cells on oral squamous epithelial cells.

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: 164/KKEPK.FKG/VIII/2016).

This study was an experimental laboratory study with a sample of 18 mice (Mus musculus) of the Swiss Webster strain, which qualifies with the calculation using the Higgins-Kleinbaum formula. Each mice was male, aged ± 2 months, weighs 25-35 grams, adapted for 1 week, and randomly divided into 3 groups; negative control group (K1), positive control group (K2), and treatment group (P).

The negative control group (K1) was soaked in water, with a time of 70% of the mice's maximum swimming capacity, 3 times per week for 12 weeks. In the 5th week 0.04 mL of oleum olivarum was induced on the top right buccal mucosa, 3 times a week for 4 weeks. The positive control group (K2) is soaked in water, with a time of 70% of the mice's maximum swimming capacity, 3 times per week for 12 weeks. In the 5th week the mice were induced by 0.08 mg of benzopyrene (Merck, Sigma-Aldrich Pte. Ltd., Singapore) / 0.04 ml oleum olivarum on the top right buccal mucosa, 3 times a week for 4 weeks. Lastly, the treatment group (P) was given physical exercise with weights weighing 3% of the mice's body weight (BW), with a time of 70% of the maximum swimming capacity, 3 times per week for 12 weeks. In the 5th week induced by 0.08 mg of benzopyrene / 0.04 ml oleum olivarum on the top right buccal mucosa, 3 times a week for 4 weeks. At the beginning of the 13th week, mice were sedated with ether, waited until unconscious. The next stage, tissue on the top right buccal mucosa was removed in each group. Staining was done using the immunohistochemistry method, and observation of squamous epithelial cells expressing caspase-3 (are calculated on a 10 field of view with an magnification 400x).[13]

Statistic: The data collected was analyzed with Oneway Analysis of Variance (Anova) on SPSS 19.0 for Windows.

Results

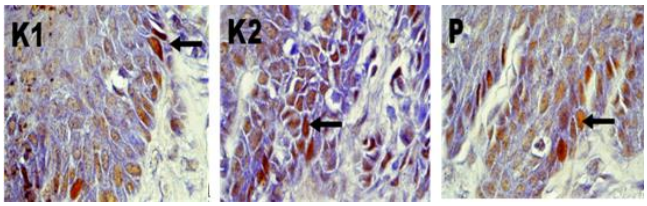

Figure 1. The expression of caspase-3 (black arrow) on K1 (a), K2 (b) and P (c) groups with immunohistochemical staining (400x magnification). Arrow (→) indicates cells expressing caspase-3.

The figure 1 showed the expression of caspase-3 by immunohistochemical slide. The mean of caspase-3 expression was found highest in group P (13.33), meanwhile in group K1 and K2 were 3.00 and 8.33, respectively (Figure 2). The cell that express caspase-3 showed brown color due to anti caspase-3 antibody absorption.

Oneway Anova test results of caspase-3 expression, shows that there are significant differences ($p < 0.01$). The Tukey Post Hoc Test

results also showed significant differences (p> 0.01) between all 3 groups, the results can be seen in Table 1.

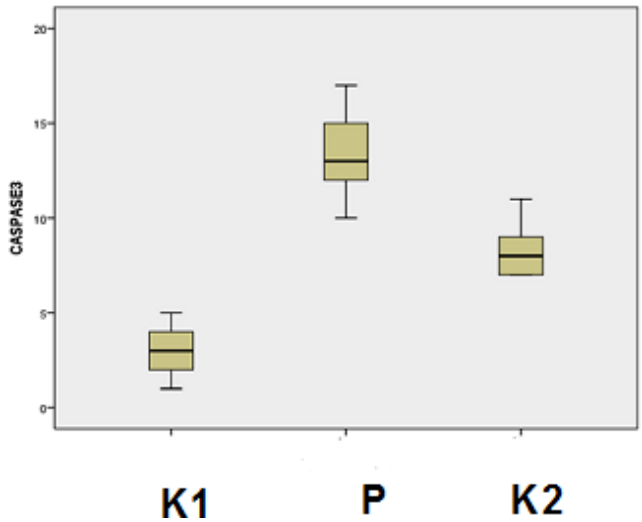

Figure 2. The average of caspase-3 expression on K1, K2 and P groups.

Group	K1	K2	P
K1	-	p = 0,000*	p = 0,000*
K2			p = 0,001*
P			-

Table 1. Tukey Post Hoc test of *caspase-3 expression on* squamous epithelial cells.
Description: * showed that the data is significant.

Discussion

The expression of caspase-3 plays an important role in the change of normal cells into cancer cells in gastric mucosa.[14] Results of the research conducted proved that moderate exercise can increase the expression of caspase-3 in prevention transformation cells on oral squamous epithelial cells. The expression of caspase-3 was higher in group P due to group P being given treatment in the form of physical exercise with weights about 3% of the mice's body weight (BW), with a time of 70% of the maximum swimming capacity, 3 times per week for 12 weeks.

Moderate exercise can increase the expression of caspase-3 because it can trigger Ca^{2+} channels opening in the membrane cell. The opening of the Ca^{2+} channels will cause an influx of Ca^{2+} into the cell cytoplasm resulting in an increase in the concentration of Ca^{2+} in the cell. The increased Ca^{2+} concentration in the cells activates signal transduction pathways involved in various cellular responses-including proliferation, transcription, contraction, exocytosis, apoptosis, immune response, and neurotransmission- one of which enables complex signal transduction Ras and Src. Active Src and Ras as signal transduction and then activate the MAPK (mitogen-Activated Protein Kinase) in the nucleus. MAPK activation causes phosphorylation of various transcription factors and act as an intermediary in the process of signal transduction from the plasma membrane to in the nucleus.[15,16]

Benzopyrene is a compounds contained in cigarettes can enter into the tissues of the body through the skin, inhalation, or through the mouth. Benzopyrene is metabolized in the liver by the cytochrome P4501A1 enzyme (CYP1A1) with the final result of the carcinogenic metabolites, that is benzo [a] pyrene-7,8-dihydrodiol 9,10-epoxide (BPDE). BPDE that enter the cell can bind to DNA, thus later will be responded by p53.[17] This bonding causes DNA mutations of p53, which can hinder the process of protein synthesis (transcription of wild p53). MAPK activation causes phosphorylation of various transcription factors and act as an intermediary in the process of signal transduction from the plasma membrane to the nucleus. One of the proteins that undergo transcription is wild p53 protein. Wild p53 is a protein that acts as a tumor suppressor gene that inhibits the occurrence of cancer.[4]

Bcl-2 protein complexes play an important role in controlling apoptosis through the mitochondrial pathways. Bcl-2 complex is divided into two, namely apoptosis suppressor gene and gene promoting apoptosis. Bcl-2 acts as a suppressor gene, and the gene is acting as promoting Bax. Stimulation barriers Bax Bcl-2 by causing the release of cytochrome-c. The release of cytochrome-c from the mitochondria to the cytosol is the initial phase of the process of apoptosis.[7,18] Cytochrome c binds to Apoptosis Protease-Activating Factor (Apaf-1). The complex formed then activates caspase-9 which is responsible for the apoptotic cascade. Activation of caspase-9 causes the activation of caspase-3. Activation of caspase-3 causes CAD (Caspase Activated DNAse) endonuclease to be active. In the process of cell proliferation CAD, equipped with complex inhibitor, namely ICAD (Inhibitor Caspase Activated DNAse). In the

process of apoptosis, caspase-3 ICAD serves to break down and release the CAD. CAD endonuclease then degrade chromosomal DNA in the nucleus and causes chromatin to condense and form apoptotic bodies.[8,19] Apoptotic bodies formed does not cause the formation of squamous epithelial cells transform cells of the oral cavity.

Conclusions

Moderate intensity of physical exercise increases caspase-3 expression on the epithelial transform cell of the oral squamous cell.

Declaration of interest

The authors report no conflict of interest.

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