Accumulation of Iron Oxide Nanoparticle and Conventional Iron Oxide in Rat Ovary and Oxidative Stress Caused by It

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Abstract

Background: Iron is one of the most essential elements of life that plays a major role in structure of the most cells of body and is potentially toxic and dangerous, because it easily participates in oxidation-reduction reactions and produces reactive oxygen species, which leads to oxidative stress; nanomaterials such as iron nanoparticles found in environmental pollution and can also be dangerous.

Objectives: The current study aimed at evaluating and comparing the effects of accumulation of conventional iron oxide and iron oxide nanoparticles in rat ovary and its relationship with serum oxidative stress.

Methods: The current experimental study was conducted on 5 groups of female rats, control, iron oxide (15 mg/kg), and iron oxide nanoparticles (5, 15, and 45 mg/kg). All rats were treated intraperitoneally for 16 days. Then, they were euthanized and their ovarian tissue was removed, and iron accumulation in the ovaries was measured by atomic absorption. Malondialdehyde (MDA), carbonyl protein, thiol protein, and total antioxidant activity were also measured in rats serum samples.

Results: According to the current study findings, iron accumulation increased significantly ($P = 0.046$) in the group that received conventional iron oxide, as compared with the control group. Also, the mean of total antioxidant activity ($4.4 \pm 294.31 \mu\text{M/L}$) and thiol protein ($3.3 \pm 381.09 \mu\text{m/mL}$) showed a significant ($P \leq 0.05$) differences in group that received conventional iron oxide, compared with the other groups, MDA and protein carbonyl had no significant ($P \geq 0.05$) difference.

Conclusions: According to the findings, conventional iron oxide particle induced more accumulation and more oxidative stress than nanoparticles.

Keywords: Iron Oxide, Oxidative Stress, Ovary, Rat

1. Background

Iron is one of the most essential elements of life and plays a major role in the structure of most of the body cells. On the other hand, iron is potentially toxic and dangerous because it easily participates in oxidation-reduction reactions and produces reactive oxygen species, which leads to a phenomenon known as oxidative stress. It is believed that oxidative stress also leads to many pathological reactions (1). In adults, iron is stored in hepatocytes and tissue macrophages in response to the need for tissue storage and flows in response to the severe needs (2). Tissues can be exposed to iron and iron compounds through different ways including in hereditary diseases such as hemochromatosis or blood transfusions in lethal thalassemia and anemia, long-term hemodialysis, medical practices such as

stem cell therapy, tissue engineering, or imaging (3) as well as the presence of iron particles in water, food, and air (4). Free iron, due to the oxidizing ability as well as the ability of producing oxygen radicals through Haber-Weiss reaction and also because of its strong bond with proteins, leads to iron accumulation that is cytotoxic (5). The basic mechanism of pathogenicity of iron overload is the peroxidative damage of cell membrane lipids. High accumulation of iron oxide particles in the cell affects the physiology of the cell, cell cytoskeleton component, and the expression of genes and production of proteins, and ultimately, reduced cell proliferation and induced cell apoptosis (6). Nowadays, the oxidative stress is mainly evaluated by measuring the oxidized biological products in the body (7). Free radicals are usually produced daily in animals and

the body neutralizes and makes them safe through certain mechanisms; with increasing age, the ability to neutralize free radicals is gradually decreased because of undisclosed reasons, which leads to the irreversible cell damages (8). According to some studies, when the size of the particle is decreased, the surface to volume ratio is increased and its chemical and biological reactions are increased too. More chemical reaction of nanomaterials leads to increase of the generation of free radicals such as reactive oxygen species (ROS), which damage cell membranes and these radicals lead to inflammation and death of cells by increasing lipid peroxidation. ROS production is observed in the diverse range of nanomaterials including metal oxide nanoparticles (9, 10). Although several studies are done on the impact of iron oxide on different tissue and oxidative stress, limited resources are available to evaluate the impact of iron oxide and conventional iron nanoparticles on body tissue including reproductive organs. Iron is an essential metal for living organisms, but if accumulated in the body, it can be very toxic. Therefore, the current experimental study aimed at designing a biochemical approach to evaluate and compare the impact of iron oxide and iron oxide nanoparticles on oxidative stress markers; also quantitatively measuring the amount of iron accumulated in the ovarian tissue to find out whether the size of particle can influence the amount of accumulation in the ovarian tissue.

2. Methods

2.1. Animal Housing

The current experimental study was approved by the ethics committee Shahid Chamran University Ahvaz, Iran (code number: 1391.207). Female Wistar rats aged 10 to 14 weeks (adult reproductive age) and weighed 200 ± 20 g were purchased from the Center of Ahvaz University Production and Breeding Laboratory Animals, and then, transferred to the laboratory of the study center. All rats were maintained under controlled light (12:12 hour light/dark cycle) and room temperature ($25 \pm 2^\circ\text{C}$), during the study and were fed with commercially plated food with free access to water.

2.2. Animal Grouping

Animals were randomly divided into 5 equal groups ($n = 5$) as follows: The first group received no treatment and was kept under nutritional and environmental condition similar to those of the other groups. The second group received conventional iron oxide 15 mg/kg intraperitoneally (ip) for 16 days (10). The third group received iron oxide nanoparticles 5 mg/kg ip for 16 days (nano 5)

(4). The fourth group received iron oxide nanoparticles 15 mg/kg ip for 16 days (nano 15). The fifth group received iron oxide nanoparticles 45 mg/kg ip for 16 days (nano 45). Iron nanoparticles were generated in the department of physics at Shahid Chamran University and the size of the nanoscale iron particles was 40 nm in diameters based on the transmission electron microscopy (TEM) measurement.

2.3. Samples Collection

At the end of the experiment (after 16 days), the rats were anaesthetized with sodium thiopental (30 mg/kg) and sacrificed. Blood samples were collected from the heart using a 2-mL syringe without using anticoagulant agents and centrifuged at 3000 rpm for 10 minutes for serum separation. The ovaries of all animals in each group were collected. To access the ovaries, the abdominal skin was cut by the scalpel incision along the midline until the pubic bone and ovaries were removed after pushing the skin and appendages. The ovaries were then homogenized and existence as well as the level of iron accumulated in ovarian tissue was determined (11, 12) by the light absorption device, iron quantitative detection kits with photometric method and software designed by Pars Azmoon Iran company.

2.4. Serum Biochemical Measurement

In the current study, the amount of malondialdehyde (MDA), carbonyl protein, thiol protein, and total antioxidant activity was measured as the evaluation criteria for oxidative stress; all of the abovementioned chemicals were purchased from Merck company (Germany). MDA reacted with thiobarbituric acid in a test tube, produced a red complex and measured by spectrophotometry. The intensity of the color is proportional to MDA levels in serum and ultimately to the oxidative stress (13). During the process of protein oxidation, active aldehydes and ketones (resulting from oxidation of amino acids side chains) reacted with nitrophenyl hydrazine and resulted in the formation of hydrazine assessed photometrically (14). To measure thiol protein (G-SH) through the Elman indicator (5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), it reacted with reduced sulfhydryl groups (G-SH) and formed a colored complex measured by spectrophotometry at 412 nm (15). The measurement of total antioxidant activity was based on the antioxidant power of the sample in reducing the ferric ion to ferrous and creating the complex assessed by photometric method (6). The ferric reducing ability of plasma (FRAP) method was used to measure the total antioxidant activity. This test was based on the ability of plasma in reducing Fe^{3+} -TPTZ to TPFZFe^{2+} at pH 3.6 and 25°C

temperature and producing dark blue color measured by spectrophotometry. The calculations were carried out using the standard curve of $\text{FeSO}_4 \cdot \text{H}_2\text{O}$. Moreover, the average differences between oxidative stress parameters were analyzed statistically. All measurements were made by a trained observer.

2.5. Data Analysis

All the analyses were performed with SPSS version 16. Groups variances were analyzed by the one-way analysis of variation (ANOVA) and the Fisher least significant difference test (LSD) to evaluate significant differences between the groups. A P value of ≤ 0.05 was considered statistically significant.

3. Results

In the current study, various concentrations of iron oxide were injected into the rats for 16 days, and then, the ovaries removed to evaluate the tissue iron concentration; ovaries were digested and iron levels were measured using a Perkin Elmer AAnalyst 700 atomic absorption spectrometer. There was a significant ($P \leq 0.05$) increase in the accumulation of ovary iron in animals receiving conventional iron oxide compared with the ones in the control group ($P = 0.046$). Also there was a significant ($P \leq 0.05$) increase in the accumulation of conventional iron oxide compared with the iron nanoparticles (15 mg/kg) ($P = 0.014$), but there was no significant difference between iron accumulation in animals receiving nanoparticles 5 and 45 mg/kg and the others ($P \geq 0.05$). The amount of accumulated iron in the ovary tissue is shown in Figure 1. Statistical analysis of the left and right ovary weights between the groups ($X \pm \text{SEM}$) showed a reduction in the weight of ovaries in all interventional groups compared with those of the control group ($P \leq 0.05$), but there was no significant difference between the weight of ovaries in animals receiving iron nanoparticles in different doses and animals receiving conventional iron oxide ($P \geq 0.05$) (Figure 2). Also, there was a reduction in the volume of ovaries, in all groups compared with those of the control group ($P \leq 0.05$), but there was no significant difference in the volume of ovaries between animals receiving iron nanoparticles in different doses and animals receiving conventional iron oxide ($P \geq 0.05$). Statistical analysis of the left and right ovary volume between different groups ($X \pm \text{SEM}$) is shown in Figure 3. Total antioxidant activity in the group receiving conventional iron oxide showed significant differences ($P \leq 0.05$) compared with those of the control group and groups receiving iron oxide nanoparticles ($P \leq 0.05$) (Table 1). Changes of serum MDA, despite the increase

in the group receiving conventional iron oxide, had no significant difference among other the study groups ($P \geq 0.05$) (Table 1). The average amount of thiol protein in the group receiving conventional iron oxide had significant difference with those of all other groups and decreased ($P \leq 0.05$) (Table 1). Changes of protein carbonyl had no significant difference in any of the groups ($P \geq 0.05$) (Table 1).

Figure 1. Mean $\pm$ SEM Iron Accumulated in the Ovary ($P \leq 0.05$)

Figure 2. Mean $\pm$ SEM Weight of the Left and Right Ovary (g) in the Study Groups ($P \leq 0.05$)

4. Discussion

The findings of the current study demonstrated the accumulation of iron in the ovary of all groups. Iron is the most abundant metal in the body and the structure of cells and establishes a strong bond with proteins leading to the accumulation of iron in tissue such as ovaries, but in the current study, the group treated with conventional iron oxide showed more iron overload than the other groups, perhaps because they are larger in size compared with iron

Table 1. Mean $\pm$ SEM of Oxidative Stress Parameters in the Study Groups^a

Groups	Index			
	Total Anti-Oxidant Activity $\mu\text{M/L}$	Malondialdehyde, μM	Protein Thiol, $\mu\text{m/mL}$	Protein Carbonyl, $\mu\text{m/mL}$
(a) Control	411.53b $\pm$ 5.8	2.61 $\pm$ 0.22	687.66bce $\pm$ 2.1	15.43 $\pm$ 0.58
(b) Iron oxide	294.31acd $\pm$ 4.4	5.44 $\pm$ 2.81	381.09ade $\pm$ 3.3	14.06 $\pm$ 1.13
(c) Nano 5 mg	383.72db $\pm$ 7	3.42 $\pm$ 0.2	465.91abd $\pm$ 3.1	15.97 $\pm$ 0.95
(d) Nano 15 mg	446.43bc $\pm$ 6.1	2.34 $\pm$ 0.25	404.19ae $\pm$ 2.1	14.29 $\pm$ 0.55
(e) Nano 45 mg	432.55b $\pm$ 4.3	3.22 $\pm$ 0.26	516.43abd $\pm$ 5.1	13.68 $\pm$ 1.58

^aThe dissimilar letters indicate significant differences between the groups ($P \leq 0.05$).

Figure 3. Mean $\pm$ SEM Volume of the Left and Right Ovary (mm³) in the Study Groups ($P \leq 0.05$)

nanoparticles, and then, due to larger size had more accumulation. Also, the current study analyses showed a reduction in the weight and volume of ovaries in all groups compared with those of the control group; Soenen et al. (3) and Morovvati et al. (16) showed that high intracellular iron oxide particles affect cell physiology and high levels of iron oxide cores affect the actin cytoskeleton, focal adhesion kinase formation, level of protein production, it can reduce cell proliferation, and induce apoptosis. Due to iron overload in ovaries, follicles grow old and shrink and then ovaries grow small and atrophy (17), which was in agreement with the results of the current study. According to the presented evidence, the formation of reactive oxygen species by iron particles caused oxidative stress that eventually had a significant negative impact on fertility and health of gametes (18). In general, there were fewer articles about the negative effects of iron oxide nanoparticles, in contrast to those of the conventional iron oxide, but the findings of the current study showed, considering similar articles, the increased oxidative stress; meanwhile, further studies are needed. Based on the current study findings, the cell damage caused by iron oxide particles was both

dose- and time-dependent. The results of the above studies were somewhat consistent with those of the current study. However, since iron oxide nanoparticles are smaller and due to their probable faster removal through the urine, their toxicity is different in tissue. Iron oxide nanoparticles can stimulate oxidative stress and cell dies after 24 hours, but despite the fact that they are immediately removed and disposed, their impact on various tissue is different (19, 20). Iron oxide nanoparticles were intraperitoneally injected into mice at different doses and it was found that the accumulation of iron oxide nanoparticles and oxidative stress increased (21). Moreover, Hoskins et al., showed that oxygen free radicals generated by iron nanoparticles increased, which ultimately led to cell death. It was expressed that iron oxide nanoparticles enter into lysosomes and by breaking down the membranes of lysosomes release from enzymes that damaged the cells (22). In addition to the changes listed for various oxidative stress factors, protein carbonyl index showed no statistically significant difference in any of the groups that was probably due to changes in the factors of chronic cases, or the other reasons associated with the creation of the intermediate elements such as Cu and Fe; and its impact on the circulatory system proteins and carbonyl group creation was very low. Researchers used different methods to assess the entire antioxidant capacity and antioxidant activity (TAA) in biological samples. The current study demonstrated that conventional iron oxide can induce more oxidative stress than nanoparticles, which was similar to a recent research done by Razi et al. (23). In the current study, there was a significant decrease in TAA in the group that received conventional iron oxide. MDA is produced via lipid peroxidation and can react with amine groups of proteins. Here, MDA is a byproduct and is directly related to the amount of damage caused by lipid peroxidation. MDA values are used as a useful indicator to assess oxidative stress, MDA in the current study showed an increase in the group that received conventional iron oxide, but the increase was not signifi-

cant with the protein carbonyl, the other index of oxidative stress; the data were according to those of the study by Doba et al. (24, 25). There are other methods to evaluate oxidative stress through the evaluation of protein oxidation such as measuring plasma thiol; thiol groups reduce due to oxidative stress and the group that received conventional iron oxide showed significant decrease in this regard. At the end, there is a great variety of mechanisms of oxidative stress due to iron overload, as it easily participates in oxidation- reduction reactions and produces reactive oxygen species that finally leads to oxidative stress. Rezaizadeh et al. reported that iron nanoparticles had antioxidant properties, which was contrary to the current study results (26). Conversion of the superoxide anion and hydrogen peroxide to active hydroxyl free radicals during the Haber-Weiss reaction leads to toxic effects and damage to the cell membranes, proteins, and DNA, which depends on the iron (8). In general, it seems that iron oxide nanoparticles has less destructive effects than the conventional sizes, and thus causes less oxidative stress. In fact, the current study suggested that extensive studies should be performed to determine the safe concentrations of such particles. The limitation of the study was that the additional oxidative markers were also measured; and the strong point of the study was using nanoparticle of iron oxide in 3 doses to obtain more complete results.

4.1. Conclusions

In conclusion, the results of the current study demonstrated that conventional iron oxide can accumulate and increase cell death more than nanoparticles. This can negatively affect the fertility of female rats. However, there are many questions about to the ways that nanoparticles enter, transmit, or excrete the organs.

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