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The study of the expression of apoptotic Bax, Bcl2 cells in the hippocampus following ischemia reperfusion model, pretreatment with nanoparticles of iron oxide in rat brain

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Abstract: Progressive cell death of nerve cells after injury to the central nervous system is known as one of the most destructive factors in nervous system diseases. Ischemia / reperfusion cerebral blood supply to the brain process the sudden loss of blood supply to the brain and then return stroke during different parts of the brain under stress. Observations have shown that reperfusion or re-supply of blood to the brain via the Radygalhay Return oxygen, mitochondrial dysfunction of neurons and activation of many signaling pathways involved in cell death key role in the development of the nervous Varz of ischemia / reperfusion brain is capable. Nanoparticles of iron oxide nanoparticles safely and with minimal cellular toxicity, which is now widely used in brain imaging and treatment of some diseases have been based on MRI. In the present study the neuroprotective effects of ischemia reperfusion model in rats brain Fe2O3 nanoparticles were studied. In this study Alfa' after ischemia / reperfusion of brain in rats treated with 5 mg / kg and 10 mg / kg apoptotic Bax and Bcl-2 gene expression levels using real time-PCR in the hippocampus region of the animals studied it placed. Results The studies showed that the ratio of Bax and Bcl-2 gene expression after pretreatment of animals with ischemia / reperfusion brain using iron oxide nanoparticles resulted in the induction of apoptosis Bax gene expression significantly (P- Value <0.01) than the control group decreased. According to the results of the above study seem to be pre-treated with iron oxide nanoparticles with high potential to protect nerve cells in the brain from stroke are important
Keywords: apoptotic genes Bax and Bcl-2, hippocampus, ischemia-reperfusion rat model of stroke, pretreatment, Nanvzra iron oxide.

INTRODUCTION

Stroke is the second leading cause of death and the leading cause of severe disability in adults reported Despite extensive research, no effective treatment for stroke that is used worldwide, have been reported.

It is important to note that after re Azbrqrary blood flow in the vessels that were previously blocked, too much oxygen free radicals (ROS) are produced, which ultimately is the reperfusion injury (6-3). Especially apoptosis after ischemia-reperfusion is one of the main reasons that lead to cell death after reperfusion is (8-7). In a study of Kin (2004) conducted on rats myocardial reperfusion myocardial injury observed that the postcond In the first few minutes of reperfusion reduced (9). The Sun and colleagues (2006) found that postcond of the rat cardiomyocytes apoptosis prevents reperfusion (10). Zhao and colleagues (2006) have for the first Bargzarsh postcond infarct size if it is used during the first hour after stroke, reduces (11). Ren et al (2008) have proven that the use of delayed postcond even up to 4 hours induction of ischemia, ischemic brain injury reduces local (7). Xing and colleagues (2008) reported that postcond apoptosis and oxidative stress injury / Rprfyvzn reduced in rats (12).

Now, using techniques such as reperfusion by thrombolysis in the first hours after the attack, as well as recombinant tissue plasminogen activator Intravenous use, including strategies used in Protect nerve cells from damage caused by ischemia, respectively (14). But the widespread use of these methods is still fundamental limitations. Factors such as Golden Time limit consumption and an increased risk of brain hemorrhage start of the constraints facing these methods are (16-15).

In recent years with the development of new approaches to solve problems in different areas of the biomedical sciences, medicine and pharmacy is available to researchers. In general, nanoparticles, particles with dimensions of 100-1 nm, depending on the size, origin and composition of these particles have different biological properties and functions of their show (18-17). However, significant barriers to entry nanoparticles in clinical practice and the widespread use of these nanoparticles in neuroscience researchers are still ahead. Among the most important problems neurotoxicity of nanoparticles and the nanoparticles can cross the blood and brain (BBB) is.).

The nanoparticles used in medicine as iron oxide nanoparticles (Fe2O3) noted that many clinical applications in imaging of the central nervous system is (23-22). Evidence from studies of iron oxide nanoparticles can protect nerve cells against oxidative stress in spinal cord lesions (25-24). It seems that iron oxide nanoparticles through the Ros and limiting factors in the induction of apoptosis in many causes

These cells are protected from death (24-25). Increased angiogenesis in the affected areas of the brain to treat stroke is the most important health policy.).

Gabri of the major problems of nanoparticle toxicity of these nanoparticles is of interest. Therefore, using the least amount of side effects of nanoparticles have the necessary for nerve cells to treat stroke. Evidence shows that iron oxide nanoparticles of tissue and cellular toxicity was low and has shown few side effects as well. Studies have found that these nanoparticles on the rat LD50 of 600mg / kg, which indicates the use of high doses of nanoparticles in the environment In vivo (28)of iron oxide nanoparticles protect the nerve cells in the brain after ischemia and reperfusion it would be efficient and safe. The aim of this study examines the effects of iron oxide nanoparticles on ischemia reperfusion model rats brain is neuroprotective.

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, Fe2O3 nanoparticles using the 100 mg of digital scales Laboratory (AND MODEL EJ303) weight and 10 cc saline solution and a temperature of -35 to 40 ° C for 5 minute suspension to be placed in the vortex.

Animals and grouping study

This study on 40 adult male Wistar albino rats (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 they were kept randomly, without any intervention. Ischemia-reperfusion induced brain after surgery and then animals, in order to study in four groups of studies are 1) healthy animals 2) control, 3) Ischemia-reperfusion model animal groups Brain pretreatment with 5 mg / kg Fe2O3 and 4) ischemia-reperfusion animal model of brain pretreatment with 10 mg / kg Fe2O3, respectively.

Ischemia-reperfusion induced brain

Ischemia-reperfusion model was created based on the instructions found in this study (29). For this purpose, the animals using a combination of drugs xylazine (now alfasan) at a concentration of 20 mg / ml ketamine (now ROTEXMEDICA) at a concentration of 50 mg / m were anesthetized and were fixed on surgical bed. Clamps were then taken to re-establish blood flow was (reperfusion)

Grooming

Rats in Group 1 animals were healthy without any intervention were examined. Ischemia-reperfusion Injury in Rats in the control group consisted of animal models that the brain 20 minutes before and 20 minutes after induction of brain injury were treated with 1cc saline intraperitoneally (29). Rats Ischemia-reperfusion Injury in Group 3 includes animal models that the brain 20 minutes before and 20 minutes after induction of brain injury with a total dose of 5 mg / kg Fe2O3 were treated intraperitoneally. Ischemia-reperfusion Injury in Rats in Group 4 includes animal models that the brain 20 minutes before and 20 minutes after induction of brain injury with a total dose of 10 mg / kg Fe2O3 were treated intraperitoneally.

Euthanasia of animals and get the hippocampu

4 days after surgery and treatment, the animals were killed with painless procedure to be molecular profiling of their hippocampus. Animals injected with a high dose combination of xylazine and ketamine were killed. The animal's head was beheaded by guillotine for rodents and animal heads after sterilization using 70% ethanol was transferred into the hood. After removing the brain hippocampus was removed and Krayvtyvb RNas, DNas free was frozen in liquid nitrogen were placed. The samples in the fridge -80 ° C for RNA extraction and cDNA synthesis were transferred.

Total RNA and cDNA synthesis

Their brain total RNA extraction kit RNA (Roche Co, 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 ° cGy

Reaction Real-time- PCR SYBER Green

Real-time- PCR test by Cycler iQ5 (Bio-Red Co, USA) was used. List of apoptotic Bax gene primer and B-cl2 as well as gene -actinβ (reference gene) is 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 sweep with a concentration of 10 µmolar with 2µl of cDNA were extracted.

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

Size	(bp)	gene sequence
136	CCAGGACGCATCCACCAAGAAGC	Forward Bax
	TGCCACACGGAAGAAGACCTCTCG	Reverse
118	GGATGACTTCTCTCGTCGCTACCGT	Forward Bcl-2
	CGAGTGAGGATGTGCATGAA	Reverse
105	GAACCCTAAGGCCAACCGTG	Forward β-actin
	AGGCATACAGGGACAACACAGC	Reverse

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 of the cDNA molecule chain is to open the door (95 ° C for 3 minutes). The second step for 35 seconds at 60 ° C Annealing temperature Bax and Bcl-2-specific primers and primers temperature of 59 ° C for β-actin process 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 (30).

To Confirm amplification of specific target genes, the reaction product using agarose gel electrophoresis (2%) were studied. In addition to outlining the melting curve (Melting curve) temperature of 50 to 99 ° C for 5 seconds per repetition rose by 1 degree

Standard curves and draw it

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

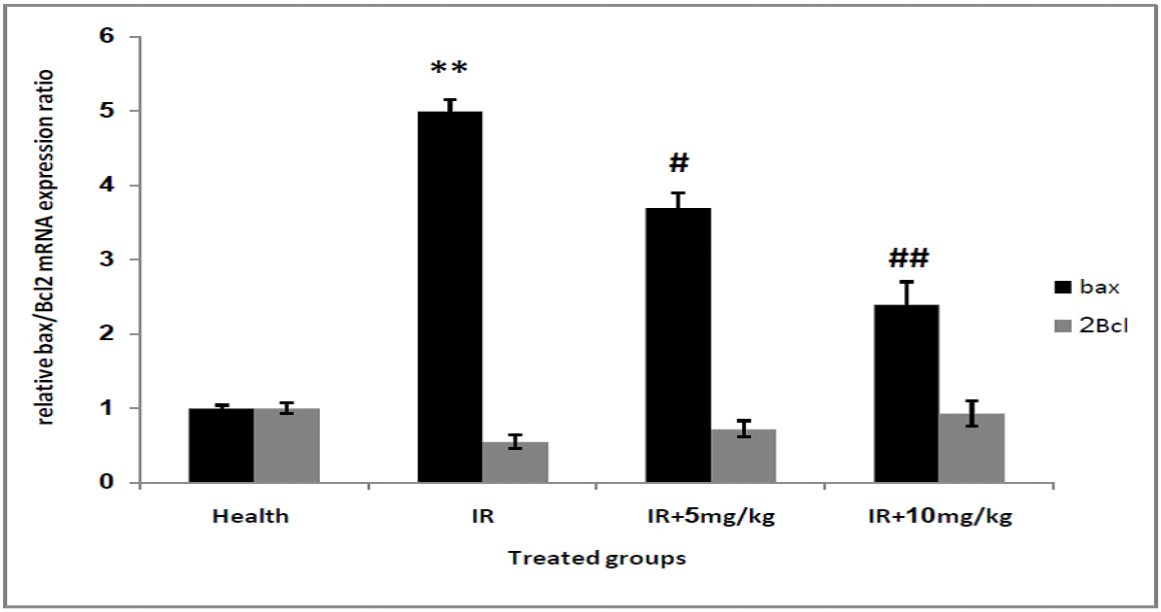

Figure 1: The expression of Bax and Bcl-2 in brain tissue samples using real time -PCR studies in healthy groups (Health), ischemia, cerebral IR: Ischemia Reperfusion)), cerebral ischemia model animal group treated with iron nanoparticles oxide (Fe2O3 nanoparticles) with different concentrations. * Indicates <0.05 P-value, ** indicates <0.01 P-value animal model of cerebral ischemia and hopes that groups compared with the control group. # Indicates <0.05 P-value, # # represents <0.01 P-value animal model of cerebral ischemia and hopes that the group treated with iron oxide nanoparticles with animal models of cerebral ischemia Ydvn treatments are compared.

Data analysis

Data were recorded in SPSS-16 software and installation gene expression analysis on samples taken from each group as compared to the threshold cycles (Livak) as well as methods Whitney - Mann was used for data analysis (31). The level of less than 0.05 was considered significant

RESULTS

Expression analysis of genes Bcl2, Bax in hippocampus brain tissue of animals treated with Fe2O3 nanoparticles using Real Time-PCR

As Figure 1 shows, the ratio of the expression of Bax and Bcl-2 we see before boarding a stroke with the use of nanoparticles Fe2O3 means that the gene expression induced apoptosis Bax significantly (P-Value < 0.01) Planning is reduced to IR. The observations also showed that the higher the dosages increased apoptotic Bax gene expression significantly in the group treated with 10 mg / kg nano-particle To 5 mg / kg significantly reduced (PV <0.05) have. In the case of anti-apoptotic gene Bcl-2 also utilizes Pretreatment of animals with stroke using Fe2O3 nanoparticles caused a slight increase in the expression of this gene .Also check the expression of Bax and Bcl-2 gene in each group showed that the amount and intensity of apoptosis in treated groups with Fe2O3 particles Planning IR group decreased significantly As well as the amount and apoptosis by increasing the dose of nanoparticles decreases.

DISCUSSION AND CONCLUSION

The aim of this study to evaluate the expression of apoptotic Bax, Bcl2 cells in the hippocampus following ischemia reperfusion model in rats treated with nanoparticles Fe2O3 the brain. The ratio of the expression of Bax and Bcl2 Evidence from using Real-time- PCR SYBER Green after pretreatment ischemia model animals Ischemic reperfusion Fe2O3 nanoparticles significantly at a dose of 5 mg / kg (P-value <0.05) and 10 mg / kg (P-value <0.01) the Bax gene expression in the hippocampus ischemia-reperfusion animals have reduced brain .

With the return of blood flow to the brain, which reduced the amount of damage to a number of factors such as the timing, extent and severity of ischemia is associated (33).

So far, extensive studies to find the mechanisms involved in neuronal cell death under ischemia and the effect of Ryprfzvzhn been done. During cerebral ischemia and reduced blood supply to the brain, intracellular calcium levels will increase dramatically. is released into the cytoplasm. Following the release of this protein complex formation Apvptvzvm, activated caspase-9, which eventually activated caspase-3-dependent apoptotic cell death pathway leads (34). For the release of cytochrome C and the formation of proteins identified in the cell death process is complex Pvptzvn will be non-recursive. It should also be expressed in that cell death The process is progressive damage to the central nervous system that over time will increase the level of injury (34).

The use of agents or drugs protect nerve cells against damage from something useful and necessary to prevent cell death and thus increase the efficiency of treatment will be completed. Today, many clinical applications Fe2O3 nanoparticles in medical imaging of the central nervous system to account for (35). Evidence from earlier studies indicate that nanoparticles can protect against oxidative stress in the nervous system injury Nerve center. Found that iron oxide nanoparticles by limiting the formation of reactive oxygen species and factors involved in apoptosis, to protect nerve cells against cell death caused by ischemia and reperfusion will be (36). The results of our study also demonstrated that Fe2O3 nanoparticles and gene expression of apoptotic Bax and performance by limiting the amount and intensity of the process of apoptosis in nerve cells reduce Hppvkamp.

Thus, according to the results of previous studies on the positive effects of Fe2O3 nanoparticles in reducing complications Ischemia and reperfusion and its low toxicity effects as well as the results of the above study that the positive result of protection Pre-treatment of neurological Fe2O3 nanoparticles on ischemia reperfusion rat model brain, it seems Pretreatment with the nanoparticles of the potential to reduce ischemic brain lesions and ultimately protect brain tissue Have a stroke.

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