

# Studies of thiamine deficient rat brains with in vivo and in vitro proton NMR spectroscopy

Haakil Lee, George Holburn, Peter R. Martin\*, and Ronald Price

Department of Radiology and \*Psychiatry, Vanderbilt University Medical Center, Nashville, TN 37232

## Introduction

Thiamine, vitamin B1, is essential for aerobic glucose metabolism. Thiamine deficiency (TD), a frequent complication of alcoholism caused by decreased thiamine absorption, may result in brain damage known as Wernicke's encephalopathy. In chronic alcoholics, neurobehavioral deficits related to TD and brain glucose metabolism have been shown to recover during abstinence. However, the mechanism(s) whereby thiamine treatment facilitates recovery of brain functions during detoxification and continued abstinence and threshold and timing of reversibility with treatment are not fully understood. Our previous work (1) suggests that TD induced by pyriethamine causes reliable reductions in brain choline containing compounds, which are reversed in a dose-dependent fashion subsequent to thiamine administration. In this work we examine the changes in brain Cho-containing compound(s) during pyriethamine-induced TD in the rat and its reversal with the administration of thiamine, followed by chemical identification of specific choline compounds derived from brain extracts.

## Experiments

In-vivo localized proton MRS experiments were performed using a 4.7T/440 Spectroscopy Imaging Systems Corporation (SISCO) imaging spectrometer. Localized proton spectra were acquired from a ROI (4x4x4 mm) inside the brain using a STEAM sequence (TR/TE:3000/68ms). Body temperature was kept at 37°C throughout the experiment using circulating heated water. Normal (n=8), TD (n=8), and thiamine treated (n=8 with 5mg/Kg and n=8 with 100mg/Kg) rats were prepared as previously reported (1). Rats were sacrificed by microwave irradiation (5kW) of the head for a duration of 2sec. The brain was removed immediately and ground in a mortar containing liquid N<sub>2</sub>. Metabolite extraction was done according to a modification of the Blight-Dyer technique (2). In vitro spectra were acquired on a DRX500 Bruker spectrometer with TR=15sec, NA=8, 45° pulse width, and TSP as an internal standard for chemical shift and concentration.

## Results

Table 1. Concentration of metabolites in rat brain. high dose: 100mg/kg thiamine treatment, low dose: 5mg/kg thiamine treatment, mean±SD (mmol/wet g)

	GPC	PC	Cho	PCr/Cr	NAA
high dose	0.35(0.10)	0.23(0.10)	0.04(0.03)	6.81(0.71)	5.81(0.76)
low dose	0.30(0.10)	0.24(0.06)	0.04(0.02)	6.17(0.65)	5.53(0.60)
TD	0.12(0.03)	0.16(0.07)	0.08(0.04)	6.15(0.84)	5.52(0.89)
normal	0.41(0.09)	0.24(0.10)	0.05(0.03)	6.51(0.77)	5.92(0.89)

In TD rats, the change in Cho-containing compounds was reliably measurable in all animals by 12 days of pyriethamine injection, while no significant change occurred in either Cr or NAA peaks. It also shows a dose dependent increase/recovery in Cho when TD rats were scanned 2 hours after administration of thiamine. GPC, PC, and choline could be easily identified by their peaks at 3.24, 3.23, and 3.21 ppm, respectively, in the brain extract spectra. Table 1 shows the concentration of metabolites in four different groups of rats, demonstrating GPC was the main component responsible for the observed decrease and recovery in Cho peak in TD rat brain.

## Discussion

TD, a frequent complication of alcoholism, contributes significantly to alcohol-induced brain damage. Thiamine its active form, thiamine pyrophosphate, is the cofactor for three enzymes involved in glucose metabolism and TD results in decreased decarboxylation by  $\alpha$ -ketoglutarate dehydrogenase and pyruvate dehydrogenase leading to failure of ATP synthesis, and in diminished transketolase activity which impairs cellular capacity to produce sufficient quantities of biosynthetic reducing equivalents. In pyriethamine-induced TD in the rat monitored over time with proton magnetic resonance spectroscopy, we have shown a decrease in brain concentration of GPC and recovery in Cho with thiamine replenishment. These findings suggest that a reduction in GPC may be relevant to the primary biochemical lesion in TD resulting from impaired glucose metabolism, and are compatible with reduced catabolism of choline metabolites (4). Consequently, this data is compatible with the hypothesis that a decrease in choline compounds is the cause of the biochemical abnormalities which precedes neuroanatomical damage characteristic of Wernicke's encephalopathy.

## References

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