International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **3(7),** 1-5, July **(2014)** *Int. Res. J. Biological Sci.*

The effects of Pulmonary administration of Fe_2O_3 Nanoparticles on the Lung Tissue in Wistar Rat

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Available online at: www.isca.in, www.isca.me
Received 6th August 2013, revised 20th February 2014, accepted 24th June 2014

Abstract

Iron oxide nanoparticles are magnetic nanoparticles have widespread application in MRI and heat therapy of cancer as contrast elements. They also used effectively to drug and gene delivery because of effective penetrating to the cells and tissues. However, these features cause Fe_2O_3 nanoparticles have toxic effects that not studied yet completely. In this study, effects of iron oxidenano particles on lung tissue in adult male wistar rats were studied. We used pulmonary inhalation method for nanoparticle administration and used ether as a helper. Our results showed administrated nanoparticles penetrated to the circulation rapidly and created serious inflammation in lung tissues. This study used two different nanoparticle doses (20 and 40 mg/kg) and two different exposing number (7 and 14 times).Histological studies show ednano particle treatment of rats cause pulmonary emphysema, interstitial hyperemia and inflammation in lung. By increasing the administrated dose lung tissue showed all of mentioned symptoms by increased intensity. Nanoparticle exposing causes presence of neutrophils, lymphocytesandeosinophils in the lung tissue that confirmed there is a serious pathologic condition.

Keywords: Fe_2O_3 nanoparticle, lung tissue, inflammation, pulmonary administration, immune system.

Introduction

Nanoparticles based on physical and chemical properties and special shape, size and surface area to volume ratio are unique for biological, medical and industrial applications. One of the most useful features of this is high surface area of nanoparticles that cause its widespread application in medical science and production of nano based drugs[1]. Magnetic nanoparticle is one of the useful nanoparticles that have above mentioned features and can be used widely in drug delivery, gene delivery and targeting[2]. But for mentioned application magnetic nanoparticle should be biocompatible and biodegradable. But published literatures showed material at the nano size has relatively greater toxicity rather than large sizes materials, because nanoparticles are highly reactive and cause oxidative stress in human and animals. Previous researches confirmed exposing to nanoparticles crate malignant brain damages in fishes[3]. Nanoparticles can pass through cell membrane easily and even pass through blood-brain barrier and blood-testes barrier [4], so it can affect all organs of the body[5]. Nanoparticles can enter the bloodstream and reach to the organs (including the brain, heart, kidneys) rapidly by blood circulation[5].

Nanoparticles between 10-100 nm size should be used for biological purpose because nanoparticles that are smaller than 10 nmexcrete by the kidneys and particles with larger size than 200 nm don't pass through the cell membrane easily and they can induced immune system asaforeign thing so removed from the body[6].

Biomedical applications of iron oxidemagnetic nanoparticles are widely than others due to biocompatibility, highstabilityandease of use. Magnetic nano particles such as Fe_3O_4 and Fe_2O_3 have more application in drug delivery[7,8]. Because of widespread application of these particles in various industries human exposing to these increased so investigation of nanoparticle role in cell growth and survival has more importance[9]. Human skin, lungs and digestive system are the most commonentry routes for nano particles and its pathogenicity[10]. Airborne nanoparticles have high mobility and canbeinhaledint other espiratory system easily[11].

Slow and direct injection of the test material into the lungs through the trachea used as an alternative to the normal ambient conditions in many studies. In this method we can sure all of nanoparticle that entered to the lung, absorbed completely[12].

This study investigated the effects of administrated gamma Fe_2O_3 nanoparticles at different dose (20 and 40 mg/kg) and different injection number (7 times injection in 14 day and 14 times in 28 day) on rats lung tissue.

Material and Methods

Nano iron oxide (Fe_2O_3) particles prepared from Pishgamane Nano Company (Iran-Mashhad). XRD results showed nano Fe_2O_3 is in crystalline phase with 20 nm size. Purification of nano Fe_2O_3 determined as 99.5 % by ICP-MS. Table 1 summarizes features of nano Fe_2O_3 used in present study. Not that properties of nanoparticle is very important in experimental

response and chemical or biological properties. XRD pattern also showed in figure 1.

Table-1
Present study, These features are more important in chemical and biological properties of Fe_2O_3 nanoparticle

Color	Brown
Morphology	Spherical
Crystallinephase	gama
Specificsurfacearea	40-80 M^2/g
Size	20 nm
purity	99.5 %

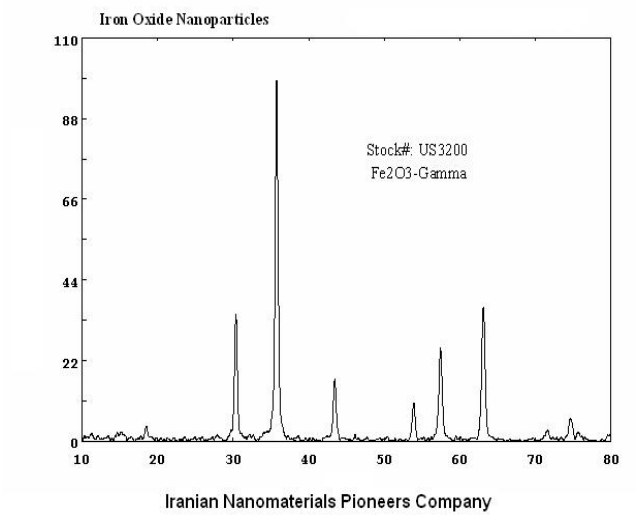


Figure-1
XRD pattern of used Fe_2O_3 nanoparticle

This study was conducted on experimental animals and we used adult male wistar rats weighting 300-250 g were estimated from the animal house of martyr portal was developed. Animals whit average age of 3-5.2 months selected. Testing carried out at temperature of 28-32 centigrade degree that day duration was 12 hours and 12 hours dark lighting. Municipal tap water was used adjusted drinking water and eating animals for food by rats (feed compression) that the company prepared feed was buying. Nanoparticle administration was done by inhalation with anestheticether. Experimental animals were randomly divided into six groups (8 rats in each group) as follows: fist control group feed by usual water and food. Second control group that referred to Placebo, administrated by 1 ml distillated water every other day by inhalation for equivalency of shock that obtained by inhalation of ether. Groups from 3rd and 4th injected by 1 ml Fe_2O_3 nanoparticles in 20 and 40 mg/kg doses respectively, administration repeated every other day. This continued until 7 times injection in 14 day.Other Groups from 5th and 6thinhaled by 1 ml Fe_2O_3 nanoparticles in 20 and 40 mg/kg doses respectively, administration repeated every other day by inhalation. This continued until 14 times injection in 28 day.

Administration technique: One of the important techniques to exposing the animals to nanoparticle is pulmonary administration or inhalation. Pulmonary administration widely used to evaluate the different materials effects in body[13]. This method also used to evaluation of toxicity potential of materials in air routes. In this method, penetration into the respiratory tract depends on the substance dose, particle shape and size and species of animals. In this method particles are breathing into the lungs without passing through the upper respiratory passages, so some of the defense mechanisms related to the upperrespiratory tract are removed[12,14]. One of the advantages of these methods is receiving whole of administrated dose without losing of this[14]. So we can sure administrated dose actually is receiving dose by host animal. Fluidity of administrated material is more important in this way due to choking possibility.

For histological studies, one day after last injection, rats anesthetized by diethyl ether and their lungs were removed and fixed by 10% natural formalin buffer. After tissue processing, the samples were blocked in cylindrical paraffin blockers and then stained by Hematoxilin- eosin [15]. It should be noted that the sample's diameter are 5-6 µm. Stained tissue samples studied by light microscope.

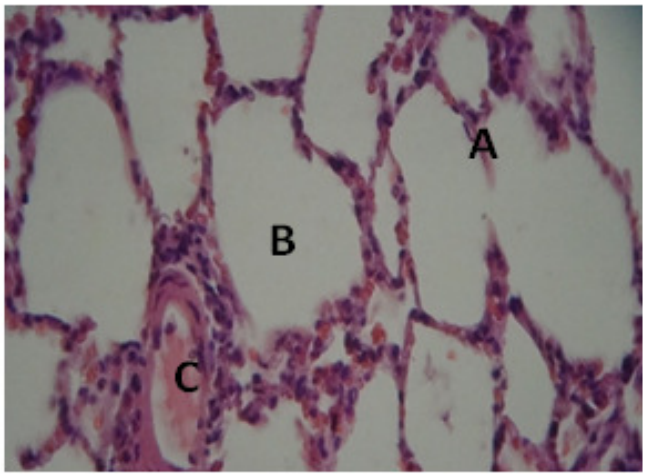
Results and Discussion

1 day after last injection and after ending the administration time course, rats killed and its lungs separated. Tissue samples treated by formalin for fixation and stained by Hematoxilin-eosin method and then studied by light microscope. Histological studies showed a hyperemia in alveolar wall capillaries with focal hemorrhage in this area (figure 2). In rats that treated by 40 mg/kg dose in 14 day hyperemia accompanied by bleeding in the lining air spaces and deposition of hemosiderin in parenchyma tissue of lung (figure 3). Histological changes observed after 7 times administration suggests that lung cell sex posed to nano particles have been irritated and the inflammation increased with increased duration to 28 days (14 time administration). Inflammation intensity depends onthedose and administration duration.

Increasing of experiment time duration to 28 day increased bleeding in the liningairspaces and hyperplasia oflymphoidfolliclesinlung. By increasing both of time duration and dose that occurred in 6th group (28 day and 40 mg/kg) bleeding in air space, losing of alveolar, changing of fat shapes, interstitial hyperemia and emphysemawas observedin lungtissue that presence of neutrophils, lymphocytesandeosinophils observed also. Presence of these immune cells indicates an abnormal and unhealthy condition. Therefore we don't observe this variation in control and placebo groups that they are healthy and untreated by nanoparticle (figure 1).

Nanoparticles have very specific chemical and physical characteristics of size, shape and high surface area to volume ratio that facilitate its medical and biological applications. This material distributed in all of body rapidly after injection by circulation and reached to the all of the organs and tissues[16].

2a



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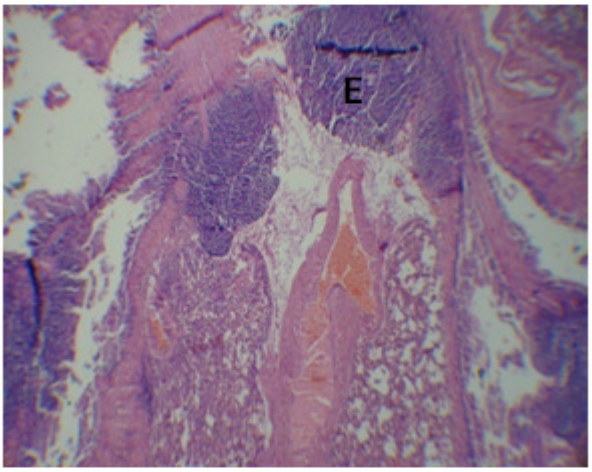

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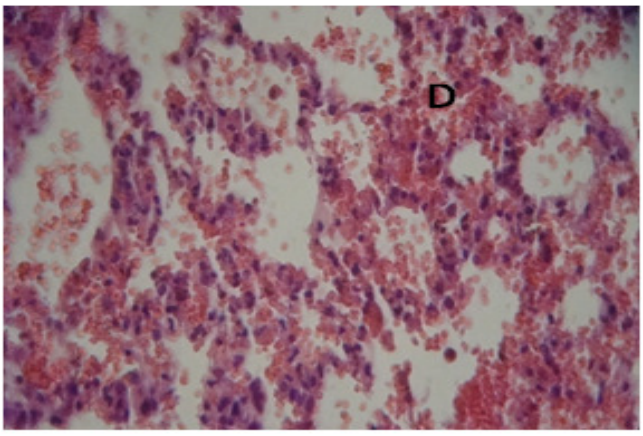

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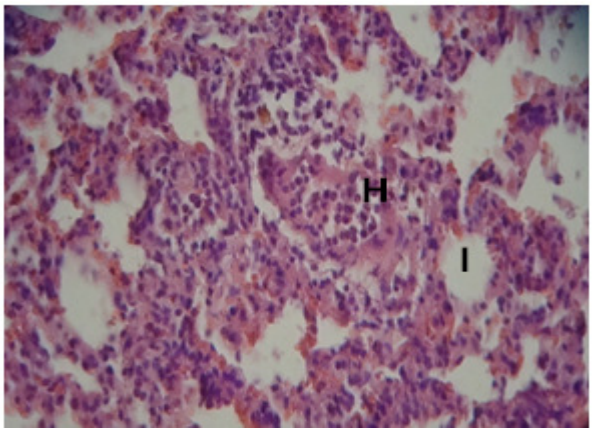

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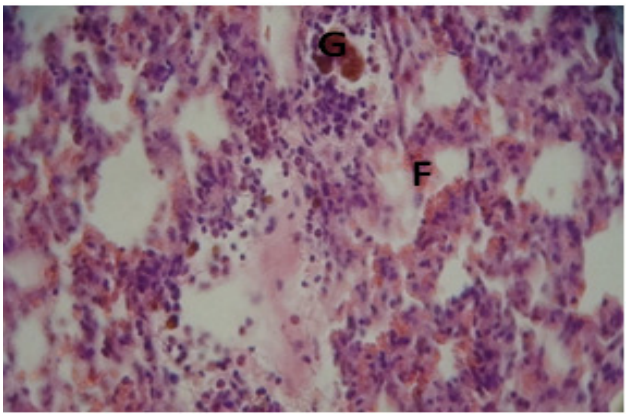

Figure-2

Light microscope studies from lung stained sample (×400). Figure 2a related to control and placebo rats, A: Alveoli, B: Pulmonary alveolar, C: Bronchioles. Figure 2b related to 3rd group that treated by 20 mg/kg and 14 days, D: Alveolar wall congestion. Figure 2c related to 4th group that treated by 40 mg/kg dose and 14 days, E: Follicular hyperplasia. Figure 2d related to 5th group that treated by 20 mg/kg dose and 28 days, F: Alveolar wall congestion, E: Sediment of hemosiderin. Figure 2e related to 6th group that treated by 40 mg/kg dose and 28 days, H: increasing of neutrophils, eosinophils and lymphocytes, I: Alveolar degeneration, the fatty degeneration.

Before its application as medicine equipment, effects of nanoparticle on environment, biocompatibility and its toxic effects on human and animals should be assessed. These particles because of small size have high surface area and they are highly reactive, it is one important reason for its toxic effects[17]. Fe_2O_3 nanoparticle due to optical, electrical and catalytic properties, have very important applications in various industries, including industrial targeting, carrier of gene delivery, bioremediation, air and water filtration and cancer treatment[18]. Therefor because of widespread use of Fe_2O_3 nanoparticle human and animal are exposing to this material[19]. Aim of our study is investigation of Fe_2O_3 exposing effects in lung tissue such as lung tissue appearance. Nano materials that are released into the environment through natural or artificial processes generally enter the body through there spiratory pathways[20] and the lung considered as the main entrance of the nano particles to the body[21]. Lung cancer is one of the bad effects of exposing to nanoparticles because they create mutation in cells that resulted to cell proliferation[12]. Present study showed lung tissue and lung cells engaged by nanoparticles because they try to remove particles and prevent them to crossing the blood circulation. By increasing nanoparticle dose or exposing time lung injuries increased and lung unable to refinement all of the nanoparticles that is due to special shape, size and ability to cross the filter. Previous studies confirm that nanoparticles can pass through cell membrane easily and even pass through blood-brain barrier and blood-testes barrier [4], so it can affect all organs of the body[22]. Nanoparticles can enter the bloodstream and reach to the organs (including the brain, heart, kidneys) rapidly by blood circulation [5] and by production of free radical create serious biological response. Later experiment showed nanoparticle by 20 nm size rather than small particles whit 250 nm size cause more infection in lung tissue. Our results agreed by there and after 7 times exposing to Fe_2O_3 nanoparticle by 20 nm size there are significant inflammation in lung tissue. This inflammation is depends on nanoparticle surface area and surface properties. Mechanism that used by nanoparticles for inflammation is direct effect on alveolarmacrophages that make phagocytic and cytoskeletal variation that all of these caused by free radicals such as OH and H_2O_2[23]. Lung injection of Fe_2O_3 Increasedcapillary permeability and bloodcoagulation timeisprolongedalso [24]. In this study by extending the exposing time (which occurred in 4th group) bleeding in the lining airs paces and lung lymphfolliclehyperplasia was observed (figure 4). By increasing the administrated dose lung tissue showed all of mentioned symptoms by increasing intensity. Allevents thatoccurred innanoparticle exposingcause presence of neutrophils, lymphocytes and eosinophils in the lung tissue (figure 5) that showed invasive behavior of nanoparticles and free radical production can induce immune system.

Not that inhalation of Fe_2O_3 only affected lung tissue and other organs such as spleen, liver and kidney don't show such symptoms [21]. Also previous experiments showed improving of body weight but weight of lungs significantly decreased. Wang and coworkers in 2010 administrated Fe_2O_3 through nose to rats and reach to same results (Pulmonary emphysema, interstitialhyperemiaandinflammation in lungcells)[23]. Our results punctuated previous results and confirm that size of administrated nanoparticle, dose and exposing time are more important factors that affected mentioned pathologic symptoms.

Particles larger than 100nm remain in intercellularspace and can't pass through the cell membrane and enteringtocirculationorremainattached to thevesselwall. Therefore particles with average size between 5 to 20 can be effectively used as a carrier to gene delivery and drug delivery.

Conclusion

This study established the harmful effects of nanoparticle exposing to lung. Our results confirm that nanoparticle that used in this study able to pass through the lung cells membrane and enter the circulation so that be effectively used as a carrier for genes and drugs.

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