PROPERTIES OF MAGNETIC NANOPARTICLES AND THEIR ROLE IN THERAPY OF NEURODEGENERATIVE DISEASES

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Abstract

Magnetic nanoparticles are widely studied as potential therapeutic vectors in a number of conditions including neurodegenerative diseases - Alzheimer's & Parkinson's disease. These diseases are known for production of aberant protein forms with β-amyloid structure. The ability to cross blood-brain barrier and the possibility of surface modification present unique opportunity to use high frequency electromagnetic hyperthermia to destroy such amyloids, which we investigated in this study. Starch covered 100 nm magnetite nanoparticles can be heated to hyperthermic temperatures by 3.5 MHz alternating electromagnetic field. Hen egg-white lysozyme amyloid fibrils were prepared in acidic conditions. Micromolar amounts of magnetic nanoparticles, ensured heating of the mixture in electromagnetic field and we evaluated effect of hyperthermia on fibrils from absorbance peak shift from 490 nm to 540 nm in Congo red spectra, which is present when the dye binds with amyloid fibrils. Up to 40-45 μL of 25 $mg \cdot mL^{-1}$ magnetic nanoparticles in 1 mL of hen egg-white amyloid fibrils did not have any considerable effect on the fibrils, but amounts larger than 50 μL exhibited ability to destroy the fibrils to such an extent that no red shift in Congo red spectra was observed.

Among other beneficial properties of magnetic nanoparticles is their ability to act as peroxidases which can also prove to be beneficial in neurodegenerative diseases. Evidence suggests involvement of ROS, to which hydrogen peroxide contributes in various ways. We investigated peroxidase activity of 100 nm starch-covered magnetite nanoparticles using o-phenylenediamine dihydrochloride as a chromogen. The results were interpreted using Michaelis-Menten kinetics.

Keywords: Magnetic nanoparticles, neurodegenerative diseases, amyloid fibrils, hyperthermia, peroxidase activity

1. INTRODUCTION

Neurodegenerative diseases are yet untreatable diseases that affect mostly people in their sixth decade and older. In Europe the overall prevalence for dementia, which is one of the main symptoms of neurodegenerative disease, is 1.55 % [1]. Women are more likely to suffer from dementia with twice as many cases documented. The two most common neurodegenerative diseases are Alzheimer's disease (AD) and Parkinson's disease (PD).

AD is progressive disease that destroys memory, ability to learn and adapt, to react and communicate in social situations. It affects neurons in amygdale and hippocampus which are responsible for short term memory, and then slowly other neurons in cerebral cortex. Microscopic changes also involve formation of senile plaques consisting of insoluble form of β-amyloid peptide in β-sheet conformation and neurofibrilary tangles consisting of hyperfosforilated τ proteins [2].

PD is mostly associated with loss of motor function, tremors, muscle rigidity and bradykinesis caused by gradual death of dopaminergic neurons in *substantia nigra*. Dementia symptoms are caused by neuronal death in basal forebrain. Insoluble forms of proteins in β-sheets, mainly α-synuclein, are present in Lewy bodies, which histologically present as eosinophilic cytoplasmic inclusions [3].

In both of these diseases involvement of ROS has been found. Many neurons can cope with increase in oxidative stress, but some neuron populations are more vulnerable to it - this phenomenon is called selective neuronal vulnerability. Specifically, the brain areas most affected in neurodegenerative diseases show different, but increased vulnerabilities towards ROS and therefore it may be involved in etiopathology of these diseases [4]. Moreover, overall brain vulnerability towards ROS is higher because brain utilizes about 20 % of respired oxygen even though it represents no more than 5 % of total body weight, contains more metal ions which accumulated there throughout life, easily oxidable polyunsaturated fatty acids and lower concentration of antioxidants and related enzymes.

Aggregated misfolded proteins that form high-ordered insoluble fibrils are common in neurodegenerative diseases. The mechanism of their formation is not yet fully understood, although there is some evidence of it being a product of genetic and environmental factors where oxidative stress is one of them. Proteins modified by ROS tend to aggregate and may act as inhibitors for proteasomal activity - a major way of removing of misfolded proteins, which may lead to subsequent protein aggregation [5].

Magnetic nanoparticles represent potential vector for several potential treatments for neurodegenerative diseases. However, brain is protected against 98 % of small molecules by blood-brain barrier, which prevents harmful substances from passing into brain. Although most magnetic nanoparticles are unable to cross the blood-brain barrier passively, several strategies such as attaching molecules for receptor or carrier mediated transport, transport mediated by external magnetic field and increase of blood-brain barrier permeability by heat generated by magnetic nanoparticles during application of alternating magnetic field have been utilized successfully [6].

All above mentioned strategies also represent the advantages of magnetic nanoparticles as therapeutic vectors for neurodegenerative diseases. Apart from the ability to functionalize their surface by ligands, they can also be modified by therapeutic agents for delivery into brain, such as dopamine to substantia nigra for Parkinson's disease. Application of alternating magnetic field in combination with magnetic nanoparticles produces hyperthermic effect upon the tissue containing the nanoparticles. In single domain naoparticles this is due to rotation and relaxation of magnetic moment within the nanoparticle - Néel relaxation. Heat can also be produced by Brownian motion and friction within environment containing the nanoparticles. For multidomain nanoparticles heat is produced via hysteresis losses within the domain walls [7].

Since hyperthermia has a really good effect on brain tumors, we decided to try this approach for insoluble misfolded protein fibrils that are common for neurodegenerative diseases. The study was conducted using hen egg-white lysozyme a well-known and accepted model for amyloids. The aim was to see if controlled hyperthermia can in any way affect amyloid fibrils and in best case scenarios if it could lead to fibril disintegration.

The other approach on using magnetic nanoparticles as therapeutic agents is their ability to act as peroxidases [8]. Since brain has generally lower concentration of antioxidant enzymes and neurodegenerative diseases are connected to ROS generation and effects, this particular property of magnetic nanoparticles can be a pro in their utilization as therapeutic vectors in these diseases.

2. EXPERIMENTAL

2.1. Heating of magnetic nanoparticles

Electromagnetic heating of 100 nm starch-covered magnetite nanoparticles with 80 $mg \cdot ml^{-1}$ of Fe_3O_4 was performed using a 3.5 MHz radiofrequency generator (GV6A, ZEZ Rychnov, Czech Republic) with a power dissipation of 6 kW. The coil-shaped and water-cooled antenna was made of 3 copper windings with a diameter of 15 cm, connected to a water-cooled resonance circuit which produced the electromagnetic field. The temperature of the suspension was measured by contactless infrared thermometer.

2.2. Hyperthermia of amyloid fibrils

Amyloid fibrils were prepared by adding 2 mg·ml^{-1} of hen egg-white lysozyme into pH 2, 100 mM phosphate buffer with 150 mM NaCl [9]. This solution was heated to 65 °C for one week. 20-60 µL of 100 nm starch-covered magnetite nanoparticles (25 mg·ml^{-1} of Fe_3O_4) were added per one milliliter of fibrils and put into 3.5 MHz alternating magnetic field until their temperature reached 42 ± 1 °C. The excess of magnetic nanoparticles was removed from solution via magnetic decantation. Congo red staining was used to evaluate presence of fibrils. 100 µL of hyperthermically treated fibril were added to 1 ml of Congo red solution prepared in pH 7, 50 mM phosphate buffer and left to incubate for 30 minutes. Spectrophotometric measurements were conducted on spectrophotometer Jenway 7315 (Staffordshire, UK).

2.3. Peroxidase activity

0.5 mg·ml^{-1} of o-phenylenediamine dihydrochloride (PDD) was dissolved in pH 5.5 phosphate-citrate buffer. 1.44 ml of PDD was mixed with 160 µl of hydrogen peroxide and 64 µl of 100 nm starch-covered magnetite nanoparticles [10]. The final concentration of hydrogen peroxide in reaction mixture was ranging from 3.37 % to 0.34 %.

3. RESULTS AND DISCUSSION

Magnetic nanoparticles subjected to and external alternating magnetic field increase their temperature as a function of time of magnetic field action. This is due to Brown and Néel relaxation processes and hysteresis losses. 100 nm starch-covered magnetite nanoparticles with 80 mg·ml^{-1} of Fe_3O_4 can reach almost 90 °C temperature in one hour as shown in **Figure 1** as compared with control sample without nanoparticles. To avoid temperatures higher than hyperthermic, final concentration of magnetic nanoparticles was adjusted to 25 mg·ml^{-1} and used in all further experiments.

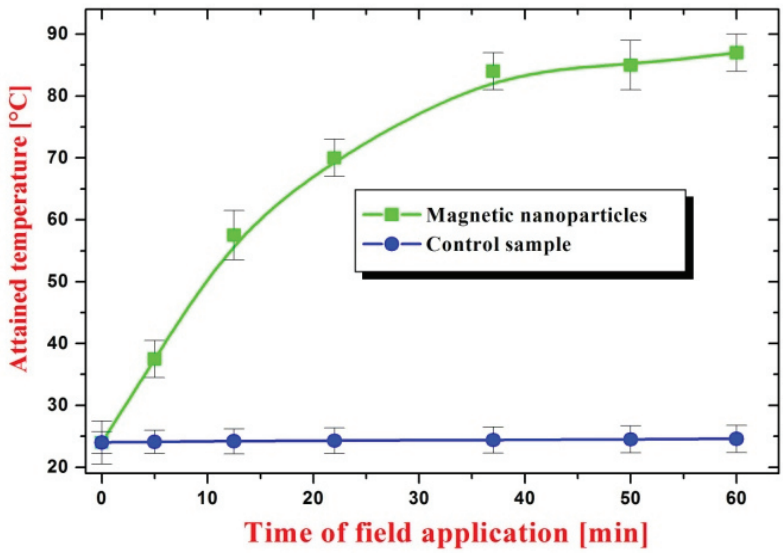

Figure 1 Heating of 100 nm starch-covered magnetic nanoparticles (80 mg·ml^{-1} of Fe) in an alternating magnetic field. Averages and standard deviations from three experiments

Hyperthermic treatment of HEWL amyloid fibrils was done with the same nanoparticles which were added in 7 different amounts in 1 ml of fibrils. Congo red staining was used for evaluation - presence of fibrils is characterized by red shift of absorption peak, where the highest difference between Congo red spectrum and Congo red-stained fibrils is at 540 nm. Absorbances of Congo red spectrum and hyperthermically non-treated fibrils at 540 nm were used as controls. As can be seen in **Figure 3** amounts of 10, 20, 30 and 40 µL of

magnetic nanoparticles had no effect on the formed fibrils, whereas 45 µL and higher showed that fibrils were not present in the solution. In this case no gradual absorbance decrease with higher concentrations of magnetic nanoparticles was observed. Usually there are several stages of absorbance decrease present before the absorbance reaches that of pure Congo red [11,12], although most of the experiments of this kind are chemical in nature. Magnetic nanoparticles affect fibrils by dissipating heat and Brownian motion under magnetic field influence, which is physical in nature. The mechanism given seems to be more gate-way like where certain concentration of magnetic nanoparticles is enough to disintegrate most of the fibrils so that almost none are detected by Congo red assay [13].

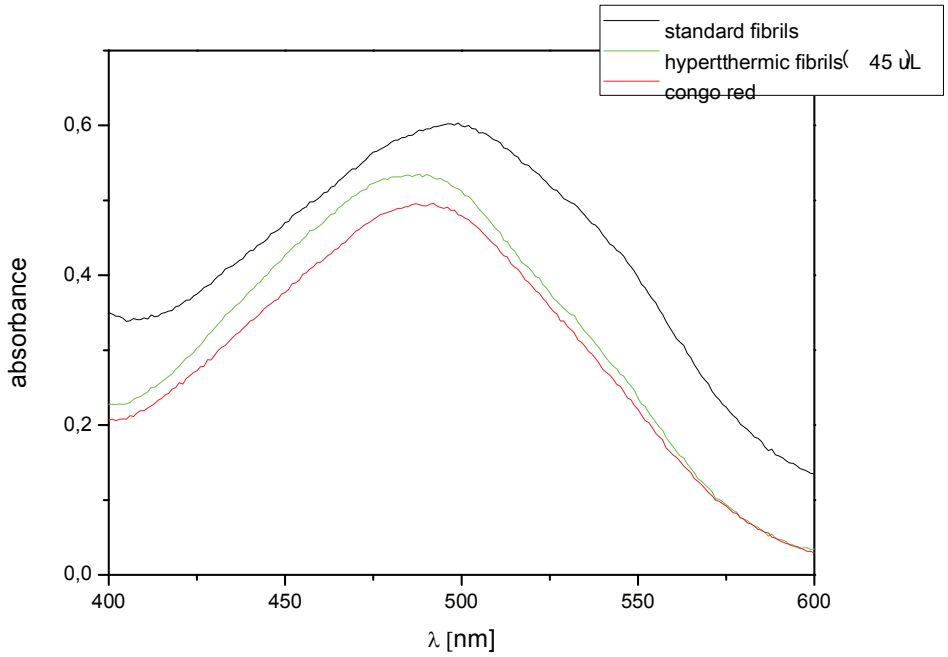

Figure 2 Absorbances of fibrils stained with Congo Red (black), Congo red stained hyperthermically treated fibrils where 45 µL of magnetic nanoparticles were used (green) and Congo red (red). Black line exhibits absorbance peak shift to red side with increased absorption at 540 nm

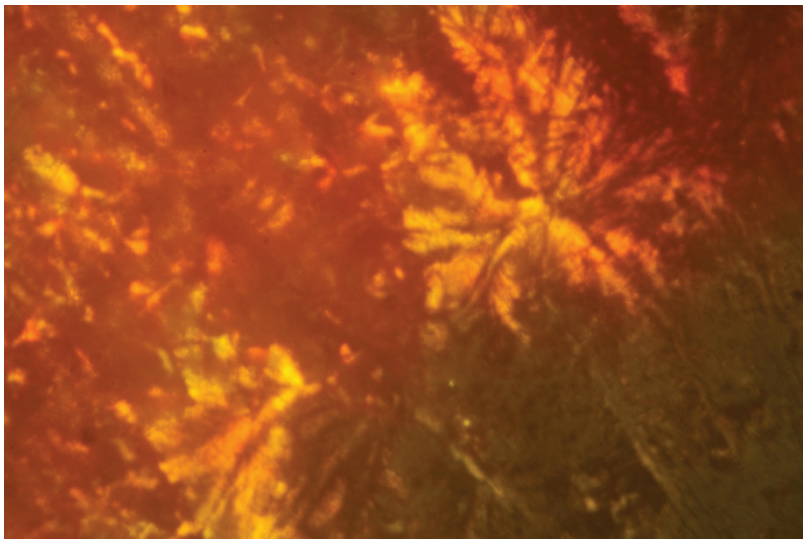

Figure 3 Experimental demonstration of amyloid-like structures using optical microscopy images of fibrils stained with Congo red under polarized light (Congo red-stained samples were examined under polarized light with a Meiji TechnoTC5400 microscope equipped with Canon EOS 400D camera

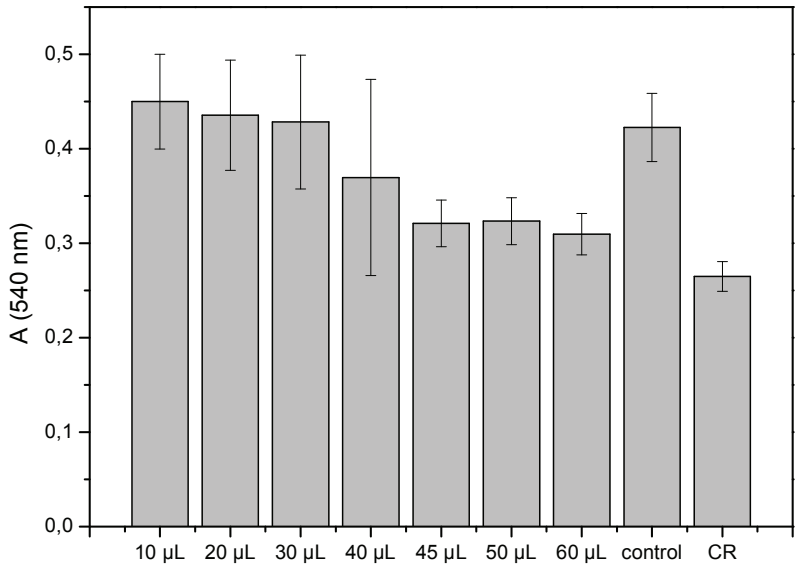

Figure 4 Absorbances at 540 nm of Congo red-stained hyperthermically treated fibrils where different amounts of magnetic nanoparticles were used, comparison with absorbances of control (standard fibrils) and pure Congo red. Averages and standard deviations from 6 experiments

Magnetic nanoparticles are able to cause oxidation of chromogen PDD in presence of hydrogen peroxide and the yellow-orange product is detectable by spectrophotometer at 450 nm. Absorbance of this solution rises with concentration of hydrogen peroxide. Michaelis-Menten kinetics fit was done to prove enzymatic character of this reaction (Hill equation with *n* fixed to 1 in Origin 8) and is depicted in **Figure 5**. The K_M value was found to be 174 mmol·L^{-1}.

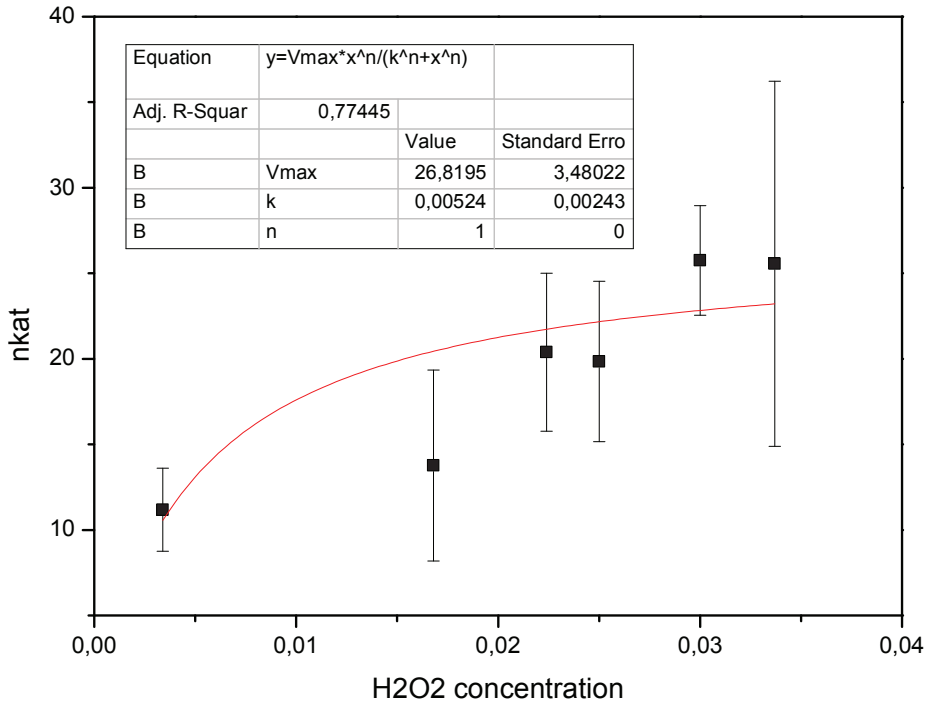

Figure 5 Michaelis-Menten type of kinetics for concentration-dependent detection of hydrogen peroxide. Averages and standard deviations for 5 experiments

4. CONCLUSION

In our previous studies we have shown that magnetic nanoparticles can be useful for delivery of various synthetic or nutraceutical antioxidants [14,15]. Our new results obtained in this paper imply that functional magnetic nanoparticles may offer a promising therapeutic approach to combat neurodegenerative diseases - first as a destructor of amyloid plaques, and also due to their inherent peroxidase activity. This shows great potential as a replacement of natural enzyme in a brain.

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