Brain Metabolism Disturbances in Case of Lung Diseases - In Vitro NMR Studies of Mice Brain Extracts

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Abstract

Metabolic changes in the central nervous system in case of disease outside the brain were investigated using ¹H MRS. Examinations were performed on mice with generated lung metastases (M2) and healthy mice (M1) for comparison. Brain extracts were obtained using the Folch method.

The results indicate altered brain metabolism in case of mice with lung metastases versus healthy ones. The main differences are observed within the spectral range of 3.19 - 3.29 ppm encompassing signals due to cholines and taurine. The metabolic changes indicate hypoxic demyelization and brain homeostasis disturbances.

Keywords: brain metabolism, ¹H NMR spectroscopy, animal model studies

Introduction

Lung cancer is the most frequent cause of morbidity among cancers. Our earlier ¹H MRS in vivo studies of patients with diagnosed non-small cell lung cancer revealed that brain metabolism is markedly disturbed [1]. However, due to non-homogeneity of the human group (differences in age, genetically and dietary determined factors, etc.) and the complexity of the living systems the in vivo results are difficult to be interpreted.

The high resolution NMR studies provide a basis for a better understanding of in vivo results and improve their clinical relevance. ¹H MRS animal model studies employing higher fields and providing a better signal to noise ratio than in human in vivo studies enable to obtain more insight into brain metabolism.

In the current work the high resolution ¹H MRS was employed to investigate the brain metabolic disturbances in mice with generated lung metastases.

Experimental procedures

Two groups of mice were studied: one with the generated lung metastases (M2) and the healthy one (M1), for the comparison. The groups contained 18 and 21 mice, respectively. The brain extracts were obtained using the Folch method[2].

The high resolution ¹H NMR spectra of hydrophilic and hydrophobic fractions were acquired using Varian Inova-300 multinuclear pulsed NMR spectrometer operating at the ¹H resonance frequency of 300 MHz.

The spectra were normalized to 1, than the integral intensities were calculated in MestreC (Mestrelab Research, Spain).

Statistical calculations were performed using the Mann-Whitney U-test. The results were statistically significant when p < 0.05.
Results

The spectral analysis of the extract hydrophilic fraction reveals the distinct reduction of the alanine and choline integral intensities in the M2 group versus M1 group (for alanine: from 0.72 in M1 to 0.58 in M2, p<0.05, and for choline: from 6.23 in M1 to 5.25 in M2, p<0.05) and the statistically important decrease of the choline/(creatine + phosphocreatine) ratio (from 1.15 in M1 to 1.04 in M2, p<0.05). Along with the quantitative differences, the qualitative ones are also seen in the range between 3.19 and 3.29 ppm (Figure 1a).

In case of the hydrophobic fraction of the extract the increase in the signals at 1.50 ppm due to -CH\(_2\) group of cholesterol and fatty acids (from 6.09 in M1 to 6.84 in M2, p<0.03), and the decrease of the signals at 3.20 ppm due to phospholipids (from 0.49 in M1 to 0.32 in M2, p<0.03) takes place (Figure 1b). The changes in the integral intensities of the other signals have no statistical importance.

Discussion of results

As reveals from the high resolution NMR studies of the water soluble fraction the cho/(creatine + phosphocreatine) ratios and the normalized integral intensities of the signals due to alanine and cholines differ in both the animal groups. The strongest differences are observed in the range of 3.19 – 3.29 ppm encompassing the signals due to cholines, and taurine. The choline containing compounds visible in the in vitro studies include free choline, glycerophosphorylcholine (GPC), and phosphorylcholine (PC) [3].
In case of the water insoluble fraction the changes in the cholesterol, fat acids, and phospholipids levels take place.

GPC and PC are known to be the intermediates in the phospholipids synthesis and degradation [4]. Their changes in combination with variation in phospholipids levels suggest disturbances in the membrane lipids metabolism.

In conclusion, the results of the in vitro model studies show distinct differences of the brain metabolites’ concentrations in the group of mice with generated lung metastases versus the healthy one, which seems to confirm the in vivo observations.

Combining the results obtained for both the hydrophilic and hydrophobic fractions enables the quantitative and qualitative changes in the hydrophilic fraction to be explained in terms of the disturbances in the membrane lipids metabolism.

References