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Different Signals of Magnetic Resonance Imaging and Ultrasound from Substantia Nigra in Parkinson's Disease and Control — Is Iron the Cause?

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Motor symptoms of Parkinson's disease are caused by a progressive degeneration of substantia nigra, a small structure located deep in the brain. The cause of this process is unknown but may be related to iron mediated oxidative stress. The aim of this study was to understand the mechanism of the change of magnetic resonance and ultrasound signals found in patients with Parkinson's disease, which were attributed by several authors to an important increase of the concentration of iron in substantia nigra. USG and MRI measurements were performed on phantoms simulating human brain to which high amounts of iron were introduced. The USG signal was unaffected by insertion of iron-loaded ferritin, while it was by insertion of glial tissue. Injections of iron-loaded ferritin and iron ions to the phantoms decreased T_2 relaxation time. Our results suggest that the observed change of the signal from Parkinsonian brains is probably due to a proliferation of glia and not to an increase of the concentration of iron.

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1. Introduction

Parkinson's disease is one of so-called neurodegenerative diseases. In these diseases nervous cells degenerate in specific brain areas. In Parkinson's disease (PD) it is substantia nigra (SN), a small structure of about 500 mg located in mesencephalon.

The causes of neurodegeneration remain unknown. One of the hypotheses is oxidative stress, in which iron plays an important role [1]. Divalent iron triggers the Fenton reaction producing free radicals [2]. The overproduction of free radicals may lead to the death of nervous cells via DNA related apoptosis or cells membrane destruction necrosis. Indeed some studies showed an important increase of the total iron concentration in Parkinsonian SN compared to control. Also the differences between PD and control found in magnetic resonance (MRI) studies and with the use of transcranial sonography (TCS) by several authors were attributed by them to this difference in the concentration of iron in SN [3–5]. However, our own Mössbauer spectroscopy studies did not show any difference in the total concentration of iron between PD and control SN [6]. According to our results most of iron in SN is located within ferritin, a protein structure composed of H and L chains of aminoacids forming a kind of shell, whose main role is the safe storage of iron [7]. On the other hand, we demonstrated a significant increase of the labile non ferritin bound iron in Parkinsonian SN. The concentration of this iron is 2000 times smaller than that of the total iron [8]. The difference in MRI consists in decrease of T_2 and the one found

by TCS is related to an increased echogenicity of SN in PD. Figure 1 shows the signal of MRI and Fig. 2 the TCS presenting substantia nigra.

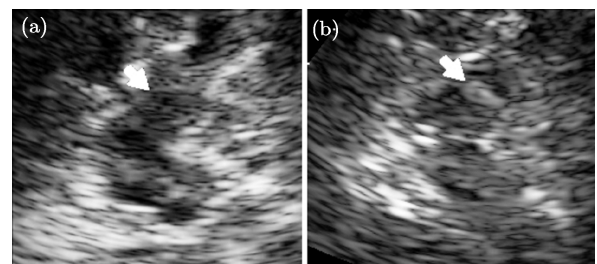


Fig. 1. Human midbrain image as depicted by TCS. (a) Healthy control, no hyperechogenicity in the anatomical localization of the SN (arrow). (b) Hyperechogenicity of the SN in the PD patient (arrow). Our own material.

Based on our studies and assuming that the difference in the TCS and MRI could not be related to the difference of the concentration of the total iron in SN we decided to perform ultrasound and MRI experiments for elucidation of those differences.

We investigated with the use of ultrasound technique and MRI the phantoms, which simulated properties of the human brain. For the TCS we used porcine brain and for MRI — a solution containing biochemical structures present in human brain.

2. Material and methods

For TCS experiment we used easily available porcine brains, whose structure may imitate the human one. In the MRI study the phantom consisting of plastic bottle

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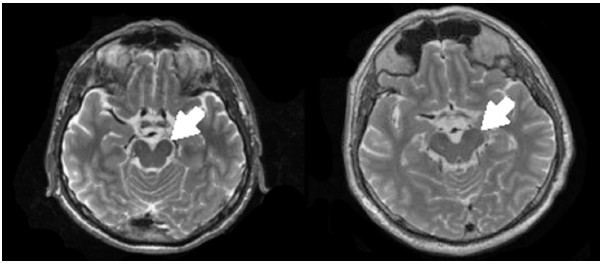


Fig. 2. T_2 — weighted image of human head. Left — healthy control. Right — PD patient. The arrows show substantia nigra. Our own material.

containing one liter of water solution of five metabolites present in human brain grey matter tissue was used. Aim of those experiments was to reproduce conditions of physical examination.

2.1. The ultrasound study

The porcine brains were used within 12 h after the death of the animal. Two experimental procedures were applied. In the first one ultrasound measurements were made before and after injections of 100 mg of ferritin containing high amount of iron (4.0 ± 0.2 mg/g). In the second experiment the examination was performed before and after insertion of glial tissue obtained from neurosurgical operations. Experiment was performed on Esaote MyLab 70XVision ultrasound scanner equipped with 13.4 MHz linear-array probe working in B mode. Echogenicity was assessed with the use of grey level scale.

2.2. MRI study

The MRI study was made in two stages. In the first one ferritin was inserted into the phantom and the transversal relaxation time (T_2) was measured as a function of the concentration of added ferritin. It is well known that in water solutions influence of ferromagnetic particles on relaxation time is much stronger than in a tissue. Therefore iron concentrations used in this part of the experiment were two hundred times smaller than those in human SN.

In the following stage of the experiment ferrous ions (which were supposed to imitate the labile iron) were injected into the same phantoms and again T_2 was measured as a function of the concentration of iron. MRI measurements were performed on phantom containing increasing ratio of labile to ferritin bound iron ranging from $r = 0$ (no labile iron) to $r = 8.6$.

Experiment was performed on General Electric 1.5 T Signal MR/I ECHOSPID 1 with a head coil. Spin echo ($T_E = 15, 30, 45, 60$ ms; $T_R = 500$ ms) pulse sequence was used. Magnetization was always measured in the centre of the phantom.

3. Results

3.1. The ultrasound study

Grey level profiles of animal model before and after ferritin infusion are shown in Fig. 3. In the area of infusion (i.e. from 55 px to 110 px) no change in echogenicity

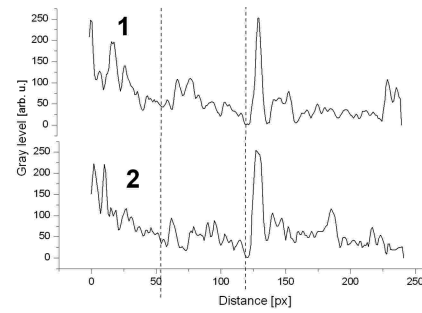


Fig. 3. Animal model echogenicity profile. 1 — plain model, 2 — the same model after ferritin injection. Highly echogenic area between 0 and 40 px is a skin and fat which are imitating skull. High (means light on picture) and narrow peak about 125 px is a boundary between two brains. Area of infusion is between two markers.

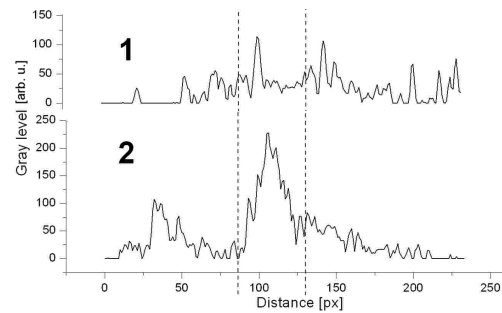


Fig. 4. Animal model echogenicity profile. 1 — plain model, 2 — the same model after glial tissue insertion. Top model layers are beyond investigated range so they are not visualised. High (grey level is 228), broad peak between 90 and 130 px corresponds to glial sample insertion area.

can be seen. Mean gray value of this area before infusion is 55 ± 21 and after 57 ± 24 .

The results of the experiment with glial tissue are shown in Fig. 4. Profile 1 corresponds to results of measurements before insertion and profile 2 corresponds to measurements after insertion of glial sample. High broad peak between 90 and 130 px on profile 2 reflects glial sample echogenicity. Mean gray level of insertion area is 39 ± 22 before and 118 ± 58 after the procedure of insertion of glial tissue.

3.2. MRI study

Results of MRI measurements are shown in Figs. 5 and 6. We present only the data related to T_2 relaxation time, as in the literature only this parameter was presented as different between Parkinson's disease and control. Relaxation times and their uncertainties were obtained from the fitting procedure described in [9].

Transversal relaxation time vs. ferritin iron concentration is shown in Fig. 5. First experimental point was obtained for plain phantom without added ferritin. Linear dependence between T_2 and ferritin iron concentration

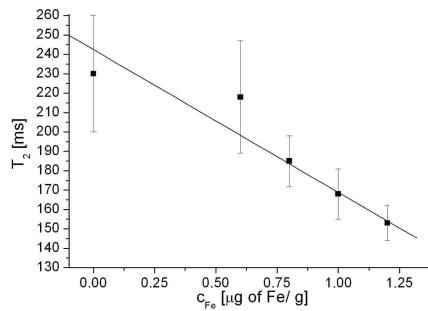


Fig. 5. Phantom transversal relaxation time vs. ferritin concentration.

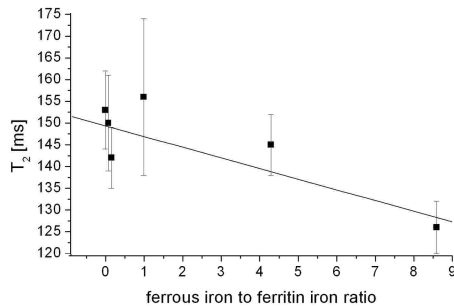


Fig. 6. Phantom transversal relaxation time vs. iron ions concentration to ferritin-like iron ratio.

can be noticed (correlation coefficient $R = -0.97$ with probability $p = 0.006$).

Transversal relaxation time vs. ferrous ions is shown in Fig. 6. Results are presented in units of ratio between ferrous iron and ferritin-like iron concentrations. Within experimental errors there is no measurable change in T_2 for r less than 1. Linear correlation could be also seen ($R = -0.87$, $p = 0.02$).

4. Discussion

From the physics point of view, if the main difference between Parkinsonian and control substantia nigra consists in an important increase (even 200%) of the concentration of iron, we should expect a change in the MRI signal (a decrease of T_2 relaxation time) but no change in the ultrasound signal. Our experiments on the human brain phantom did confirm this. However, one should keep in mind the results of studies using MRI published in the literature. Although the authors present the decrease of T_2 in PD as an important one, a more careful

look at the numbers may provoke some doubts. Antonini et al. have found the T_2 for PD patients as 67.5 ± 2.9 and 70.6 ± 3.7 for controls [10]. Such a small change could not be attributed to an increase of the concentration of iron exceeding tens of percent. Therefore one should look for another explanation of this decrease of T_2 in PD.

The results of our experiments suggest the possibility of an involvement of the proliferation of glial cells as a cause of hyperechogenicity of Parkinsonian SN. It could also be that the same change of the structure of SN in the disease causes the observed small decrease of T_2 found in MRI studies in patients with PD. This hypothesis needs, however, confirmation in experimental studies.

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