Monitoring of monocyte infiltration into the brain during experimental allergic encephalomyelitis by magnetic resonance imaging

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Abstract

MRI was used to measure the time dependence of BBB leakage and cerebral monocyte infiltration in a rat model of acute experimental autoimmune encephalomyelitis (EAE) in which transient neurological deficits develop from 11 to 17 days after immunization. Animals showed maximal BBB-leakage, explored with quantitative GDTPA enhanced T1W-imaging, 11 days after immunization. Monocyte infiltration was maximal, explored with quantitative USPIO enhanced T2-imaging and immunohistochemistry, 14 days after immunization (peak of the disease). We conclude that these techniques are sensitive to different aspects of disease processes occurring in EAE and they may be valuable tools to evaluate neuropathological status in MS.

Introduction

During multiple sclerosis, lymphocytes and monocytes gain entry to the central nervous system and form perivascular infiltrates, which is accompanied by enhanced permeability of the blood-brain barrier (BBB) [1]. Nowadays, MRI is the imaging modality for visualization of neuropathological changes in MS patients. Especially GDTPA enhanced MRI is a powerful diagnostic tool used in MS patients to detect a defective function of the BBB and new lesion formation [2]. Recently, ultra small iron particles (USPIO) have been shown to allow the detection of cerebral infiltration of cells of the mononuclear system [3]. Here we compare the use of GDTPA and USPIO enhanced imaging to elucidate the temporal events of BBB-opening and mononuclear cell infiltration in an acute model of experimental allergic encephalomyelitis (EAE) in the rat.

Methods

Animals: Acute EAE was induced in 8-11 week old male Lewis rats (n=40, 190-210g) by immunization with 20 µg guinea pig MBP in complete Freund’s adjuvant (CFA) [4]. Controls (n=10) were immunized without MBP. Neurological aberrations were scored daily and graded as 0: no EAE; 0.5, partial loss of tail tonus; 1, complete loss of tail tonus; 2, hind limb paraparesis; 3, hind limb paralysis; 4, death. MRI experiments were done at day 9, 11, 14 (peak of disease) and 17. CFA-controls were measured at day 14. After each MR-session spinal cord and brain tissue was collected and prepared for immunohistochemistry and electron microscopy. In every group 6 animals were prepared for USPIO imaging and 4 animals for GDTPA imaging.

MRI: MRI-data (35x0.75 mm slices; FOV 3.5x3.5 cm²; matrix 128x128, nt=2) were collected on a 4.7 T horizontal bore Varian NMR spectrometer. During experiments animals were mechanically ventilated with halothane (1%) in N2O/O2 (70/30). From all animals subjected to BBB status measurement the following MRI data sets were collected. Quantitative T2 maps which were the result of a mono-exponential fit of four heart-rate triggered (90°-pulse) multi-echo images (TR= 5000 ms; TE = 17.5 - 87.5 ms). GDTPA enhancement maps (GE-T1W) which were calculated from two T1W images (TR=1500, TE=60 ms) before and after a bolus of 0.5mmol/kg GdDTPA (i.v., 10 min in circulation) with GE-T1W =100*(( T1Wpost GdDTPA- T1Wpre GDTPA)/ T1Wpre GDTPA). Pixel-intensities thus display the % increase of the signal intensity of the T1W image due to GdDTPA leakage. Animals subjected to cellular infiltration studies received an i.v. bolus of USPIO 7228 (600 µmol/kg, Guerbet, France) 24 hrs prior to the MRI experiments. For these animals only quantitative T2 images were collected as described above.

Data-evaluation: Representative ROI’s were defined in the spinal cord, brainstem, basal ganglia and cortex. For all calculation pixels arising from ventricles were excluded by including only those pixels with T2<70 ms. As an objective measurement for cellular infiltration we defined in the T2 images of USPIO loaded CFA control animals at day 14 an area-specific threshold, which was the mean T2 value minus 2 x standard deviation. The percentage pixels below this threshold for a specific area was calculated in the USPIO loaded EAE animals.

Statistics: Data (presented as mean±SEM) were evaluated by one-way ANOVA, followed by bonferroni multiple comparison method t-test procedure (vs CFA-controls). P<0.05 was considered statistically significant.

Results

From 11 days after immunization, USPIO’s started to accumulate in the brain as was observed on T2W images (figure 1). Especially areas in the brainstem and basal ganglia showed massive accumulation of USPIO’s. The accumulation of USPIO’s paralleled with clinical score and monocyte infiltration as assessed by immunohistochemistry (figure 2). The results suggest that leakage of GDTPA for spinal cord and brainstem was maximal before the maximal clinical score was reached (day 14, ns figure 2). It is known in this model that the cortex remains free from lesion formation, which was in agreement with the absence of an increment of GDTPA leakage and absence of USPIO accumulation throughout the experimental time in this area. CFA-control values were comparable with day 9 values of the EAE animals.

Discussion

The results in this study suggest that in this model leakage of the BBB precedes the cerebral infiltration of monocytes. This sequential pattern has been shown to occur in EAE with invasive-techniques and has been suggested to occur in humans with MS [1]. In conclusion GDTPA- and USPIO-enhanced imaging represent valuable complimentary tools in the evaluation of the neuropathological status of animal models of multiple sclerosis and could prove useful in future clinical studies of human MS patients.


Figure 1. T2W images (TE=60ms) of animals that were acquired 24 hours after an i.v. bolus of 600µmol/kg 7228. Cerebral USPIO accumulation is most pronounced at the height of the disease.

Figure 2. Clinical score a) and the area of tissue showing accumulation of USPIO’s (- - - - , left axis) and leakage of GdDTPA (-O-, right axis) in b) spinal cord, c) brainstem, d) basal ganglia and e) cortex during the progression of EAE. * P <0.05 vs CFA-control.