The study of the expression of Bax and Bcl-2 in liver and kidney rats after chronic administration of different doses of iron oxide nanoparticles

Sanaz Homayoun Hamedani[1], Lila pishkar[2*], Mojtaba Falahati[3]

1. Department of Biology, Islamshahr Branch, Islamic Azad University, Islamshahr, iran
2. Department of Biology, Islamshahr Branch, Islamic Azad University, Islamshahr, iran
3. Department of Nanotechnology, Faculty of Advance Science and Technology, Islamic Azad University of Pharmaceutical Sciences(IAUPS),Tehran, Iran

Abstract: With the arrival of nanotechnology in the medical field and increasing the use of these compounds in the treatment and diagnosis of diseases whole, recognizing the interaction of nanoparticles with different organisms is crucial. Iron oxide nanoparticles are widely used in the field of diagnosis and treatment of many behalf. Many studies, including iron nanoparticles oxide nanoparticles on healthy, low-power-induced toxicity introduce body, but a careful examination of the molecular toxicity of these nanoparticles on the liver and head as the most important organs are interacting with iron oxide nanoparticles required Is. In this study, 40 male rats Wistar divided into 4 groups of study as control group, animals treated with 10 mg / kg particles of iron oxide for a week, the animals treated with 20 mg / kg of iron nanoparticles oxide for a week and the animals treated with 40 mg / kg of iron oxide nanoparticles were used for a week. The liver and kidneys of animals at day 7 after initiation of injection were extracted under sterile conditions and then the expression of Bax and Bcl-2 genes using real time-PCR in the tissues were measured. More than a dose of 5 mg (PV <0.05), respectively. Bax gene expression also increased at all doses 20 and 40 mg (P-V <0.01) significantly more of the dose of 5 mg (P-V <0.05), respectively. The results of this study suggest that the chronic use of high doses of iron nanoparticles induced cell death process in the liver and all living beings possess.
Keywords: Chronic, iron oxide nanoparticles, Bax and Bci-2, hepatic and renal toxicity, rat

INTRODUCTION

Using nanoparticles in biotechnology, materials science and biology are incorporated. The unique properties of nanoparticles has made them widely used in medical applications (1). Biological molecules such as proteins, nucleic acids similar in size to the nanoparticles, as well as some of their behavioral characteristics such as magnetic behavior or fluorescence due to the increasing use of medical and biological sciences is (2,3).

Magnetic nanoparticles range in the field of biomedical applications because chemical levels are correct. When nanoparticles are Enter blood. Their interactions with blood proteins, so it is inevitable to reduce these interactions iron oxide nanoparticles are coated with specific molecules to biological interactions . On the other hand biocompatibility obtain optimal physiological environment. Magnetic nanoparticles in the body of the organism also has a variety of applications including drug carriers, agents in MRI imaging agent is induced blood clot (6-4). To date iron oxide due to biological and chemical stability of the production process is relatively simple efforts to implement the nanoparticles of magnetite (Fe_3O_4) and Magmyt, (γ-Fe_2O_3) in medicine has attracted the most attention. However, there are still numerous questions in relation to these nanoparticles, including magnetic is adequate? These substances are toxic if administered dose? Is there coverage? The interaction of particles coated with bodily fluids, biomolecules or cells? What this combination of materials can induce cell death in mammalian tissues of the body including the liver, kidneys and brain are responsible?

Naqvi and his colleagues murine macrophage cell line during treatment with iron oxide nanoparticles Super Magnet observed that long-term use of low doses of iron oxide nanoparticles (200- 25 µg / ml) as well as short-term treatment (6 hours) of high doses the nanoparticles (500-300 µg / ml) did not apply oxidative stress and cytotoxicity (7). However, they pointed out that the use of low doses of iron oxide nanoparticles reliability will be more a lack of cytotoxicity in advance. Mueller and his colleagues also In vitro study on the effects of the toxicity of nanoparticles of iron oxide (Ferumoxtran-10) with doses of 1 mg / ml and 10 mg / ml of the drug acute toxicity with human leukocytes were not significant, they also observed that no inflammatory cytokine activity and not by the nanoparticles cells (8).

The results of the study showed that the use of iron oxide nanoparticles in order to design and manufacture of drugs, not only in terms of the toxicity of the drugs are safe but also inflammatory responses and cytokine-induced leukocyte observed. In vitro study phase, however they had been lack of similarity between the terms of In vitro and

physiological conditions such as major problem was studied. Baratyl colleagues examined mitochondrial toxicity in a study of iron oxide nanoparticles on the mitochondria of the heart, lung, liver, kidney and brain observed that the presence of doses of 100, 200, 300 and 500 µg / ml Fe3O4 with no tissue mitochondria respiratory chain dysfunction and induces mitochondrial oxidative stress in mitochondria were observed. Find them too, like enjoying the view Naqvi and Muller noted that iron oxide nanoparticles can function with minimal cytotoxicity to cells exert their physiological (9)

In a study to evaluate the pharmacokinetics and toxicity colleagues Vzyldr iron oxide nanoparticles of iron oxide nanoparticles observed that low dose (mg / kg1) to rats in addition to any animals had blood tissue toxicity. They also fit the pattern either by bonds methods of prescribing and taking these nanoparticles observed that the half-life of about 3 hours is the nanoparticles in vitro models (10)

However, the problem was the lack of toxicity of these nanoparticles at high doses, because they need to evaluate the pharmacokinetics of iron oxide nanoparticles utilizes a higher dose safe without any toxicity, tissue and blood, to changes in blood serum concentration of the nanoparticles to different time periods animals review.

Although the various studies ever conducted to examine the cytotoxicity of nanoparticles tissue iron oxide (Fe2O3) In vivo and In vitro conditions, but have done any comprehensive study to predict the toxicity of this substance at higher doses and serial is required. Considering the above studies aim of this study can be used to predict comprehensive and widespread apoptosis and toxicity of the liver, kidneys and brain nanoparticles of iron oxide (Fe2O3) in chronic phase.

MATERIALS AND WAYS

Iron oxide nanoparticles and nanoparticle suspension Fe2O3 iron oxide nanoparticles in a size around 20nm prepared for the company Sigma-Aldrich (747424-1Sigma Aldrich CO: ML) was purchased. In order to prepare a suspension of nanoparticles Fe2O3, the amount of 100mg Aznanvzrh Fe2O3 with the digital scale laboratory (AND MODEL EJ303) Vznkrdhv in 10 cc of saline at a temperature of -35 to 40 degrees Celsius solution for 5 minutes to prepare a suspension in the vortex placed there.

Animals and grouping study

A study on 40 adult albino race Lbynvvystar adult male (6-8 weeks) with a weight range of 200-250 g were obtained from Pasteur Institute of Iran. Animal animals in standard laboratory conditions with 12 hours of light and 12 hours dark cycle in a temperature range of 22-24 ° C were studied in compliance with all ethical principles. Animals then transferred to laboratory animals, in order to acclimatize to the conditions the animals were kept home without intervention. The animals in the study were randomly divided into four groups of studies are: 1) control group consisted of 10 rats healthy, 2) dose of 10 mg / kg: including 10 rats that Badvz 10 mg / kg daily for 1 week intraperitoneally were treated, 3) group dose of 20 mg / kg: including 10 rats with a dose of 20 mg / kg daily for 1 week intraperitoneally were treated and 4) a dose of 40 mg / kg: including 10 rats with a dose of 40 mg / kg daily for 1 week were treated intraperitoneally, were grouped.

Grooming

Fe2O3 nanoparticles animals intraperitoneally (Intraperitoneal, IP) was transferred to the animals (11). Animal control group consisted of healthy rats (Health) that for one week using a 1 cc of normal saline (solvent nanoparticles) were treated for IP. Animal Groups 3, 4 and 5 were animals that with the dosage of 10 mg / kg, 20 mg / kg and 40 mg / kg using Fe2O3 nanoparticles for Injection IP were treated for 1 week.

Anesthesia and get samples of liver and kidney

In order to induce anesthesia drug combinations xylazin (now alfasan) Baghlzt 20 mg / ml Vktamyn (now ROTEXMEDICA) at a concentration of 50 mg / m was used (12). For this purpose, the combined ratio of 1: 5 (5 ml + 1 ml ketamine xylazine) was prepared as stocks and the amount of 110 mg / kg based on the weight of each animal they intraperitoneally (IP) was injected. Then the abdominal hair was shaved animals and using the iodine was completely disinfected. In order to prevent contamination of animal tissue into the hood laminar class 2 (model KG-A100) were transferred after opening the abdomen animals lobe great all the animals and all the All the animals were removed and cryotherapy tubes were placed was frozen in liquid nitrogen. Then -80 ° C freezer samples to extract RNA and cDNA synthesis were transferred.

Total RNA and cDNA synthesis

Liver and kidney total RNA extraction kits animals using RNA (RocheCo, Germany) according to the instructions included in the kit were derived. cDNA synthesis kit using cDNA (fermentas CO, Lithuania) according to the instructions specified in order to perform real time- PCR kit were obtained and were transferred to the fridge -20 ° C.

Reaction Real-time- PCRSYBER Green

Real-time- PCR test by Cycler iQ5 (Bio-Red Co, USA) was used. Apoptotic genes Bax primer list and B-cl2 as well as gene -actinβ (reference gene) has been reported in Table 1. Real-time- PCR process in this study is a double glass tubes with a final volume of 25µl was 96. In the process of 12.5 µl of QuantiFast SYBR Green PCR Kit (Qiagen, Europe) with 9.5 µl of distilled water, 0.5µl of each primer at a concentration of 10 µ sweep with 2µl of cDNA were extracted.

Table 1: type, size and sequences of primers used in the study

Gene	Sequences		Size) bp(
Bax	Forward	CCAGGACGCATCCACCAAGAAGC	136
	Reverse	TGCCACACGGAAGAAGACCTCTCG	
Bcl-2	Forward	GGATGACTTCTCTCGTCGCTACCGT	118
	Reverse	CGAGTGAGGATGTGCATGAA	

Application response time and temperature

In order to carry out the reaction the reaction temperature was divided into three stages as described above: the first step is the denaturation temperature to the opening of two elderly cDNA molecule chains (95 ° C for 3 minutes). The second step for 35 seconds at 60 ° C Annealing temperature primers specific for Bax and Bcl-2 took place. And the third stage at a temperature of 72 ° C for 3 minutes was considered as an extension temperature. This process is distinct and consecutive cycles were performed in 40 (13).
In order to confirm amplification of specific target genes, the reaction product using 2% agarose gel electrophoresis were studied. In addition to outlining the melting curve (Melting curve) temperature of 50 to 99 ° C for 5 seconds at each iteration Santy¬Grad increased by 1 degree.

Standard curves and draw it

The reaction efficiency of PCR (PCR efficiency) were evaluated based on the standard curve. were used as controls. As well as the proliferation curve (Amplification Curve) were plotted for each response analysis compares data based on the threshold cycle (Ct Value) different groups and a control group were studied.

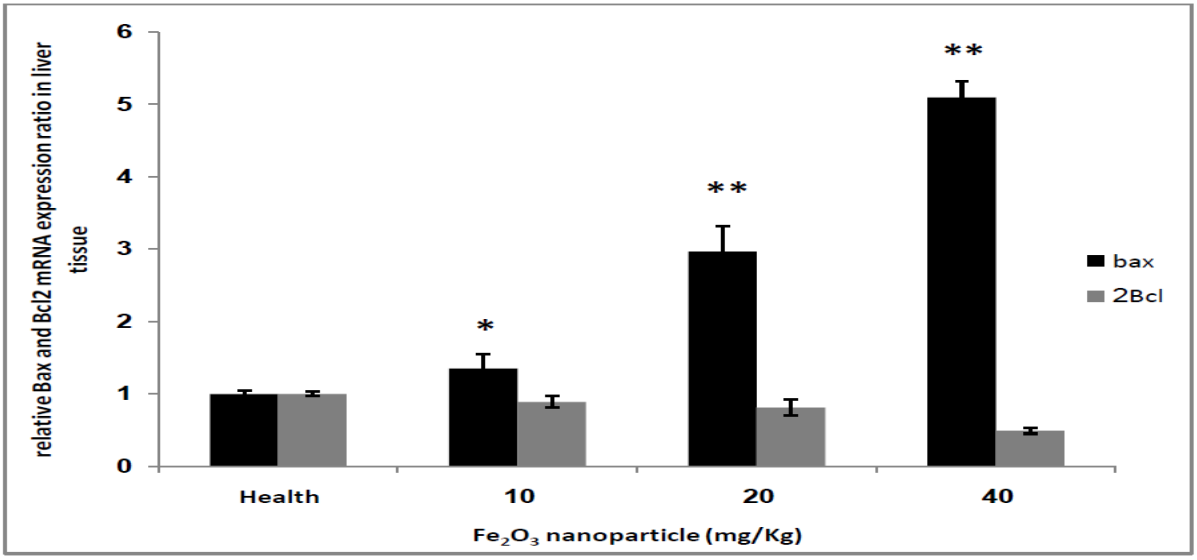

Figure 1: The expression of Bax and Bcl-2 Liver tissue samples using real time -PCR sex groups controlled studies (Health) Treatment with nanoparticles of iron oxide (Fe2O3 nanoparticles) with different concentrations. * Indicates <0.05 P-value, ** indicates <0.01 P-value is, only the group of animals treated with nanoparticles of iron oxide (Fe2O3 nanoparticles) compared to the control group.

Data analysis

Data were recorded in SPSS-16 software and the relative expression of genes by comparing the threshold cycles of samples of each group as 2 ^ ΔΔCT (Livak) as well as methods for data analysis Whitney- Mann was used (14). The level of less than 0.05 was considered significant.

Results

Fe2O3 nanoparticles effects on the expression of apoptotic genes in the liver of animals Also according to Figure 1 is specified using nanoparticles of iron oxide significantly in three doses of 10 mg / kg (PV <0.05), 20 mg / kg and 40 mg / kg (PV <0.01) than the expression of Bax who cause death inducer known to be increased in the liver of animals compared to controls. An increase in the expression of Bax to Bcl-2 gene increasing the dose increased dramatically go down so that the toxicity of nanoparticles in doses, 20 mg / kg and 40 mg / kg (PV <0.01) dramatically more than the dose 10 mg / kg (PV < 0.05).

Fe2O3 nanoparticles effects on the expression of apoptotic genes in renal tissue of animals

By Fig. 2, identified by the use of nanoparticles of iron oxide, both doses of 20 mg / kg and 40 mg / kg significantly (PV <0.01) than the level of gene expression of Bax who factor-induced apoptosis known in the context of all of the control group (Health) increase Is. Informants were also found at a dose of 10 mg / kg of Bax and Bcl-2 gene expression levels were not significantly increased.

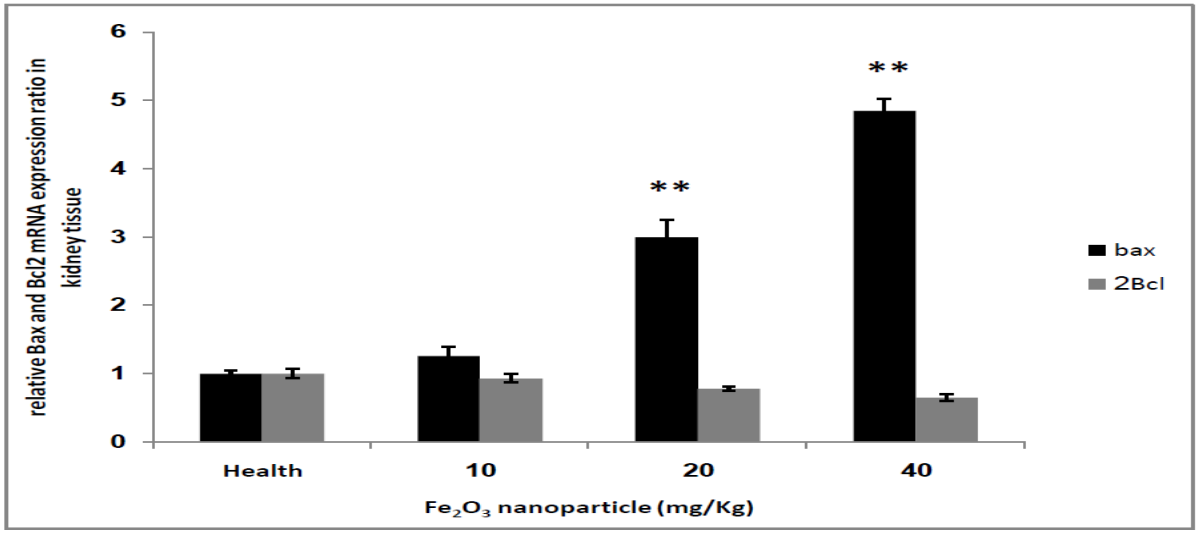

Figure 2: The ratio of Bax and Bcl-2 gene expression in kidney tissue samples using real time -PCR sex groups controlled studies (Health) and treated with nanoparticles of iron oxide (Fe2O3 nanoparticles) with different concentrations. * Indicates <0.05 P-value, ** indicates <0.01 P-value is, only the group of animals treated with nanoparticles of iron oxide (Fe2O3 nanoparticles) compared to the control group

Discussion and conclusion

The purpose of the study follows the apoptotic effects of chronic use of iron oxide nanoparticles on the liver and kidneys in rats. The results mapping exercise molecular methods Real-Time PCR on samples of liver tissue on the expression levels of genes involved in apoptosis showed that the ratio of how much of global gene expression of apoptotic Bax on genes anti-apoptotic Bcl-2 were significantly higher in all three treatment groups with nanoparticles compared to the control group pleasant whose value P group, 10 mg / kg of P <0.05 and another two against P <0.01, respectively (Figure 1).

The results of molecular investigations also confirm the lack of renal toxicity of Fe2O3 nanoparticles at a dose of 10 mg / kg, respectively. Accordingly, it was noticed that the ratio of the expression of apoptotic Bax compared to protein anti-apoptotic Bcl-2 Groups of 20 mg / kg and 40 mg / kg significantly compared to the control group has changed (P <0.05), while the proportion of animals Group 10 mg / kg was seen (Figure 2)

At present, the development of nanotechnology increasing the productive use of these technologies at the global level, due to the application of the materials used in cancer treatment (15), reduction of microbial infections of the skin and burns, as well as to prevent the accumulation of bacteria from the surface of various tools such as prostheses, were used (16). In this regard, Fe2O3 nanoparticles frequency applications to biomedical sciences have themselves. Therefore, evaluating the toxicity of these nanoparticles has been done (17-19). Fe2O3 nanoparticles

results of the study showed that doses above 10 mg / kg and higher than the chronic form of nanoparticles with high potential to induce apoptosis in liver and kidney extension. So in summary, the present study indicated that keeps utilizes nanoparticles to treat or diagnose diseases on an ongoing basis (chronic) high potential to cause damage to some organs such as the kidneys and liver have shown. Therefore recommends that an extensive study to find safe doses without the least capable of inducing cell death in various tissues of Fe2O3 nanoparticles to be done.

References

Alizadeh, Ali Mohammad, Majid Sadeghizadeh, Farhood Najafi, Sussan K. Ardestani, Vahid Erfani-Moghadam, Mahmood Khaniki, Arezou Rezaei et al. "Encapsulation of curcumin in diblock copolymer micelles for cancer therapy." BioMed research international 2015 (2015).

Baratli, Yosra, Anne-Laure Charles, Valérie Wolff, Lotfi Ben Tahar, Leila Smiri, Jamal Bouitbir, Joffrey Zoll et al. "Impact of iron oxide nanoparticles on brain, heart, lung, liver and kidneys mitochondrial respiratory chain complexes activities and coupling." Toxicology in Vitro 27, no. 8 (2013): 2142-2148

Barkalina N, Charalambous C, Jones C, Coward K. Nanotechnology in reproductive medicine: emerging applications of nanomaterials. Nanomedicine: Nanotechnology, Biology and Medicine. 2014 Jul 31;10(5):e921-38.

Boisseau P, Loubaton B. Nanomedicine, nanotechnology in medicine. Comptes Rendus Physique. 2011 Sep 30;12(7):620-36.

Harrison, Benjamin S., and Anthony Atala. "Carbon nanotube applications for tissue engineering." Biomaterials 28, no. 2 (2007): 344-353.

Isanejad, Amin, Ali Mohammad Alizadeh, Sadegh Amani Shalamzari, Hamid Khodayari, Saeed Khodayari, Vahid Khori, and Najmeh Khojastehnjad. "MicroRNA-206, let-7a and microRNA-21 pathways involved in the anti-angiogenesis effects of the interval exercise training and hormone therapy in breast cancer." Life sciences 151 (2016): 30-40.

Jain, R. K., and T. Stylianopoulos. "Delivering nanomedicine to solid tumors. Nature reviews Clinical oncology. 2010 Nov; 7 (11): 653–64."

Jurgons, R., C. Seliger, A. Hilpert, L. Trahms, S. Odenbach, and C. Alexiou. "Drug loaded magnetic nanoparticles for cancer therapy." Journal of Physics: Condensed Matter 18, no. 38 (2006): S2893.

Khori, Vahid, Sadegh Amani Shalamzari, Amin Isanejad, Ali Mohammad Alizadeh, Shaban Alizadeh, Saeed Khodayari, Hamid Khodayari et al. "Effects of exercise training together with tamoxifen in reducing mammary tumor burden in mice: Possible underlying pathway of miR-21." European journal of pharmacology 765 (2015): 179-187.

Mody VV, Cox A, Shah S, Singh A, Bevins W, Parihar H. Magnetic nanoparticle drug delivery systems for targeting tumor. Applied Nanoscience. 2014 Apr 1;4(4):385-92.

Mohsenikia, Maryam, Ali Mohammad Alizadeh, Saeed Khodayari, Hamid Khodayari, Aliasghar Karimi, Mina Zamani, Saleh Azizian, and Mohammad Ali Mohagheghi. "The protective and therapeutic effects of alpha-solanine on mice breast cancer." European journal of pharmacology 718, no. 1 (2013): 1-9.

Müller, Karin, Jeremy N. Skepper, Mihaly Posfai, Rikin Trivedi, Simon Howarth, Claire Corot, Eric Lancelot, Paul W. Thompson, Andrew P. Brown, and Jonathan H. Gillard. "Effect of ultrasmall superparamagnetic iron oxide nanoparticles (Ferumoxtran-10) on human monocyte-macrophages in vitro." Biomaterials 28, no. 9 (2007): 1629-1642

Murase K. Development of a method for measuring blood coagulation using superparamagnetic iron oxide nanoparticles and an alternating magnetic field. arXiv preprint arXiv:1605.00255. 2016 May 1.

Naqvi S, Mohammad Samim, M. Z. Abdin, Farhan Jalees Ahmed, A. N. Maitra, C. K. Prashant, and Amit KD. "Concentration-dependent toxicity of iron oxide nanoparticles mediated by increased oxidative stress." International journal of nanomedicine 5 (2010): 983-995.

Pavlovic M, Mayfield J, Balint B. Nanotechnology and Its Application in Medicine. InHandbook of Medical and Healthcare Technologies 2013 (pp. 181-205). Springer New York.

Shin TH, Choi Y, Kim S, Cheon J. Recent advances in magnetic nanoparticle-based multi-modal imaging. Chemical Society Reviews. 2015;44(14):4501-16.

Shiri, Sadaf, Ali Mohammad Alizadeh, Behzad Baradaran, Baharak Farhanghi, Dariush Shanehbandi, Saeed Khodayari, Hamid Khodayari, and Abbas Tavassoli. "Dendrosomal curcumin suppresses metastatic breast cancer in mice by changing m1/m2 macrophage balance in the tumor microenvironment." Asian Pacific journal of cancer prevention: APJCP 16, no. 9 (2014): 3917-3922.

Tan, Aaron, Jayakumar Rajadas, and Alexander M. Seifalian. "Exosomes as nano-theranostic delivery platforms for gene therapy." Advanced drug delivery reviews 65, no. 3 (2013): 357-367

Weissleder, Ret al, D. D. Stark, B. L. Engelstad, B. R. Bacon, C. C. Compton, D. L. White, P. Jacobs, and J. Lewis. "Superparamagnetic iron oxide: pharmacokinetics and toxicity." American Journal of Roentgenology 152, no. 1 (1989): 167-17.