Short-term metabolic changes in rat brain after intravenous CDP-Choline administration studied by means of localised 31P and 1H MR spectroscopy in vivo

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INTRODUCTION. It was shown by Grieb et al. [1] that CDP-choline (CDPCho) given in daily doses of 500 mg/kg for five days produced significant protection of CA1 neurons against delayed post-ischemic death in gerbils. Similar reports were published by Rao et al. [11]. Sato et al. [12] reported lack of protection of CA1 in the rat after intravenous injection of CDPCho at doses of 76.1 and 152 mg/kg. This study was designed to clear some aspects of thesis reported by Grieb et al. [1] using ^{1}H and ^{31}P MR spectroscopy *in vivo*. The aim was to monitor concentrations of choline (Cho) and inorganic phosphate (Pi) in the rat brain after CDPCho administration. Only a few similar studies were performed in humans with oral choline and in animals with different choline-containing compounds using ^{1}H MRS, but the results contradicted each other [2-5]. So far no reports of using ^{31}P MRS *in vivo* for studying short-term metabolite changes (i.e. during few hours after administration) after CDP-choline administration were encountered in scientific papers. The only ^{31}P MRS long-term study (i.e. during few days of choline administration) was performed on humans by Babb et al. [13].

METHODS. The *in vivo* MR spectroscopy was performed using 4.7T MR research system with MARAN DRX console from Resonance Instruments and MR probe based on double-tuned (^{1}H - *200 MHz* and ^{31}P - *81 MHz*) eliptical head surface coil. Spatial localization was performed by means of surface coil.

^{1}H MR signals were acquired with water suppression sequence based on spin-locking pulses [7] with TR/TE of 1s/200ms. EXORCYCLE [8] phase cycling for additional water suppression was used. ^{31}P MR signals were acquired with excitation-acquisition (FID) sequence with TR of 5s. Total acquisition time was 8.5 minutes for every analysed ^{1}H MR signal, and 9.5 minutes for every ^{31}P signal.

20 different healthy male *Wistar* rats of body weight 250-280 g in four groups were examined in this study. All the rats were cannulated before the experiment. Solution of CDPCho was administered intravenously in two subsequent doses of *1 g/kg* each, given every hour after starting MR signal acquisition. For control study saline was administered the same way like CDPCho. Volume of each dose of saline had exactly the same volume as CDPCho and was 0.25ml. Anaesthesia was induced by chloral hydrate (*500 mg/kg intraperitoneally*), and was maintained with a mixture of halothane (*1.5%*) with air (*40%*) and oxygen O_2 (*60%*). ECG, breath and temperature of rats were monitored during the whole examination. The total duration of a complete single examination was about 2 hours. Reference signal was acquired before any intravenous injection. MR signal acquisition started 2 minutes after each subject was injected CDPCho and was repeated every 10 minutes.

Time series of MR signals were analysed in time domain with jMRUI software package [9,10]. ^{1}H MR signals were deconvoluted using unsuppressed water signal as a reference prior to signal analysis. Deconvoluted signals were then fitted in time domain using AMARES method. ^{31}P MR signals were fitted using AMARES utilizing prior knowledge and estimated amplitudes of signal components were T_1 corrected. Relative metabolite concentrations Cho/Cr, Cho/NAA, PCr/Pi, PCr/ATP and Pi/ATP were calculated. Metabolite ratios were compared between the measurements taken after each CDPCho injection and the reference. Results were averaged over the group (5 animals in every group). Statistical analysis inside the group were performed using non-parametric signed-rank Wilcoxon test with significance level of $p<0.05$. Statistical analysis between groups was performed using Mann-Whitney U test.

RESULTS. ^{1}H-MRS study: Statistically significant ($p<0.05$) increase of the Cho/Cr ratio after first and second dose of CDPCho were observed (see Fig. 1). The average Cho/Cr values were 0.834 before (reference) and 0.926 after first injection, as well as 0.834 before first and 0.956 after second injection of CDPCho. There was also a statistically significant ($p<0.05$) increase of the Cho/NAA ratio after first injection of CDPCho and statistically not significant ($p>0.05$) increase after second injection. The average Cho/NAA values were 0.364 before and 0.400 after first dose, as well as 0.364 before first and 0.380 after second dose of CDPCho.

^{31}P-MRS study: Pi/ATP increased from 0.292 (reference) to 0.447 after first injection and to 0.473 after second injection of CDPCho (see Fig. 1). Both of these changes were statistically significant ($p<0.05$). The PCr/Pi ratio decreased from 0.943 (reference) to 0.908 after first and to 0.890 after second dose of CDPCho. However there was no statistical significance ($p>0.05$) in this case. In addition, statistically significant ($p<0.05$) differences were observed for the Pi/ATP ratios between control and CDPCho group. The average Pi/ATP values were 0,304 in the control and 0.422 in the CDPCho group after first injection, as well as 0.314 in control and 0.546 in CDPCho group after second injection.

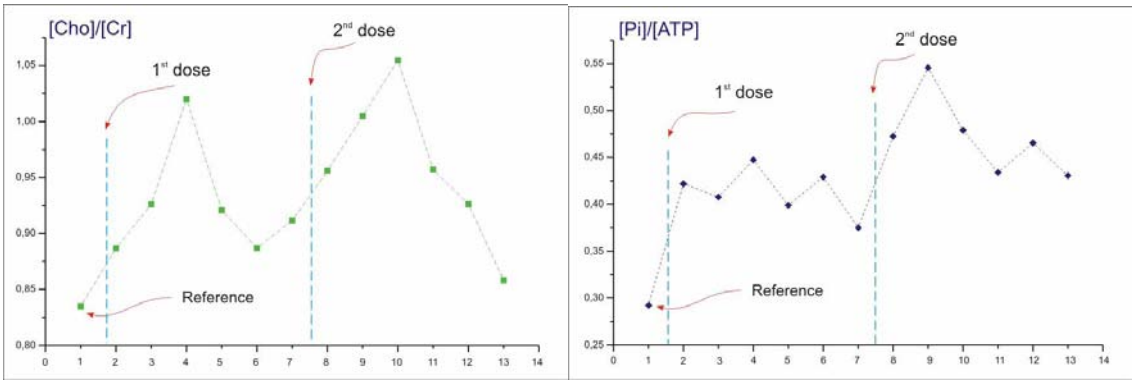

Fig 1. Average metabolite ratios over five animals during the CDP-Choline experiments. *Timescale: 1 - reference taken before any CDPCho injection; 2- measurement started 2 min. after first CDPCho injection; 3,4,5,6,7 - consecutive measurements started every 10 min.; 8 - measurement started 2 min. after second CDPCho injection; 9,10,11,12,13 - consecutive measurements started every 10 min.*

Conclusions

Short-term increase of Pi/ATP and Cho/Cr in rat brain after intravenous CDP-Choline administration in doses of 1 g/kg were detected using ^{31}P and ^{1}H MRS *in vivo*. Statistically significant increase of Pi/ATP was visible immediately after the first CDPCho administration while significant increase in Cho/Cr ratio was clearly visible after the second administration. No changes of Pi/ATP and Cho/Cr were detected for a control group. 31P MRS *in vivo* seems to be complementary to 1H MRS and more sensitive than 1H MRS for studying metabolic changes in such short-term studies. Obtained results should help to explain mechanisms underlying neuroprotective properties of CDP-Choline.

References

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