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The effect of pre-treated with nanoparticles of iron oxide (Fe2O3) to reduce the level of NF-kB gene expression in rats model brain Ischemia-reperfusion.

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Abstract: Introduction :Studies the role of NF-kB signaling pathway in the development of nerve cell death caused by ischemia reperfusion, as well as the development of inflammation in nerve tissue after a brain injury is. In this regard, the nanoparticles of iron oxide (Fe2O3) protect neurons in the central nervous tissue against oxidative stress has been proved. The aim of this study was to investigate the effect of pre-treatment of Fe2O3 nanoparticles on NF-kB gene expression is reduced.Methods :During the study, 40 Wistar albino rats were randomly divided into four groups, control group pre treated with doses 5 mg / kg and 10 mg / kg respectively. Animals pre-treated and control groups underwent common carotid artery occlusion for 20 minutes with cerebral ischemia and reperfusion was established by re-opening the arteries. were treated only by saline. 48 hours after induction of ischemia - reperfusion in brain tissue, animal euthanasia and animal brain hippocampus tissue was extracted and the expression of NF-kB was evaluated using the Real time- PCR techniques.Results:The results of the study showed was that relative gene expression NF-kB in the context of the hippocampus of animals in both dose 5 mg / kg and 10 mg / kg significantly compared to the control group decreased (P-V <0.05). This decreased expression was significantly associated with increased doses of relationship (P-V <0.05).
Key words: Pretreatment, iron oxide nanoparticles, NF-kB, cerebral ischemia reperfusion, rat.

INTRODUCTION

Ischemia-reperfusion brain is one of the biggest health problems known global communities. This factor has been the most important reason is that after myocardial infarction, stroke and cancer, the third leading cause of death in Western countries is allocated. Cerebral ischemia leads to movement disorders, sensory and behavioral disorders, especially vision, speech disorders (aphasia), learning disabilities (Spatial learning) is (1 and 2). Accordingly, ischemia reperfusion (IR) is a temporary reduction of blood supply to the return of blood flow to part of the brain that During the damaged part of the brain is temporarily loses its natural function (3). Long-term lack of oxygen and nutrients, leads to the return of blood flow and oxygen to the tissue, high levels of harmful agents such as free radicals and reactive oxygen species (ROS) produced by mitochondria electron transport chain complexes (3, 4). This can impact on the activity of signaling pathways that ultimately led to the immune cells to the site of injury, and ultimately stimulate production of inflammatory factors induce cell apoptosis (apoptosis) is (3). Therefore, a correct understanding of the cellular processes involved in brain damage caused by ischemia and reperfusion can provide appropriate solutions to prevent and treat this disease.

Inflammation, considered as the second harmful mechanism in the ischemia reperfusion brain process. Several studies on the process and expanding the role of inflammatory damage to brain tissue is examined after ischemia (5). The results of the studies show that it would be Tumor Necrotic Factor- alpha (TNF-α) and Interleukin-1 beta (IL-1β) As the most important inflammatory factors involved in the occurrence of ischemic damage leads to activation of the signaling pathway-dependent factor

Factor-κB Nuclear (NF-kB) is (3 and 6). Has been shown that NF-kB activity leads to decreased expression of anti-apoptotic factors such as Bcl-2 and increased expression of apoptosis factors such as p53 and Bax and It also has a positive effect on the expression of inflammatory factors in the site of vessel injury (3 and 7). Therefore, it is thought that the use of new therapeutic agents and methods of reducing the expression and function of NF-kB is based on a good strategy for reducing ischemia-reperfusion-induced nerve damage brain.

In the current situation, several studies for the use of nanoparticles to prevent and treat a range of diseases is in progress (8 and 9). The evidence from studies be nanoparticles of iron oxide (Fe2O3) in protecting nerve cells against oxidative stress and the development of spinal cord injury by controlling ROS and limiting factors involved in

the induction of apoptosis. It has been shown that these nanoparticles can inhibit the expression of inflammatory cytokines in human immune cells are (10 and 11). Thus, according to the results of previous studies, The purpose of this study was to investigate the effect of pre-treatment of iron oxide nanoparticles on NF-kB gene expression was reduced. The results of the study showed that brain tissue of rats with cerebral ischemia reperfusion NF-kB gene expression levels were significantly decreased compared to control group ($P<0.05$).

MATERIALS AND METHODS

Materials

Nanoparticles Fe2O3, xylazine and ketamine were purchased from Sigma company(Sigma-Aldrich Co. St Louis, MO, USA). RNA extraction kits from Roche company(Roche Co, Germany) , CDNA synthesis kit were purchased from Fermentas Company(Fermentas CO, Lithuania) And SYBR Green Real-time PCR Kits company were purchased from Kyvtn company (Qiagen, Europe).

Animals

This study on 40 male albino Wistar rats (6-8 weeks) with a weight range of 200-250 g were purchased from Pasteur Institute of Iran. Animals in standard laboratory conditions of 12 hours of light and 12 hours of darkness, in a temperature range of 22-24 ° C and were studied in compliance with all ethical principles (12).

Study groups

A week after the keeping of animals kept in the room for adaptation, animals were randomly and equally in to four groups: 1. Group of healthy animals, 2. Group of Control, including ischemia reperfusion animal model of cerebral pre-treated with solvent, 3. Groups of animals model brain Ischemia-reperfusion Pretreated by 5 mg / kg Fe2O3, 4. Groups of animals model brain Ischemia-reperfusion Pretreated by 10 mg / kg Fe2O3 Were divided.

Brain Ischemia-reperfusion induced

`Brain Ischemia-reperfusion induced to animals, Rats using intraperitoneal injection of xylazine and ketamine combination of drugs with the final dose 110 mg / kg were anesthetized (13) and were fixed on the surgery bed. After shaving the area of surgery and sterilize the area, A vertical incision in the neck skin of the animal (lower jaw slightly lower than the top of the sternum) was created. With the skin and muscles along the front of the trachea, both common carotid arteries were exposed. After removing the vagus nerve, carotid arteries using microsurgery surgical clamps were blocked for 20 minutes. Re blood flow (reperfusion) was established by opening the clamp. The resulting gaps using a sterile silk suture was stitched and finally the animals to recover and prevent hypothermia doubt were transferred to a warm place (14).

Suspension of nanoparticles Fe2O3: Fe2O3 nanoparticles about 20 nanometers in size that are ready were purchased from Sigma-Aldrich company, Weighing 100 mg using a digital scale laboratory (AND MODEL EJ303) and has been solved In 10 mL of saline at a temperature of 40-35 ° C and For 5 minutes to prepare the solution was placed in the vortex.

Treatment of animals

Rats in Group 1 animals were healthy without any intervention were examined. the control animals that were under brain Ischemia-reperfusion the control animals, 20 minutes before and 20 minutes after induction of brain injury each time with 0.5 ML of saline intraperitoneally were treated. the Group 3 of animals, including brain Ischemia-reperfusion model of rats pre-treated under Fe2O3 nanoparticle with a general dosage 5 mg / kg that 20 minutes before and 20 minutes after induction of brain damage per 0.5 Ml of solution contains half the final dosage for intraperitoneal injection. The Group 4 of animals under pre-treated Fe2O3 nanoparticle with a general dosage 10 mg / kg that 20 minutes before and 20 minutes after induction of brain damage per 5.0 ML of solution contains half the final dosage for intraperitoneal injection (14).

Euthanasia And extracting the hippocampus of the animals brain

4 days after surgery and treatment, animals with injected high doses of xylazine and ketamine were killed combining high dose. Then animal's head was beheaded by guillotine for rodents And after sterilization using 70% ethanol was transferred into the hood. After complete removal of the brain, the hippocampus region of the brain areas removed and Krayvtyvb RNas, DNas free were And frozen in liquid nitrogen and store -80 ° C were transferred to a refrigerator (15 and 16).

Measurement of gene expression

First, total RNA was extracted from animals brain tissue Then cDNA synthesis using the kits was performed according to specific instructions of kit and then, In order to do real time- PCR was transferred to the -20 ° C fridge. Real-time- PCR test by Cycler iQ5 (Bio-Red Co, USA) was used. NF-κB gene primers list and also β-actin gene is reported in Table 1. Real-time- PCR process in this study repeated twice in 96 glass tubes with a 25 µl final volume took place. In the process of 12.5 µl QuantiFast SYBR Green PCR Kit with 5.9 µl deionized water,0.5 µl of each sweep primers with 10 µmolar concentration With 2 µl of cDNA was used.

The polymerase chain reaction was carried out according to the protocols: 1. denaturation, Including a temperature of 95 ° C for 3 minutes. 2. Annealing, Include a temperature of 60 ° C for 35 seconds for primers NF-κB and temperature of 59 ° C for 35 seconds for primers β-actin process took place.3. extension, At 72 ° C for 3 minutes. The final number was 40 cycles.

In order to confirm amplification of specific target genes, the reaction product by using agarose gel electrophoresis (2%) were studied. In addition to outlining the melting temperature curve (Melting curve) from 50 to 99 ° C for 5 seconds per repetition increased by 1 degree. Finally, the reaction efficiency of PCR (PCR efficiency) were evaluated based on the standard curve. For this purpose cDNA with appropriate concentrations are chosen and then different concentrations based on the logarithm of the concentration of cDNA (horizontal axis) and cycle threshold (vertical axis, ct1) for each gene was drawn from samples without cDNA template as control was used As well as the proliferation curve (Amplification Curve) were plotted for each response that took analysis data based on the compares threshold cycle (Ct Value) different groups with control group (17).

Table 1. Type, size and sequences of primers used in the study

Product Size (bp)	Sequence	Gene name	
175	ACCTGAGTCTTCTGGACCGCTG	Forward	NF-κB
	CCAGCCTTCTCCCAAGAGTCGT	Reverse	
105	GAACCCTAAGGCCAACCGTG	Forward	β-actin
	AGGCATACAGGGACAACACAGC	Reverse	

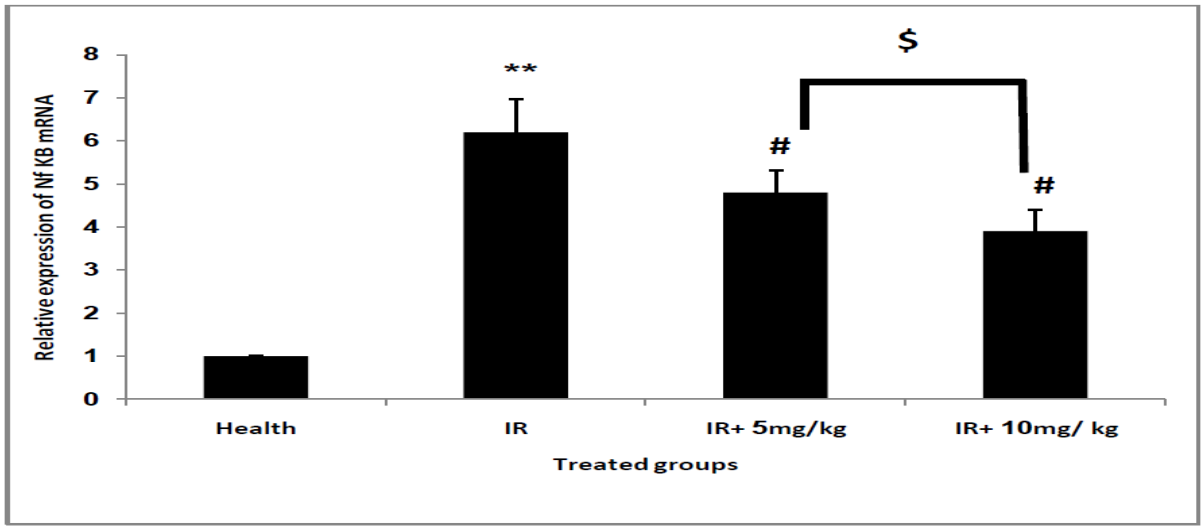

chart 1: Nf-kB gene expression in brain tissue samples by using Real time -PCR in the healthy Studies groups (Health), control groups (IR: Ischemia Reperfusion), cerebral ischemia animals groups treated with nanoparticles of iron oxide (Fe2O3 nanoparticles) with different concentrations. * Indicates P-value <0.05, ** indicates P-value <0.01, that animals model of cerebral ischemia group compared with the control group. # Indicates P-value <0.05, # # indicates P-value <0.01, that the animals model of cerebral ischemia disease treated with iron oxide nanoparticles compared with animals model of cerebral ischemia with no treatment.

$ indicates P-value <0.05, that the animals model of cerebral ischemia disease treated nanoparticles of iron oxide (Fe2O3 nanoparticles) are compared with different concentrations.

Data analysis

Data were recorded in SPSS-16 software and Evaluation of relative gene expression by comparing the threshold cycles of samples from each group as 2 ^ ΔΔ CT (Livak) as well as methods Whitney - Mann was used for data analysis. The level of less than 0.05 was considered statistically significant (18).

RESULTS

The results of the Evaluation of gene expression showed that the relative gene expression of NF-kB in Group IR, 48 hours after induction of ischemia reperfusion significantly (P-V <0.01) compared to the control group animals has increased.

It was also found that the use of pre-treated with Fe2O3 nanoparticles, 48 hours after brain injury induced, is reduced the relative gene expression of NF-kB in the hippocampus tissues of animals in both dose 5 mg / kg and 10 mg / kg significantly compared to IR group (PV <0.05).

The results of the relative gene expression of NF-kB showed that Pre-treated with increasing doses of Fe2O3 nanoparticles Perhaps decreased the inflammation amount of the lower dose and IR group.

DISCUSSION

The aim of this study was to evaluate the effects of pretreatment Fe2O3 nanoparticles and inhibition of NF-κB in brain ischemia reperfusion model of rats had. The results of the genomics research demonstrate that Pretreatment of Fe2O3 nanoparticles significantly to the performance NF-κB factor as the key factor involved in the control of NF-κB signaling pathway in mouse brain tissue been under ischemia reperfusion cerebral. Accordingly, the results of Real-time PCR on the NFκB gene expression in different groups of animals, showed a significant decrease in expression of the gene in the treatment groups compared to IR group (P <0.05). The significant difference in the results between the two groups IR + 5 mg / kg and IR + 10 mg / kg proportion was NFκB gene expression (P <0.05) (Figure 1).

Ischemia (reduced blood supply) brain is one of the biggest health problems known to the international community. Cerebral ischemia is the most important reason of stroke that after myocardial infarction and cancer, the third leading cause of death in Western countries is accounted for that result of incidence of movement disorders, vision, speech, learning and behavioral disorders. (19 and 20). reperfusion Lesions is created as a result of the return of blood flow to tissue after ischemia. The lack of oxygen and nutrients, which creates special status as a result reperfusion and returning circulation leads to inflammation and oxidative Lesions caused by oxidative stress. The return of blood flow cause the return of oxygen to the cells and damage caused by release of free radicals. This can affect the signaling pathways and caused programmed cell death (Apoptosis) (3).

Inflammation considered as the second harmful mechanism in the ischemia and stroke process. Must be expressed that During Several studies the role of inflammatory factors on the process and expanding brain tissue damage after ischemic has been investigated. With tissue ischemia, as a result of increased expression of molecules involved in cellular connectivity such as ICAM, E-selectin and P-selectin on vascular endothelial cells, inflammatory process begins (3 and 21). During this process, As a result of influence white blood cells into the brain tissue and their interaction with micro-glial cells and the secretion of inflammatory cytokines, The expansion of the damage and cell death will significantly increase (22). increased levels of inflammatory cytokines Leads to stimulate and activation of NF-κB signaling pathway is dependent on the NF-κB factor. Must be expressed that NF-κB Leads to the expression of inflammatory cytokines involved in the nervous system. Found that, NF-kB activity Leads to decreased expression of anti-apoptotic factors such as Bcl-2 and increased expression of apoptosis factors such as p53 and Bax is (3). The use of inhibitors of inflammation is one of the importance leading ways to reduce nerve damage caused by ischemia and reperfusion (IR) in the central nervous system. The results of the above study was that demonstrate that Pretreatment of Fe2O3 nanoparticles significantly decreased level of performance NF-κB signaling pathway in nerve cells that As a result of this, can protect nerve tissue against the spread of neurological damage resulting from the inflammation.

Among the nanoparticles used in medicine can be noted that Fe2O3 oxide nanoparticles that capable of many clinical applications in imaging of the central nervous system. Evidence from studies indicate Fe2O3 nanoparticles can protect nerve cells against oxidative stress is spinal cord injury. It seams, Fe2O3 nanoparticles through the Ros pathway and limiting factors involved in the induction of apoptosis is to protect the cells from death (10 and 11). Well known that These nanoparticles can inhibit the expression of inflammatory cytokines in human immune cells. But the study was not to be evaluated doses of iron oxide to inhibit inflammation after ischemia and reperfusion brain.

According to the results of previous studies on the protective effect of Fe2O3 nanoparticles on cells of the central nervous system, The results of the above study implies on the High power of pre-treatment Fe2O3 nanoparticles on inhibition of NF-κB inflammatory pathway.

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