

Correlation of motor coordination with in vivo metabolite ratios in lysophosphatidyl choline induced demyelination model of multiple sclerosis: A sequential in-vivo proton MRS study

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Introduction: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder characterized by the presence of multifocal, white matter lesions in the central nervous system (CNS) which alter the tissue biochemistry and function. MR techniques (MRI and MRS) serve as an indispensable tool to gain insight into the disease characteristics. While MRI gives anatomical indication of the disease pathology, MRS provides information about the tissue metabolism and biochemical function related to the disease process and serves as a tool to evaluate the severity of the disease and understand its pathogenesis. Such studies are however difficult in humans and hence experimental model is necessary for sequential evaluation of the changes in metabolic function during the various stages of disease progression. Changes in metabolite ratios during different stages of de- and re-myelination in an animal model have been reported [1]. Several studies on MS patients have shown the association between neuronal dysfunction and changes in metabolite levels [2]. Hence, the objective of the present study was to study the correlation between changes in the motor activity with in-vivo metabolite ratios in an experimental model of demyelination at different stages of the de- and re-myelination.

Materials and Methods: Focal demyelination was induced in the internal capsule (ic) region of Wistar rats (n=5; body wt: 200-250 mg) by the stereotaxic injection of 0.2 µl of 1% lysophosphatidyl choline. Sequential single voxel MRS was carried out at 4.7 T (BIOSPEC Bruker BioSpin MRI GmbH, Germany) using the following parameters: TR/TE = 3000/20 ms, average = 512 and spectral width = 2000 Hz at different stages of the disease namely, pre acute (day 3), sub-acute (days 5 and/or 7), acute (between days 9 to 12) and remyelination (days 15, 20 and 26). Metabolite ratios were determined using LC model and the values are reported as mean ± standard deviation. The demyelinated rats were subjected to motor co-ordination tests (rotarod test, grip test, foot fault test and actophotometer test) to assess the changes in the motor activity during the different stages of demyelination (i.e days 6 and 11) and remyelination (days 22 and 26). A baseline study on day 0 prior to lesion creation in these rats served as control. The results of the motor performance test were reported as mean ± standard deviation. For foot fault test the % error was calculated using (number of foot fault steps/number of paired steps) x 100]. Correlation between the four motor coordination tests and metabolite ratios was assessed using Pearson correlation.

Results: Spectra obtained from ic area of rat brain showed resonances from metabolites such as N-acetyl aspartate (NAA), choline (Cho), creatine/phosphocreatine (Cr/PCr), glutamate/glutamine and lipid (see Fig. 1). During demyelination i.e. from day 3 till day 11, NAA/ Cr increased while Cho/Cr decreased (Figure 1) compared to controls (Table 1). The values subsequently reversed during remyelination (from day 15 onwards) and reached near normal values by day 26.

Photoactometer test: The activity on photoactometer significantly decreased during demyelination (day 6 to day 11) and subsequently increased during remyelination (day 22 and day 26; Table 1). Strong positive correlation was observed between photoactometer test and NAA/Cr (r = 0.93) while a negative correlation was observed with Cho/Cr (r = -0.52).

Rotarod test: Activity on rotarod, i.e. the time spent on the rotor decreased significantly from day 0 till day 11 and subsequently increased from day 20 reaching normal value by day 26 (Table 1). Positive correlation was observed with NAA/Cr (r = 0.99) (Figure 2).

Foot fault test: Significant increase in the % error in foot fault was observed on day 11 compared with day 0. On day 26, the error % reduced and was similar to that observed for control rats (Table 1). A negative correlation was observed with NAA/Cr (r = -0.73) and Cho/Cr showed a positive correlation with foot fault test (r = 0.38).

Grip Test: The mean score for grip test significantly decreased from day 0 to day 6 till day 11 (Table 1) and by day 26 the values considerably increased. Pearson correlation showed a positive correlation with NAA/Cr (r = 0.47) and a negative correlation with Cho/Cr (r = -0.82).

Discussion: Our data showed that the motor activity decreases with the reduction in NAA/Cr during the progression of demyelination and subsequently increased during remyelination. In contrast, Cho/Cr increased with decrease of motor activity during demyelination and reversed during remyelination. During the progression of the lesion, temporary destruction of the myelin occurs leading to neuronal damage. In this study, LPC injection was focused on the ic area, which consists mainly of motor fibers, hence any damage to the fibers in this region, such as demyelination, will reflect on the motor activity. Since NAA is considered a marker for neuronal integrity, decreased NAA can be correlated with the disability. As expected, in our study, NAA/Cr showed a strong positive correlation with rotarod and photoactometer activity as well as with grip strength while a strong negative correlation was observed between NAA/Cr and foot fault test. This correlation indicates that as demyelination progresses, due to myelin damage resulting in reduced NAA, the activity on rotarod and photoactometer decreases, grip strength weakens and the foot fault error increases. Studies have shown association of NAA levels with neuronal dysfunction [2] and its increase with improvement of disability in MS patients [3] suggesting that NAA levels may also reflect improvements in neuronal energetics and remyelination. Reduced NAA leading to neuronal disability have also been observed in a rat model of ischemia which returned to normal values with the improvement of neurological function [4]. Cho is present in myelin sheath and in membranes as a polar head group of lipids and serves as an indicator of inflammatory process as in demyelination. Hence, damage to the myelin sheath leads to increased Cho/Cr due loss of membrane structure which in turn leads to decreased motor coordination. Cho/Cr showed a negative correlation with rotarod and photoactometer activity and grip strength and positive correlation with foot fault test. Thus, our study aids in understanding the biochemical alterations occurring during the various stages of de- and remyelination which is responsible for motor incoordination.

References: [1] Degaonkar MN et al. NMR Biomed. 2002; 15: 293-300[2] Ruiz-Pena JL et al. BMC Neurology 2004; 4: 8. [3] De Stefano N et al Magn Reson Med. 1995; 34: 721-727. [4] Van der Zijden JP et al. Brain 2008; 131: 2209-2219.

	day 0 (n = 5)	day 6 (n = 5)	day 11 (n = 4)	day 22 (n = 4)	day 26 (n = 3)
NAA/Cr	1.70±0.25	1.39±0.56* (day 5)	1.22±0.15	1.67±0.18* (day 20)	1.68±0.62
Cho/Cr	0.58±0.39	0.68±0.50* (day 5)	1.22±0.17	0.94±0.14* (day 20)	0.85±0.24
Rotarod test (ms)	144.0±30.5	78.8±23.2	65.0±10.9	108.8±20.1	125.3±25.0
Grip strength test	4.6±0.5	3.6±0.5	2.5±0.6	2.8±0.5	3.7±0.6
Photoactometer (ms)	140.2±59.9	84.2±52.3	61.2±19.7	89.3±7.8	117.7±12.7
Foot fault (% error)	20.3±6.6	49.4±4.5	57.5±15.6	44.1±10.2	19.7±4.3

Table 1: Motor activity and metabolite ratios at various stages of de- and re-myelination *denotes the day MRI was done.

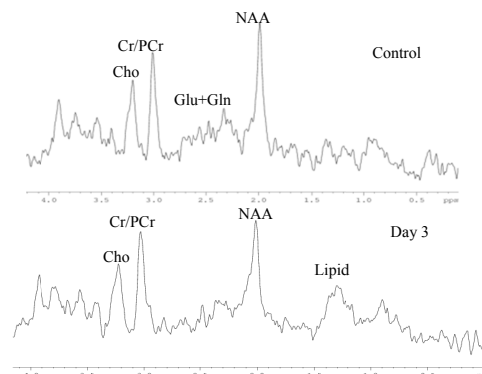


Figure 1: In vivo ¹H MR spectra from control and day 3 demyelination from rat brain

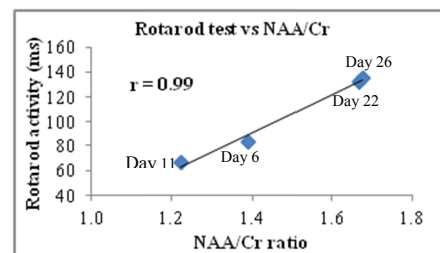


Figure 2: Correlation plot between rotarod test and NAA/Cr