Sequential correlation of relaxation times with motor functions in an experimental model of demyelination

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Introduction: Multiple sclerosis (MS) is a demyelinating disease characterized by neurological disabilities. The pathological features include inflammation, axonal loss, edema, parenchymal cellular infiltration and gliosis which tend to alter the water content which in turn affects the relaxation rates of these tissues. Conventional MR techniques show limited specificity of the underlying tissue damage and discrimination of the different stages of MS lesion. Relaxation measurements serve as a useful tool for the early detection of demyelination as well as monitor the changes associated with the lesion progression and regression. Hence measurements of relaxation times have been used as markers for monitoring the disease pathology in MS [1]. However, a detailed knowledge of the disease with respect to lesion development, progression and regression is essential in order to establish correlations with the functional disability [2]. Since such studies are difficult in humans, experimental animal models have been developed to understand the pathophysiology of the disease beginning from its onset in a detailed manner. Determination of relaxation times may allow clear demarcation of the lesion and thus provide information about the development during the early evolution of the lesion [3]. The objective of this study was to evaluate the changes in the relaxation times and correlate the results with motor coordination during the various stages of de- and re-myelination.

Materials and methods: Focal demyelination was stereotaxically induced in the internal capsule (ic) area of the brain of Wistar rats (body weight: 200-250 g) by the injection of 1% LPC. The lesion was identified at on T2-weighted (T2W) images using RARE (rapid acquisition with relaxation enhancement) sequence at 4.7 T. T2-mapping was carried out using multi-slice multi-echo (MSME) sequence with 16 echoes (TE =25 to 400 ms, with an increment of 25 ms), TR = 5 sec, slice thickness = 2 mm with no gap while T1 relaxation was measured using a spin echo sequence using the following parameters: number of TRs = 10 (500 to 5000 ms), TE = 20 ms, slice thickness = 2 mm with no gap. T1 and T2 values were calculated by selecting uniform circular ROIs of 4 pixels (area = 0.0039 cm^2) in the lesion area as well as the unaffected contralateral ic area on the respective maps generated. In addition to relaxation studies, the demyelinated rats (n=5) were subjected to four motor coordination tests (rotarod, grip test, foot fault and actophotometer) to assess changes in the motor activity during days 6 and 11 of demyelination and during remyelination on days 22 and 26. For control studies, a baseline study was carried out in these rats prior to lesion creation. The results of the motor performance test were reported as mean ± standard deviation. For foot fault test percentage (%) error was calculated as the number of foot fault steps/number of paired steps) and an average of the % errors was reported. Pearson correlation was carried out to assess correlation between motor tests with relaxation times.

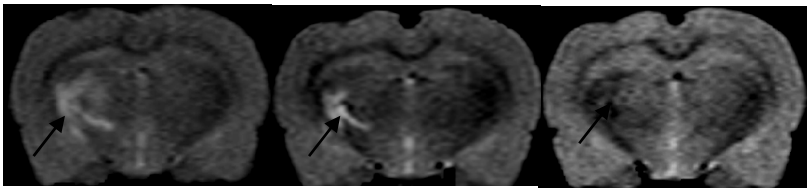

Figure 1: *Sequential T2W axial images of demyelinated rat on days 7, 11 and 26.*

Results: Our data revealed that T1 and T2 relaxation times progressively decreased during demyelination. The values subsequently increased during remyelination and reached normal values on day 26.

Photoactometer test: Significant decrease in activity on photoactometer was observed from days 6 to 11 compared to day 0. The activity subsequently increased during remyelination (day 20 and day 26; Table 1). A negative correlation was observed between photoactometer test with T1 (r = -0.94) and T2 (r = -0.99).

Rotarod test: Activity on rotarod decreased significantly from day 0 till day 11 and subsequently increased on day 20 reaching normal value by day 26 (Table 1). A negative correlation was observed between T1(r = -0.98) and T2 (r = -0.98) values and rotarod activity.

Foot fault test: % error in foot fault increased from day 0 till day 11 and subsequently decreased from day 20 (Table 1). Positive correlation was observed between % foot fault error and T1(r = 0.89) and T2 values (r = 0.97).

Grip Test: Mean score for grip test significantly decreased from day 0 to day 6 till day 11 (Table 1) and by day 26 the values increased. A negative correlation observed between grip strength T1 (r = -0.55) and T2 values (r = -0.62).

	day 0 (n = 5)	day 6 (n = 5)	day 11 (n = 4)	day 22 (n = 4)	day 26 (n = 3)
T2 values (ms)	66.4±4.4	92.6±6.4* (day 5)	103.8±14.0	83.6±10.6* (day 20)	68.3±9.4
T1 values (ms)	1275.1±112.7	1470.9±81.4* (day 5)	1618.2±119.3	1335.3±90.1* (day 20)	1261.4±143.9
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: *Relaxation times and motor activities at various stages of de- and re-myelination. * denotes the day MRI done*

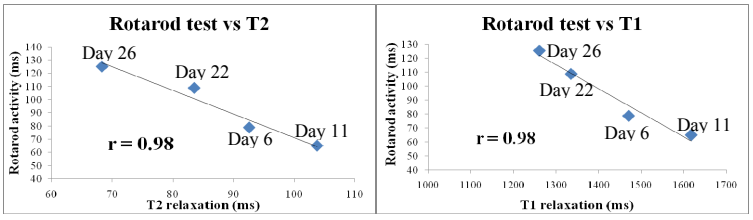

Figure 2: *Correlation plot between lesion volume and rotarod test and relaxation times*

Discussion: Longitudinal evaluation of the relaxation times at various stages of de- and re-myelination revealed a gradual increase in T1 and T2 during de-myelination and a subsequent decrease in these values during remyelination. The increase in relaxation times is attributed to the inflammatory response that occurs during myelin breakdown leading to an infiltration by the inflammatory cells in and around the lesion. Subsequent increase occurs due to alteration in the water content within tissues and changes in free water mobility. As a result, signal conduction along the fibers is disrupted. Since the fibers that pass through the ic area are motor axons, damage to these fibers would affect motor activity in the rats. This way change in relaxation times can be correlated with alterations in motor functioning. Our study showed a decrease in activity on rotarod, photoactometer and grip strength and increase in foot fault with increase in relaxation times indicating progression of demyelination. Subsequently, the rotarod and photoactometer activity and grip strength improved while foot fault error reduced during remyelination as the signal conduction along the motor fibers is restored resulting in improvement in motor functions. During this period, the relaxation times also decreased and reached near normal values by day 26 since the inflammatory responses disappeared and the extracellular water content reduced. Expectedly, rotarod and photoactometer activity and grip strength showed a negative correlation with relaxation times while foot fault test showed a positive correlation. Evidences exist that correlates MRI measures correlate with neurological disability in MS patients [2]. Thus our study demonstrates that relaxation parameters aid in understanding the mechanisms underlying the pathophysiology of demyelination.

References: [1] Larsson HB et al. Magn Reson Med. 1988; 7: 43-55. [2] Zivadinov R et al. J Neuroimaging 2005; 15: 10S-21S. [3] Degaonkar MN et al. Magn Reson Imaging 2005; 23: 69-73.