Original Article
Effects of cigarette smoke on aerobic capacity and serum MDA content and SOD activity of animal

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Received July 22, 2014; Accepted August 26, 2014; Epub November 15, 2014; Published November 30, 2014

Abstract: Objective: Study the effects of cigarette smoke on aerobic capacity, serum MDA content and SOD activity of animal. Methods: 60 male mice are randomly divided into mild smoking group, heavy smoking group, and control group, and the exhausted swimming time, serum SOD activity and MDA content of the three groups of mice are respectively measured before and after the experiment. Results: After the experiment, the exhausted swimming time for the control group, mild smoking and heavy smoking groups is respectively 276.57 min, 215.57 min and 176.54 min, and the serum SOD activities for the three objects are 216.46 U/mL, 169.16 U/mL and 154.91 U/mL, and the MDA contents are respectively 16.41 mol/mL, 22.31 mol/mL and 23.55 mol/mL. According to the comparison, it is found that compared with the control group and pre-intervention, the exhausted swimming time and serum SOD activity of the smoking group decreases obviously, and its MDA content rises sharply, and the difference has significance (P < 0.05), moreover, the heavy smoking group has more obvious changes than the mild group. Conclusion: Cigarette smoke can significantly weaken the aerobic capacity and fatigue resistance of mice, and the more the smoking time is longer, the more the harmful effect is more serious, this is related to the SOD activity drops and MDA content rises due to smoking.

Keywords: Cigarette, aerobic exercise, superoxide dismutase (SOD), malonaldehyde (MDA), mouse

Introduction
Smoking is harmful to all organs of human body, and cigarette has become the global No. 2 killer after hypertension. According to the statistics of world health organization, there are about 1.3 billion smokers around the world, and 5 million people die from the diseases linked to smoking. There are about 350 million smokers in China, the smoking rate of male exceeds 60%. At the same time, there are 540 million nonsmokers suffering from the danger of secondhand smoke exposure. However, the harm on passive smokers has not aroused enough attention [1, 2]. Smoke produced in the process of smoking, of which above 90% (tar, nicotine, carbon monoxide and other harmful substances) directly diffuses into air, people have clearly known the dangers of active smoking to human health, but the cigarette smoke dangers on other passive groups should also arouse people’s attention. At present, for the effects of smoking on human health, relevant results are mostly prone to the studies of human pulmonary function, nervous system and heart and cerebral vessels, and other aspects, but there are fewer studies on human aerobic endurance and fatigue resistance [3]. This study analyzes the effects of smoking or passive smoking on aerobic capacity, serum MDA content and SOD activity of mouse via observing the exhausted swimming time, serum SOD activity and MDA content of the passive-smoking mouse, which provides reference frame for the studies on the discussion of the smoking or passive smoking on aerobic endurance and fatigue resistance mechanism of sportsman.

Materials and methods

Materials
Experimental subject: 60 Kunming mice, clean, No.: SCXK (Henan) 2005-0001, certification No. of animal House: Yu Medical Animal No. 4104022, male, weight: 24 ± 3 g (purchased from animal feeding centre of medical school of
Zhengzhou University), feeding in each cage, free diet, good indoor air ventilation, and the room temperature is controlled about 24 degrees centigrade. Toxicant exposure materials: Passive smoking toxicant exposure cabinet (developed by Environmental Health Institute of Military Medical Sciences), glass material, volume is 100 cm × 100 cm × 70 cm = 0.7 m³ (700 L). The toxicant exposure cabinet is connected with smoking device. The LK-3 gas detection alarm apparatus is used for monitoring the concentration of CO, CO₂ and O₂. Oxygen bottle is used for feeding oxygen, the flow rate is 0.5 L/min. Red Flag Canal cigarette (produced by CTHIC Industrial Company, tar yield is 15 mg, and the nicotine amount is 1.2 mg), EDTA-2K anticoagulation, SOD kit and MDA kit (purchased from Nanjing Jian Cheng Bioengineering technology Co., LTD).

Methods

Animal grouping: 60 male mice are randomly divided into control group (no smoking) and experimental group (smoking), where the experimental group includes mild smoking group and heavy smoking group, all mice in control group do not participate in contamination test. Toxicant exposure method: Mice in mild smoking group smoke 2 times a day, starting from 8:00 a.m., and 4:00 p.m. for 14 days continuously, passive smoking is carried out on cigarettes. Each group carries out load-free adaptive swimming training for 6 days together with the control group (swimming time gradually increases from the beginning of 20 min), after having a rest 1 day, exhausted swimming is carried out, the mice is controlled to maintain the swimming state, the exhaustion standard is that the mice do not return to the water surface after sinking 10 s, and the exhausted swimming time is recorded.

Tissue sampling and processing

1.5 to 2.5 mL of tail blood is respectively extracted from the 3 groups of mice before experiment, placed into a refrigerator for staying overnight at 4 degrees centigrade, and then centrifuged with 3000 r/min for 10 min to obtain serum. The xanthine oxidase method and thiobarbituric acid method are respectively used to test the SOD activity and MDA content for each group of mice before experiment. After having the exhausted swimming, and recovery for 24 h, namely, 1.5 to 2.5 mL of tail blood is respectively extracted from the 3 groups of mice after experiment. Methods same as the pre-experiment are respectively used to test the SOD activity and MDA content for each group of mice after experiment.

Data processing

SPSS13.0 software is adopted to process data, (X ± s) is used for representing, the variance analysis is used for the comparison among multiple groups, if overall difference has Dunnett-t test is adopted to carry out pairwise comparison, P < 0.05 refers to that differences are statistically significant.

Results

The changes of weight, exhausted swimming time, serum SOD activity and MDA content are as shown in Tables 1-3.

Table 1 shows that there is no significant difference for the average weight of the experimental group and control group before experiment (P >
Cigarette smoke on aerobic capacity and antioxidants

Table 1. Weight comparison between experimental group and control group before and after intervention (n = 20, x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>Case number (piece)</th>
<th>Average weight (g)</th>
<th>Before experiment</th>
<th>After experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>20</td>
<td>24.24 ± 3.20</td>
<td>27.56 ± 3.40</td>
<td></td>
</tr>
<tr>
<td>Mild smoking group</td>
<td>20</td>
<td>23.75 ± 2.95</td>
<td>26.48 ± 3.48</td>
<td></td>
</tr>
<tr>
<td>Heavy smoking group</td>
<td>20</td>
<td>24.17 ± 3.12</td>
<td>25.57 ± 3.27*</td>
<td></td>
</tr>
</tbody>
</table>

Note: comparison between after and before experiment, *P < 0.05.

Table 2. Exhausted swimming time index comparison between experimental group and control group before and after intervention (n = 20, x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>Case number (piece)</th>
<th>Mean time of exhausted swimming (min)</th>
<th>Before experiment</th>
<th>After experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>20</td>
<td>253.14 ± 45.60</td>
<td>276.57 ± 45.97</td>
<td></td>
</tr>
<tr>
<td>Mild smoking group</td>
<td>20</td>
<td>253.57 ± 38.97</td>
<td>215.57 ± 29.49\textsuperscript{a,b}</td>
<td></td>
</tr>
<tr>
<td>Heavy smoking group</td>
<td>20</td>
<td>252.39 ± 47.83</td>
<td>176.54 ± 21.79\textsuperscript{a,b}</td>
<td></td>
</tr>
</tbody>
</table>

Note: compared with the pre-intervention, \textsuperscript{a}P < 0.05, compared with the control group, \textsuperscript{b}P < 0.05.

According to Tables 2 and 3, there is no significant difference for each index of the experimental group and control group before experiment (P > 0.05), and no statistical significance. But after interference, compared with the values of pre-interference and the control group, the mice exhausted swimming time, serum SOD activity for each experimental group decrease relatively, and the MDA content increases significantly. Moreover, compared with the control group and pre-interference, there is significant difference on the mild and heavy smoking groups (P < 0.05). Compared with the mild smoking group, each indicator of heavy smoking group changes obviously (P < 0.05). The results show that passive smoking has adverse effects on the aerobic ability and antioxidant ability of healthy mouse, and the longer the smoking time is, the more serious the adverse effects are.

Discussion

Aerobic capacity refers to the athletic ability that the body provides energy via aerobic metabolism for a long time. Studies have proved that the tar and other harmful substances in smoke can be attached to the trachea, bronchi and alveoli surface of involuntary smoker, and can increase the oxygen radical released by alveolar macrophages and neutrophile granulocyte in blood, thus to increase the adhesion of leukocyte and endothelial cells, develop inflammatory response, and finally to cause lung tissue breakdown.

0.05), meeting the characteristics and requirements of random grouping. After passive smoking, the weight of the heavy smoking group is obviously lower than the control group (P < 0.05), difference has statistical significance, and the mild smoking group is slightly lower than the control group (P > 0.05), namely the difference has no statistical significance.

Nicotine can cause elevation of blood pressure, strong beating of the heart, Heart Pump Function drops, body oxygen consumption increasing and the like, so as to decrease the aerobic capacity [4-6]. The results are in conformity with the above: after 14 d of passive smoking, the sustained aerobic ability and fatigue resistance of the mice drop, the exhausted swimming time shortens. The reason may be the various degrees of mice lung tissue damages due to smoking, and function drop of heart and muscles. In addition, other concerned studies also show that under equal conditions, the increase of mice weight will increase the swimming load, add energy consumption per unit time, and will shorten the exhausted swimming time [7, 8]. According to the weight data of mice before and after the experiment, the increase of the control group is relatively obvious, its effects on the exhausted swimming time of mice should be higher than the 2 groups of experimental groups, it shows that the control group should have stronger exhausted swimming ability from another perspective.

In this study, the changes of mice serum SOD activity and MDA content are the strong evidence to explain the above phenomena. In recent years, free radical theory provides new theoretical basis for the production and recovery of sports fatigue, arousing more and more attention. The oxygen free radical and its induced lipid peroxide have strong toxic effects.
Cigarette smoke on aerobic capacity and antioxidants

**Table 3. Serum SOD activity and MDA content indexes comparison between experimental group and control group before and after intervention (n = 20, x ± s)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Case number (piece)</th>
<th>MDA (mol/mL) Before experiment</th>
<th>MDA (mol/mL) After experiment</th>
<th>SOD (U/mL) Before experiment</th>
<th>SOD (U/mL) After experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>20</td>
<td>18.24 ± 4.61</td>
<td>16.41 ± 3.47</td>
<td>187.55 ± 18.86</td>
<td>216.46 ± 15.95</td>
</tr>
<tr>
<td>Mild smoking group</td>
<td>20</td>
<td>18.59 ± 3.12</td>
<td>22.31 ± 3.31&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>187.34 ± 21.20</td>
<td>169.16 ± 24.80&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heavy smoking group</td>
<td>20</td>
<td>18.37 ± 3.84</td>
<td>23.55 ± 2.37&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>188.76 ± 18.74</td>
<td>154.91 ± 36.37&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Note: compared with the pre-intervention, *P* < 0.05, compared with the control group, *P* < 0.05.

on living organism: changing the lipid micro-environment of membrane receptors, ion channels, and membrane proteins, affecting the functions of cell membrane [9, 10]. MDA is the final decomposition product of the lipid peroxidation, and it is parallel to the reaction extent of lipid peroxidation in vivo, therefore, MDA is usually used as the index to assess the degree of the lipid peroxidation for free radical [11]. At the same time, there are enzymes and non-enzymes substances for eliminating oxygen free radicals in vivo, mainly including SOD, CAT, and the like, vitamin E and other non-enzymes substances, these maintain the dynamic balance of free radical generation and elimination in vivo via own ways [12, 13]. So to speak, the serum SOD activity and MDA content reflect the body’s overall oxidation and oxidation state. In this experiment, the MDA content of the mice with passive smoking for 14 d is significantly higher than the control group, but the SOD activity to eliminate the free radicals is obviously lower than the control group. It reveals that the passive smoking plays a promoting role on the lipid peroxidation in vivo, at the same time, consumes large amounts of antioxidant enzymes and inhibits the activity of antioxidant enzymes, affecting the cell function of multi-parts. Finally, the work abilities for blood, cardiopulmonary, muscle decrease, thus to accelerate the development of the fatigue process. Correlational studies also prove that: the LPO of smoker is significantly higher than non-smoker, and the SOD activity decreases significantly more than the non-smoker [14, 15]. Smoking can aggravate the lipid peroxidation of the body in vivo, the oxidation and antioxidant balance disorders, MDA increases, so as to affect the normal physiological function of cells [16-18]. The SOD activity and MDA content measured in this experiment are the recovery situation after exhausted swimming 24 hours, it shows that it is difficult to recover the damages of harmful substances in cigarette smoke on body, so, smoking or passive smoking not only affects athletic ability and accelerates the development of fatigue, but also affects the fatigue elimination and functional recovery after sports.

**Conclusion**

Cigarette smoke can reduce the serum SOD activity of healthy mice, and increase the MDA content, this is a significant reason resulting in the drops of mice aerobic ability and fatigue resistance, and delaying fatigue elimination and functional recovery. Moreover, the longer the smoking time is, the more serious the adverse effect is.

**Acknowledgements**

This work was supported by Science and Technology Department of Henan Province (132400411286).

**Disclosure of conflict of interest**

None.

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Cigarette smoke on aerobic capacity and antioxidants


