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Protective role of Creatine in chronic hepatic encephalopathy developing brain: in vivo longitudinal ^{1}H and ^{31}P-MRS study

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Synopsis

Type-C hepatic encephalopathy (CHE) is a complication of chronic liver disease (CLD). It is known that children are more affected by CLD than adult patients. Bile duct ligated rat (BDL) is a model of CLD-induced CHE. It was shown that rats having acquired CLD as pups display more profound neurometabolic disturbances than adults. Cr-treatment showed a positive effect in youngP21 BDL-rats resulting in less pronounced metabolic changes. Our aim was to test whether Cr-supplementation dampens the changes observed in CHE in a longitudinal model of CLD acquired in early childhood-P15 and if these changes are similar to those in P21-rats.

Introduction

Type-C hepatic encephalopathy (CHE) is a complication of chronic liver disease (CLD), difficult to diagnose in its early stages. It is known that children are more affected by CLD and its related toxic accumulation of ammonium (NH4+) and glutamine (Gln) than adult patients, with long-lasting cognitive deficits after liver transplantation[1–4].

The bile duct ligated rat (BDL) is a model of CLD-induced CHE validated in the adult and developing brain[5,6]. It has been shown that rats having acquired CLD as pups display more profound neurometabolic disturbances than adults with the same disease (more important Gln increase, stronger osmotic and antioxidant response, stronger decrease of total-creatine(tCr))[6]. A decrease in tCr has been shown in developing rat brain-cells aggregates after NH_4^+ exposure, due to downregulation of Cr synthesis[7]. As such, exploring methods to efficiently sustain Cr concentration in CHE may have potentially far-reaching clinical implications.

Recently differences in brain metabolic changes between post-natal 15(P15) and post-natal 21(P21) operated BDL-rats have been shown, suggesting that age of disease onset and its coincidence with neurodevelopmental processes play an important role and may result in different vulnerability to the disease[8]. Moreover, NH_4^+-induced impaired axonal growth was shown to be rescued by Cr-treatment in organotypic 3D brain cell cultures[9] and P21 BDL-rats treated with Cr showed less pronounced metabolic changes[10].

Therefore, we hypothesized that high Cr diet might be beneficial in CHE in P15 BDL-rats. Our aim was to test whether oral Cr supplementation dampens the neurometabolic changes observed in CHE in a longitudinal model of CLD acquired in childhood and if these changes are similar to those in P21 rats.

Methods

BDL surgery was performed on 6 male Wistar rats at P15. BDL rats were compared with sham(n=6) operated animals at the same age to consider the ongoing brain development and were divided into 4 groups: BDL(n=3), BDL+Cr(n=3), sham(n=3), sham+Cr(n=3). Rats from treated groups received high Cr supplemented diet with a concentration of 40g/kg.

1H-MRS and 31P-MRS and blood tests were preformed longitudinally every two weeks(week2-4-6).

MRS experiments were done using a 9.4T-system(Varian/Magnex Scientific) together with home-built coil (quadrature ^{1}H-loops with a single ^{31}P-loop). ^{1}H-MRS spectra were acquired in hippocampus (2x2.8x2mm^3) using the SPECIAL sequence (TE=2.8ms)[11]. First and second order shims were adjusted using FASTMAP[12]. ^{31}P-MRS spectra were acquired using a non-selective AHP pulse for excitation, localized by OVS(x,z) and 1D-ISIS(y) (TR=8s, 384avg.), WALTZ-16 for NOE and ^{1}H-decoupling in VOI=5x9x9mm^3. ^{31}P-MR spectra were quantified using AMARES(jMRUI)[13] and normalized for each rat using its PCr concentration from ^{1}H-MRS acquired in VOI=4x7.5x6.5mm^3 centered in ^{31}P-VOI.

Results and discussion

All BDL-operated rats showed increase in plasma-bilirubin and blood-NH_4^+ validating the presence of CLD. Some improvements in the neurometabolic profile were noticed for BDL rats under Cr-treatment when looking at the differences Sham/BDL vs Sham+Cr/BDL+Cr. Cr-treatment seemed to restore the decrease in Cr and tCr causing a higher Cr in BDL+Cr(+13% at week6). Of note, all treated rats seem to show higher Cr concentrations in the brain following treatment, probably due to the more permeable and immature BBB at this age. Decrease in Asc concentration is a recently shown hallmark in HE (here not yet significant due to small n) and as previously shown[8] appeared later in P15-BDL rats(week6) compared to P21-BDL rats(week4). Cr-treatment restored Asc in BDL rats emphasizing the antioxidant role of Cr[14]. Treatment seems to have a positive effect on other osmolytes(Ins,Tau and tCho) which appear to have a less significant decrease in BDL+Cr than in BDL even though Gln is as high in treated rats as in non-treated (Fig.1).

This study confirmed a previously shown delayed increase in glutamine(Gln), as a consequence of NH_4^+ detoxification, for P15-BDL rats probably caused by immature glutamine synthetase[15] enzyme at a very early age delaying the NH_4^+ detoxification. There was no effect on Gln due to Cr-treatment for the P15-BDL rats(Fig.1), although a positive impact of Cr-treatment was seen only at week8 in the P21 rats[10]. Because of high impact of NH_4^+ on an immature brain, P15-BDL rats didn't survive so late in the disease.

Treated BDL rats showed a stable PCr concentration at week4(ns, BDL+Cr-vs-Sham+Cr) compared to BDL without treatment which displayed a significant decrease (*p<0.05, BDL-vs-Sham), with this effect diminishing at week6. Treatment had no effect on ATP, contrary to P21 results which showed a positive effect of Cr for both PCr and ATP at week6[10]. However, treated BDL rats have a more stable tNAD pool (similar to sham/sham+Cr) compared to non-treated ones. Higher variations in NADH and NAD+/NADH ratios indicate a more unstable redox state for non-treated BDL rats indicating an increased oxidative stress (Fig.3).

Conclusion

Our preliminary results showed an improved neurometabolic profile due to Cr supplementation emphasizing the antioxidant role of Cr. We know that there is a different vulnerability to the disease depending on age in CHE and Cr-supplementation seemed to confirm this vulnerability by a different response between P15 and P21-BDL rats (Gln, PCr, ATP). The positive effect on Asc and other osmolytes point towards the need of combinatorial

treatments in CHE. Additional studies are required to confirm our results and to investigate if these differences in neurometabolism due to Cr-supplementation translate also into different neurological outcome.

Acknowledgements

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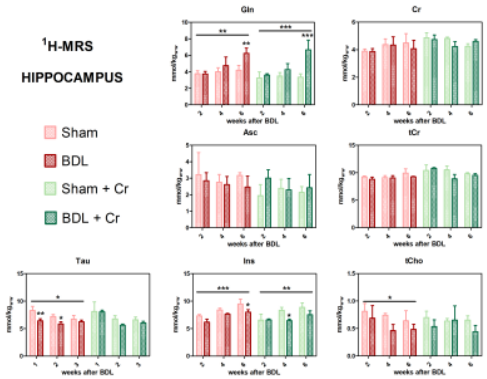
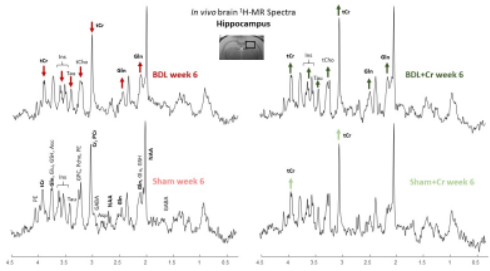
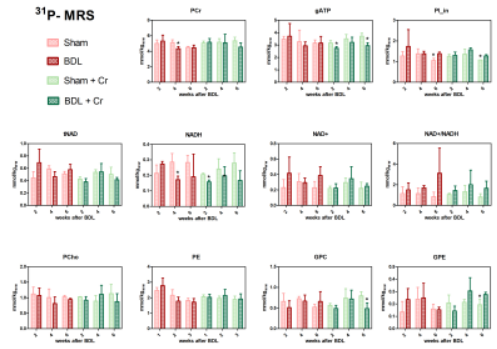
Figures



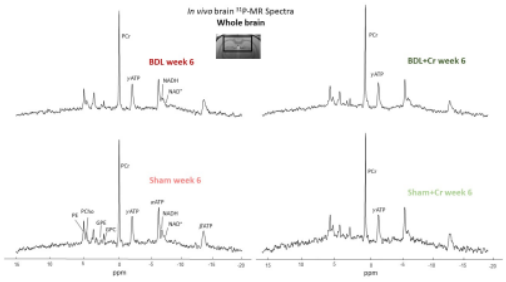
Brain metabolic changes measured longitudinally by ^{1}H-MRS in hippocampus at week2, week4 and week8 after BDL. Rats which received Cr treatment (shown in green) show higher Cr concentrations in the brain. Treatment restored Asc in the BDL rats and had a positive effect on the osmolytes (Ins, Tau and tCho). Diseased rats where always compared with sham operated rats (sham vs. BDL and sham+Cr vs. BDL+Cr) using 2-way ANOVA with Bonferroni correction (*p<0.05, **-p<0.01, ***-p<0.001, ****-p<0.0001).



In vivo ^{1}H-MR spectra from hippocampus (VOI=2x2.8x2mm^{3}) acquired at week 6 after BDL or sham surgery, each spectrum is from one animal. Spectra clearly show increased tCr in both BDL+Cr and Sham+Cr. The positive effect of Cr treatment on other osmolytes (Ins, Tau and tCho) is also visible on BDL+Cr spectrum.



. Brain metabolic changes measured by ^{31}P-MRS measured in the whole brain longitudinally (week2, week4 and week6). Treated rats showed stable PCr at week4 compared to a decrease in the non-treated and treatment had no effect on ATP. Variations of tNAD, NADH and NAD+/NADH smaller in treated animals indicating a more balanced redox state. Diseased animals were compared with the corresponding sham operated animals using the unpaired students t-test (*p<0.05, **-p<0.01, ***-p<0.001, ****-p<0.0001).



In vivo ^{31}P-MR spectra from whole brain (VOI=5x9x9mm^{3}) acquired at week 6 after BDL or sham surgery, each spectrum is from one animal.

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