ORIGINAL ARTICLE

The Decreased of p53 Mutant Expression on Squamous Cell Epithelial of Oral in *Mus musculus* by Moderate Intensity of Exercise

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ABSTRACT

Introduction: Cancer is a deadly disease, caused by benzopyrene which found in cigarettes. The moderate intensity of exercise can affect cancer growth, but how the role it to p53 mutant is not clear. This research proved the expression of p53 mutant in *Mus musculus* by moderate intensity of exercise. **Method:** Eighteen animals, were divided into three groups namely, group A, injected by oleum olivarium in the upper buccal mucosa and not given moderate intensity of exercise and benzopyrene; group B, injected benzopyrenes in the same regio, not given moderate intensity of exercise; group C injected benzopyrenes in same region, given moderate intensity of exercise. Moderate intensity of exercise given 3 times every week for 3 months. Oleum olivarum and benzopyrene injection, was given 3 times every week for 1 month, starting at week 5. At the beginning of the 13th week, a bend was taken in the right upper buccal mucosa, then the tissue was processed, painted by immunohistochemical methods. p53 mutant expression was determined by looking at preparations, using an Olympus light microscope with a magnification of 400x. p53 mutan expression was observed in 10 different visual field, then the mean was taken. **Result:** There were differences in p53 mutant expression in the three groups (p= 0.000), and the highest expression was in group B. **Conclusion:** The moderate intensity of exercise could decrease p53 mutant expression on squamous cell epithelial of oral *Mus musculus*.

Keywords: : p53 mutant, Squamous cell epithelial of oral, *Mus musculus*, Moderate intensity of exercise

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INTRODUCTION

Cancer is the disease with high rate of mortalitiy, caused by abnormal growth of cells that are not controlled, so that it can damage the surrounding cells. According to the World Health Organization (WHO), in 2018 there were 18.1 million new cases of cancer, with a mortality rate of 9.8 million. This massive cancer attack, makes WHO predict cancer will be make a number one of mortality cause in the world at the end of this century. Cancer will be the biggest barrier for humans to increase life expectancy. The results of this report were obtained after researchers analyzed data from 185 countries in the world by looking deeper into 36 types of cancer (1) .

In Indonesia, Riset Kesehatan Dasar (Riskesdas) 2018 shows that the prevalenoe of Non- Communicable Diseases has increased compared to the Riskesdas 2013, including cancer, stroke, chronic kidney disease, diabetes mellitus, and hypertension. Cancer prevalence rise from 1.4% (2013) to 1.8% (2018); the prevalence of stroke rises from 7% to 10.9%; and chronic kidney disease increased from 2% to 3.8%. Based on examination of blood sugar, diabetes mellitus rises from 6.9% to 8.5%; and the results of blood pressure measurements, hypertension rise from 25.8% to 34.1% (2).

One of the most common cancer found is head and neck (3). Oral cancer is a cancer with the highest mortality rate among all types of malignancies (4). About 95% of oral cancers are oral squamous cell carcinomas (OSCC) (5). In the global world, cases of head and neck cancer are more than 550,000 cases, and the number of deaths annually is 380,000 (6). In America, every year around 63,000 Americans suffer from head and neck cancer (7). Meanwhile in Europe, in 2012, there were around 250,000 cases and 63,500 of them died (8).

Currently oral cancer attacks many age groups under 40 years, compared to three decades ago (9). This cancer

is found three times more common in men than women (10). The most frequent tumor sites were oral cavity tumors (35.37%), and the most common histological type was OSCC (96.7% of cases). The prognosis for head and neck cancer depends on the stage (5).

Risk factors for OSCC is smoking and alcohol consumption, virus and bacterial infection, lack of nutrition, irritation of the denture, and immunosuppression (4,11). Cigarettes are the highest risk factor for the development of OSCC (12). China and India have the highest lifters of smokers in the world, each with 307 million and 106 million smokers, out of a total of 1.1 billion smokers among adults, followed by Indonesia with 74 million, as indicated by figures in the WHO report, India also has 200 millions of 367 million smokeless tobacco users (13,14).

During this time the actions taken on cancer patients are curative actions, as well as actions to improve the quality of life, especially patients who are at an advanced stage. Study by Irmawati et al proved the moderate intensity of exercise can enhance Bax and caspase-3 expression, also decrease Bcl-2 expression by benzopyrene- induced mice (15,16). But whether moderate intensity of exercise can affect the expression of p53 mutant proteins is still unknown. The aims of this research to prove the moderate intensity of exercise can reduce p53 mutant expression, as a parameter of malignancy.

MATERIALS AND METHODS

Ethical Clearance

This study was approved by Ethic Committee in Faculty of Dental Medicine, Universitas Airlangga, Indonesia with registration number 164/KKEPKWG/VIII/2016.

Sample

The research was an experimental, use a randomized block design. The study population was male, Mus musculus, 8 weeks old, 25-35 in weight. The sample size of each group in this study was six, so the total sample for the three groups were eighteen mices. Before the experimental was done, all mice being acclimatized for 1 week.

Research procedures

In the experiment group, the moderate intensity of exercise defined as the animal was given swimming activity, with 3% of weight in load, time used was 70% of the maximal swimming capacity, for 3 times every week in frequency, during 3 months. The negative and positive control group defined as the animal was given water contacted, with a same time and frequency with experiment group.

All of animals are groupped into three groups, as follows:
1. Group A, negative control group and at week 5, oleum olivarum as much as 0.08 ml, injected in the upper right of buccal mucosa, 3 times a week during 1 month.
2. Group B, positive control group and at week 5 induced benzopyrene as much as 0.08 mg (Merck, Sigma- Aldrich Re. Ltd., Singapore) dissolved in oleum olivarum with volume 0.04 ml within the right upper mucosa of buccal, 3 times a week during 1 month.
3. Group C, animal was given continuous moderate exercise and at week 5 induced 0.08 mg benzopyrene in the 0.04 ml oleum olivarum, in the upper right mucosa of buccal, 3 times a week during 1 month.

All groups, on the first day of the l3th week all mice were ether anesthetized, waited till passed out, and the upper right of buccal mucosa was taken, then the mice were sacrificed. Then the p53 mutant expression was examined (15).

Immunohistochemistry staining

Immunohistochemistry staining was used to determine the p53 expression. The polyclonal antibody of p53 was used (*anti-p53 antibody ab131442, polyclonal, Abcam*). The p53 mutant expression was measured by counting the number of cells that gave a positive reaction to anti p53 mutant, after staining using immunohistochemistry methods. Calculations were carried out in ten different fields of view using a 400x magnification light microscope (16).

The collected data was then carried out by Kruskal Wallis test, followed by the Mann-Whitney test. The level of significance used in this statistical test was 0.05.

RESULTS

Figure 1 shows the expression of the p53 mutant by immunohistochemistry slide. The cell that expressed p53 mutant showed brown color. The p53 mutant expression in group B (l4.75+1.57) was highest compared group A (2.58+0.80) and group C (6.67+I .57), respectively ($p = 0.000$) (Figure 2).

DISCUSSION

Increased incidence of cancer (including OSCC) cannot be denied. This is inseparable from the increasing consumption of cigarettes every year. Cancer is a condition where cell can grow continuously , uncontrolled, and do not undergo apoptosis. Cancer cells can cause damage to surrounding biological tissue, and can metastasis to other tissues through blood vessel or lymph vessel.

The highest risk factors for OSCC is cigarettes, which is contained benzopyrene (11). Benzopyrene is a waste product which is a combustion of organic material and carcinogenic. Benzopyrene can be delivered through the air, found in cigarettes smoke, motor vehicle fumes, and smoke from burning forest.

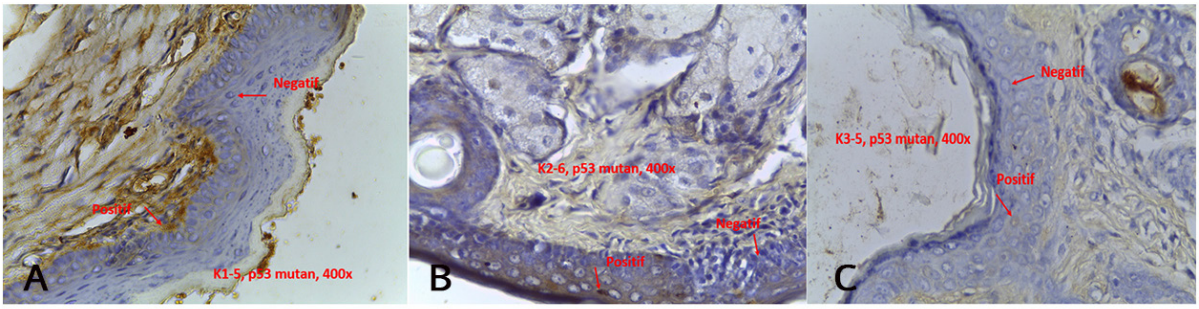

Figure 1: The expression of p53 mutant (positive) in group A, B, and C with immunohistochemistry staining

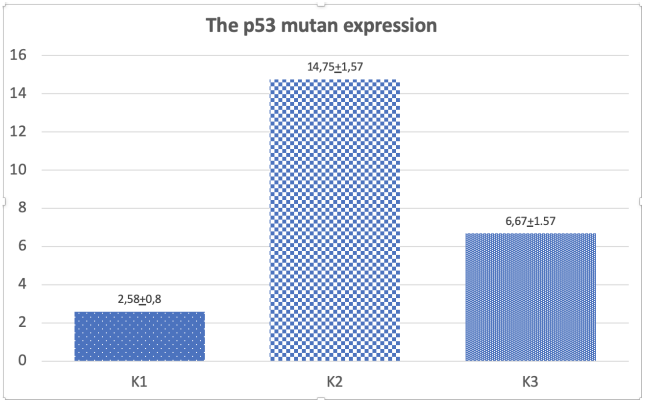

Figure 2: The expression of p 53 mutant in group B (K2) highest, followed by group C (K3) dan group A (K1)

People who do not smoke, but are exposed to cigarettes smoke for a long time, the same as those who breathe air filled with pollutants. In a study conducted on rat animals, benzopyrene was rapidly distributed to various tissues including kidney, small intestine, trachea, stomach, testis, liver and esophagus. Benzopyrene is metabolized by the cytochrome P450 lA 1 (CYP1A1) enzyme to produce a number of metabolites, and the metabolite believed to be carcinogenic is Benzopyrene 7.8 diol-9,10-epoxide (BPDE). The acute effects of benzopyrene are rare, while long-term exposure results in chest pain, respiratory tract irritation, coughing, dermatitis, decreased immune system, hepatotoxicity, and tumors (17). In this study benzopyrene was used as an ingredient to made transformed cell animal models.

The benzopyrene is chemical agent that cause a cancer. The cancer occurs because changes in genes such as mutations. When a mutated gene is found in a cell in the body, there are two mechanisms to protect the mutated cell. The first mechanism, the body will try to repair the mutated gene by using repair genes, one of which is heat shock protein 70 (Hsp70). Kowalchuck et al 2013., said in animal model (treadmill 0.5 m/s) with the maximal intensity exercise can improve Hsp70 and Hsp90 expression when compared to exercise with slight intensity (treadmill 0.25 m/s) (18,19).

In this research showed that moderate intensity of exercise able to reduce p53 mutant expression (Group C) (6.67+1.57) when compared to group B (14.75±1.57) (although p53 mutant expression in group C cannot be the same or lower than group A), expression of p53 mutant found in group A can occur due to exposure to cigarette smoke passively in experimental animals, so it is still possible for gene mutations in the p53 gene to produce small amounts of p53 mutant. Lesser p53 mutant expression can be caused because the amount of p53 which is maintained normal (wild p53) is higher. This can occur because moderate intensity of exercise can trigger the transcription of p53 mRNA, which is followed by translation and synthesis of normal p53 protein (wild p53). While in group B, high p53 mutant expression can be caused due to the induction of carcinogenic benzopyrene material continuously resulting in disruption of homeostasis in the body, which is a disharmony between the rate of wild p 53 synthesis and the rate of attack of carcinogenic substances that cause defects in the gene of p53.

In humans, the p53 gene is consist of 11 exons, located on the short arm of chromosome 17, and expressed on all body tissues. Under normal circumstances, p53 consists of a tetramer, meaning that 4 identical copies are assembled to form active p53 molecules. Therefore, if there is a defect in one of the 4 subunits (generally point mutation), it will weaken the function of p53; if 1 cell has 1 wild allele p53 and the other allele is defect, most of the functions of p53 will be disrupted. This means that p53 can only function properly if all four subunits are normal (a characteristic feature of p53 that distinguishes it from other suppressor genes, which are usually in the form of free molecules) (20).

More than 50% of tumors in humans contain p53 mutant and 95% of p53 mutations are scattered throughout exon encoding the p53 gene, especially in the middle, the binding region of p53 is specific. These mutations damage the entire conformation of the specific domain (of DNA sequences) and result in the loss of the ability to bind to specific DNA sequences. Because p53 is a number of genes involved in the cessation of the cell

cycle and apoptosis, the wild p53 which becomes p53 mutant loses an important function to maintain genome integrity. In addition, p53 mutant is longer than wild p53 and is predominantly negative for wild p53 (21).

The greater the number of wild p53 which mutates into p53 mutant, the function of p53 to stop the cell cycle in phase G1 and the function of p53 to initiate apoptosis cannot occur. This will result in the formation of genes that are defective continuously and uncontrolled cell division which is a feature of the occurrence of malignancy (cancer). The expression of p53 mutant on group C cannot be equal or lower when compared to group A, this can be possible because the repair gene of Hsp70 expression is not sufficient to repair the p53 gene that has a defect. The second possibility is that the damage to the p53 gene is too severe (in the unrepairable category). The results showed that endurance exercise can reduce p53 mutant mitochondrial in mice that do a treadmill 1.5 m/minute (in 45 minutes) for 6 months and its similar study that conducted with Safdar et al (22).

CONCLUSION

The moderate intensity of exercise could decrease p53 mutant expression on oral squamous cell Mus musculus.

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