The effect of aerobic exercise on hepatotoxicity induced by intratracheal instillation of iron oxide nanoparticles in Wistar rats

Azadeh Vasili1*, Gholamreza Sharifi[2], Mohammad Faramarzi[3], Ali Noori[4], Shora Yazdanshenas[5].

[1]Department of Exercise Physiology, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran, [2]Department of Exercise Physiology, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran, [3]Department of Exercise Physiology, Shahrekord University, Shahrekord, Iran, [4]Department of Biology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran, [5]Department of Microbiology, Science and Research Branch, Islamic Azad University, Tehran, Iran
Fax: (+98311) 2658569
E-mail: vasili.mgh@gmail.com

Abstract: Iron oxide nanoparticles are significant due to their unique physicochemical properties and environmental characteristics. They are found as ultrafine particles in ambient air. After inhalation, these particles move from the lung to phagocytosis tissues, especially the liver. The present study investigates the effect of concurrent aerobic exercise and iron oxide nanoparticles on liver enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and histological hepatic appearance. 48 Wistar rats were randomly divided into six equal groups: experimental 1 (aerobic exercise), experimental 2 (nanoparticle, anesthesia), experimental 3 (aerobic exercise, nanoparticles, anesthesia), placebo 4 (distilled water, anesthesia), placebo 5 (aerobic exercise, anesthesia), and control group. In groups 2 and 3, 40mg/kg_{bw} iron oxide nanoparticles were injected via intratracheal installation every other day for 14 days. Groups 1, 3, and 4 ran on a treadmill 30 minutes every day with the intensity of 35%-40%V_{O2max} (maximal oxygen consumption). ALT increased in group 1 but decreased in groups 2 and 3, AST was not significant in any of the groups, and ALP reduced significantly in groups 2 and 3 ($P<0.05$). Histological examination of the liver showed that, in groups 2 and 3, hepatic cells were damaged and congestion, inflammation, mononuclear cell infiltration, and ballooning degeneration occurred. Tissue injuries in group 3 were less than group 2. These findings indicate that hepatotoxicity is caused by iron oxide nanoparticles; however, low-intensity aerobic exercise can decrease the damage somewhat.
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Introduction

Special physicochemical characteristics of nanoparticles (NPs), including size, high specific surface area, reactivity, and shape, facilitate the entry of these substances into the organisms' bodies and their movement into tissues, cells and even cell organelles, although it may not be possible for larger particles to enter [1 and 2]. Iron oxide nanoparticles (IONPs) naturally are found as ultrafine particles in ambient air [3]. Magnetites or maghemites are the most common types of IONPs that are produced from traffic, industry, and power stations [3 and 5]. NPs that spread in the environment through natural ways or engineered processes may be suspended in a gas as a nanoaerosol and enter the body through respiratory pathways [6 and 7]. Despite the importance of the respiratory effects of NPs, only a small number of studies have examined these effects [8 and 9]. Precipitated NPs can overcome pulmonary tissue barriers and move out of the respiratory system. NPs pass through the respiratory epithelium, enter the circulatory system, and spread [10, 11 and 12] throughout the body. Heart, liver, and kidney tissues, are affected by nanoparticles [13 and 14]. IONPs enter via endocytosis into Kupffer cells, sinusoids and macrophages in the spleen (mono nuclear phagocyte system (MPS)) [15].

IONPs lead to an increase in the permeability of endothelial cells in human macrophages through ROS (Reactive Oxygen Species) production and can cause inflammation [5 and 16]. These particles are then integrated into the hepatocytes by the bloodstream and cause reduction in mitochondrial activity and morphological changes in rat hepatic cells [5 and 16]. Also, they induce oxidative stress response in

hepatocytes by generating free radicals and cause hepatic cell death [17]. In addition, IONPs' reaction with proteins and enzymes in hepatocytes cause structural change and will ultimately cause the liver cells to die[18].

Changes in biochemical parameters, including serum bilirubin (TBIL), alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate aminotransferase (AST), and other hepatic pathological factors, provide ways and means for the evaluation of NPSs' effects on liver function, usually causing peripheral and central venous disorders and typically causing patchy necrosis of the liver [11].

Because of the vital role of the liver in maintaining homeostasis and energy supply, it is necessary to take these organs (heart, liver, and kidney) into consideration when we evaluate the changes resulting from exercise and physical activity [19] and [20]. During exercise, several events occur to prepare skeletal muscles to use energy and accelerate the metabolism of glucose and fatty acids, increasing fat oxidation and reducing blood triglycerides and cholesterol and, subsequently, increasing the concentration of HDL serum. Also, during physical activity some mechanisms are activated that can affect hepatic cells [21] and [22]. Therefore, regular exercise causes changes such as gene expression in hepatocytes, protection from hyperglycemia, and hepatic steatosis. Additionally, it improves some metabolic parameters in patients with fatty liver [23] and [24]. Different intensities of exercise have impacted the liver greatly and lead to some other effects [25]. Regular exercise causes reduction in reactive oxygen species (ROS) through anti-oxidation system compatibility [26] and [27].

Exercise can temporarily increase liver function tests, but researchers still must carry out studies in order to reveal how exercise leads to an increase in the amount of biochemical factors [28]. Despite the importance of examining the effects of physical activity on the absorption of inhaled NPs and their effects on the liver disorders, no study has been done in this field. Also, the respiratory effects of the nanoparticles commonly used in industry have not been fuly studied; hence their potential for hazardous effects on human health remain unclear [8]. Therefore, this study aims at determining the effect of low-intensity aerobic exercise on hepatotoxicity induced by intratracheal instillation of IONPs.

Method

Iron oxide nanoparticles; γ -Fe_2O_3

IONPs were prepared from US Research Nanomaterials, Inc. (Houston, USA). XRD results as regards nano γ-Fe2O3 are in crystalline phase with 20nm size. Purification of nano γ-Fe2O3 was determined as 99.5% by using ICP-MS.

Animals

Forty-eight male Wistar rats were obtained from the Pasteur Institute in Tehran, Iran. The animal studies were performed in accordance with the protocol of care and use of experimental animals. This study was approved by the Regional Committee for Medical Research Ethics of the Vice Chancellor for Research, Isfahan University of Medical Sciences (Reference Number: 492044). Laboratory animals were kept in a 12-h light/dark cycle at a temperature of 22-24˚C and a relative humidity of 50±5% (in the Veterinary Clinic of DVM Shahriyar Adibi, Isfahan, Iran). The rats' food and water was controlled (commercial rat chow) and they were protected from possible stress. Adult male Wistar rats with an average weight of 250-300g were randomly divided into 6 groups (8 rats per group) as follows: experimental group (1): ran on the treadmill with an intensity of 35%-40%VO2max over 14 days; experimental group (2): 40mg/kgbw IONPs injected seven times (every other day), each time anesthetizing them with ether; experimental group (3): 40mg/kgbw IONPs injected seven times every other day, each time being anesthetized with ether, and ran on the treadmill during the study's 14 days with an intensity of 35%-40%VO2max; placebo group)4(: injected 7 times with distilled water every other day each time with anesthesia; placebo group (5): ran on treadmill with an intensity of 35%-40%VO2max during the 14 days being anesthetized with ether 7 times (every other day). The control group had the same environmental experimental conditions but without the exercise, injection, or anesthesia.

Intratracheal instillation method

The method used in this study was intratracheal instillation. This method was applied to examine the toxic effects of inhaled NPs and its advantage was receiving the full injection dose in the host's respiratory tract [29, 30] and [31]. In intratracheal instillation, for every injection, a 0.1 ml dose of 100mg/ml distilled water and IONP suspension solution was injected into the rat's trachea. The required dosage was 40mg/kgbw. Administration was repeated 7 times every other day during a 14 day period. At each injection, the rats were anesthetized by inhalation of ether and weighed.

Training protocol

Male Wistar rats were trained to do low-intensity aerobic exercise in accordance with the protocol [32]. With an intensity of approximately 35%-40%VO2max, they ran on a motor-driven treadmill designed for rats (MTM-5720, Proteus, Taiwan) for 30 minutes each day for 2 weeks. The exercise sequence was as follows: exercise for 5 minutes at a speed of 6m/min, exercise for 5 minutes at a speed of 9 m/min and exercise for 20 minutes at a speed of 12 m/min with a running intensity of 35%-40%VO2max. To eliminate the exercise machine as a factor, both placebo groups and the experimental group (2) were placed on a nonoperational treadmill for 30 minutes per day for two weeks. In order to reduce stress in the animals and to make them familiar with the conditions of the exercise, the running groups had a pre-adaptation period of one week during which they ran 15 minutes a day on a treadmill at a speed of 9m/min.

Histopathology examination and Enzymology method

Twenty-four hours after the last exercise session, the animals were sacrificed according to animal protection regulations, their blood samples were centrifuged at 3000rpm for 15 minutes, and the serum was given to biochemical auto analyzer (Hitachi-717, Germany-Japan) to measure the amount of enzymes (ALT, AST, ALP). Liver enzymes were measured by using enzymatic kits (Greiner, Germany) with IFCC method. Statistical analyses were performed using SPSS, results were analyzed using the T-test, one-way Anova, and then means were compared with the Duncan test. The level of significance was set at $p<0.05$. For histopathology examination, the liver was removed and the tissue sections were fixed in 10% formalin for hematoxylin-eosinophil staining. Hepatic tissue sections were examined by light microscope.

Results

Evaluation of serum levels of ALT, AST and ALP

In the experimental group (1) exercise affected and increased the levels of ALT; in the experimental groups (2) and (3) injection of pulmonary IONPs reduced ALT levels to a meaningful degree. Furthermore, results of the Duncan test indicated that in experimental group (3), IONPs reduced ALT independently, but the effect of exercise intervention depressed the effect of NPs on enzyme ALT, and experimental group 3 ranked slightly better than experimental group 2. This result indicates that the training intensity in group 3 was affected by inhalation of IONPs during the two week period ($P<0.05$). Results are shown of the Duncan test are shown in table (3). Evaluation of serum levels of AST showed no significant differences in any of the groups ($P<0.05$). Results are in table (2). No significant difference of serum levels of ALP were seen in experimental group 1. Serum levels of ALP decreased in experimental groups 2 and 3, and so both groups had low level Duncan test values in the same category ($P<0.05$).

Histopathological Evaluation

Microscope slides prepared from liver tissue showed that, compared with the control group, the experimental group (1) had no change in hepatic cells. In the experimental group (2) and experimental group (3), inflammation and mononuclear cell infiltration in centrilobular hepatocytes and also increased inflammation and spotty mononuclear cell infiltration in liver parenchyma that underlie necrotic spots was observed. There was no difference in the severity of injury between these two groups. In the experimental group (2) and experimental group (3), centrilobular venous congestion and centrilobular sinusoid congestion was observed, but the intensity of congestion in the experimental group (2) was more than that of the experimental group (3). In the experimental group (2), ballooning degeneration in preportal hepatocytes was observed, but in the experimental group (3) this disorder were not seen. The structure of hepatic cells in group 3 was far healthier and had fewer tissue abnormalities than the experimental group (2), and macrophage cell density was reduced in this group as well, so the severity of the lesions was less than in experimental group (2).

Discussion

Most studies in nanotoxicology have emphasized the fact that NPs, based on their chemical composition, shape, size, surface chemistry, surface charge, and specific surface area, cause different effects and responses in different parts of the organism's body [33]. Previous research confirmed the movement of IONPs from the lungs to the liver and damage to liver tissue [34]. The results of this study showed that aerobic exercise could not prevent the decrease in serum levels of ALT and ALP in the experimental group (3). However, the statistical analysis indicates that exercise led to the greatest changes in the serum level of ALT enzyme, as the NPs had a suppressive effect on the enzyme and training had an increasing effect on it. However, if group 3 more closely approaches the control group, the NPs have a greater effect on the serum levels of this enzyme. Studies have shown that exercise has

effects on the peroxidation of fatty liver cells, overload on the liver cells, or hypoxic liver cells, and reduced energy supplies to increase the amount of enzyme ALT [35, 36 and 37] In addition, ALP is a marker enzyme for the plasma membrane reticoloendoplasmic system [38], so damaged membrane cells may affect ALP activity, and NPs have the potential to disable ALP or to prevent its removal. Especially low levels of serum of ALP are also an indicator of liver dysfunction [34]. On the other hand ALP enzyme is not produced by the muscles [24, 28 and 39]. In addition, stress in hepatocytes, followed by aerobic exercise of low intensity, cannot change the values of these enzymes originating in the liver. Hence, the reduction in the amount of the serum levels of this enzyme in the experimental groups (3) and (2) are in line with each other. The results of the present study do not show significant changes in serum AST levels in the experimental group (2) which therefore showed no severe damage to the hepatocytes and non-stimulated mitochondrial liver tissues, since had there been more severe damage in hepatocytes and damage to mitochondria, there would have been release of AST and the ratio of AST/ALT would have risen [40]. In fact, a large increase in the levels of mitochondrial AST causes a massive increase in liver necrosis [41], but in this study we did not see necrosis of liver tissue. Since the AST in liver cells, heart, muscle, leukocytes and erythrocytes exists [42], no significant changes in the experimental group (1) and experimental group (3) in this study indicates no effect of exercise on these cells. In terms of histology, in the current study, mononuclear cell infiltration in centrilobular hepatocytes, spotty mononuclear cell infiltration in liver parenchyma, centrilobular venous congestion, centrilobular sinusoid congestion, and ballooning degeneration in preportal hepatocytes were reported. Researchers claimed that the increased permeability of IONPs and accumulated NPs in tissues, proinflammatory factors, and macrophage oxidative stress are the main reasons for changes in liver tissue [34, 43 and 44]. But, as can be seen in the experimental group (3), the extent of damage to the liver is reduced, indicating that aerobic exercise reduces NPs' effects. One of the possible reasons is that, after exercise, we see nanoparticle effects on liver disorders, hepatic blood flow and reduced contact NPs with the cells of the liver. Exercise reduces blood flow to the visceral area, thus reducing the body's absorption of drugs in the gastrointestinal system [45]. Exercise also affects the amount of available medicine cell receptors which have a pronounced effect on the activity of drug kinetics [46]. Another mechanism that is a common topic in exercise physiology and toxicity of nanoparticles is the mechanism of oxidative stress on the cell surface and its rising with NPs and falling with aerobic exercise. In fact, aerobic exercise reduces the effect of nanoparticles in the experimental group 3 due to reduction in the level of oxidative stress. Studies have shown that the most important factor-induced hepatic toxicity of NPs is oxidative stress [46 and 47]. After 12 hours of induction of IONPs, an increase in the rate of superoxide dismutase (SOD) and decline in the glutathione peroxidase (GSH) levels in cells were observed [48]. This is in contrast to how gentle and regular exercise contributes to strengthen the ability of antioxidants to reduce oxidative damage and increase resistance to oxidative stress [49]. Regular exercise increases antioxidant enzymes, DNA repair proteins, and mitochondrial electron carrier proteins that regulate the production of ROS [21]. Also, after both endurance and aerobic exercise an increase in mitochondrial ROS hepatocytes has not been seen due to an increase in antioxidant defenses, such as increased levels of GSH and NQO-1 enzyme [50]. In fact short-term aerobic exercise can delay serum apoptosis factors by decreasing oxidative stress factors and increasing anti-oxidant defenses [51 and 52]. Submaximal exercises also strengthen cytochrome P450 as a crucial factor in removing xenobiotic factors from the liver [53] so as to increase the capacity of the cytochrome P450 enzymes in rats through increased mRNA synthesis [54]. The low-intensity aerobic exercise (35%-40%VO2max) used in the present study increases the antioxidant system in experimental group (3) and so the amount of tissue abnormalities compared to experimental group (2) is reduced. In summary, our findings suggest that low-intensity aerobic exercise (35%-40%VO2max) over a period of 14 days significantly affected hepatotoxicity induced by intratracheal instillation (7 times injected 40m/kgbw dose) and somewhat reduced toxic effects of IONPs. However, since aerobic exercise induces many physiological processes in the body, evaluation of the interaction between aerobic exercise and nanoparticles' effects on organisms' function needs further study.

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Conflicts of interest

All authors declare no conflicts of interest

Corresponding author:
Azadeh Vasili
MSC, Department of Exercise Physiology, Khorasgan (Isfahan) Branch, Islamic Azad University,
Isfahan, Iran.
Fax: (+98311) 2658569
E-mail: vasili.mgh@gmail.com

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