Diffusion and perfusion in over-expression human AChE-transgenic mice: comparison with DNA microarray

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Synopsis

The couplings between gene expression and water diffusion in brain are unknown. In this study wild type control (N=19) and over-expressing human acetylcholinesterase (hAChE-Tg) mice (N=20) were used. Chronic excess of synaptic acetylcholinesterase (AChE-S) causes statistically significant suppression of the ADC of water measured in the brain of (hAChE-Tg) as compared with control mice. Contrast-enhanced perfusion MRI indicated poor brain signal recovery following gadolinium-DTPA. DNA microarray analyses unraveled over-expression of numerous ion channels, transporters and cell adhesion proteins. Our study chronic excess of AChE-S may play a central role(s) in the brain's maintenance of balanced water diffusion and ion transport.

Introduction

Transgenic animals are currently used to study different pathologies and the consequence of abnormal gene expression. Here we used T_1-, T_2- and diffusion-weighted MRI of wild type (wt) mice and are expressing human acetylcholinesterase (hAChE) in comparison with their gene expression obtaind from DNA microarray analysis.

Methods

The MRI experiments were performed on an 8.4 T spectrometer (Bruker, Germany) using a home built surface coil of 15x10 mm. The MRI protocol included T_1-weighted images, T_2-weighted images and a series of diffusion-weighted MR images. The diffusion protocol was performed on 8 wt controls and 8 over expressing human AChE transgenic (hAChE-Tg) mice. The multi-slice T_1- and T_2-weighted images were acquired using the spin-echo sequence with a field of view (FOV) of 2.5x2.5 cm, matrix dimensions of 256x128 and two averages. The repetition time and echo time (TR/TE) of the T_1- and T_2-weighted MRI images were 600/20 and 3000/50 ms, respectively. The diffusion weighted images were acquired using the stimulated-echo diffusion pulse sequence with the following parameters: TR=1800 ms, TE=41.6 ms, δ=8ms, Δ=100ms, two averages, 5 slices of 1.35 mm thickness with a 0.2mm gap and FOV and matrix dimensions as given above. The diffusion gradient strength, G, was incremented from 0 to 9.23 gauss/cm in 4 steps giving a maximal *b* value of 3.79×10^5 s cm^{-2}.

The perfusion MRI protocol a series of gradient echo images (T_2^* images) pre and post the Gd-DTPA injection. T_1-weighted images were acquired also before and after the injection of the contrast agent. The series of T_2*-weighted images were single slice (1.35 mm thick) gradient echo MR images acquired with TR=60 ms, TE=15 ms, one average, a field of view of 2.50x2.50 cm, and a matrix dimensions of 64x32. The total acquisition time per image in this series was 1 sec. At least sixty gradient echo images were acquired in each study. After the 10^{th} images, 0.42 mmole/Kg of Gd-DTPA was injected through the tail vein. The perfusion protocol was performed on 5 wt controls and 4 (hAChE-Tg) mice.

For the DNA microarray analysis the PFCs from four wt control and four transgenic (hAChE-Tg) animals were dissected on ice and their total RNA was extracted using the RNeasy mini kit (Qiagen, Hilden, Germany). MgU74Av2 Affymetrix mouse microarrays were used. Body temperature of wt controls (N=2) and transgenic (AChE-ty) (N=4) mice were measured using a chromel/alumel probe connected to a TES-1310 digital thermometer.

Results

The T_1- and T_2-weighted images of the control and transgenic mice did not show any observable morphological differences as shown in Figure 1. Figure 1 shows the apparent diffusion coefficient (ADC) maps of one slice in the brain of representative control and transgenic mice. When the ADC was compared between two groups in six different anatomic regions in the brain (cingulate cortex, motor cortex, somatosensory cortex, piriform cortex, striatum and preoptic area), significant differences were found in the motor cortex ($P < 0.03$), somatosensory cortex ($P < 0.04$), piriform cortex ($P < 0.001$) and striatum ($P < 0.009$). However, no significant difference was observed in the cingulate ($P > 0.09$) and preoptic area ($P > 0.1$). Even when the average ADC for the entire brain (ADC_{brain}) was computed for the two groups, in an automated way, we found a small but still statistically significant decrease in the averaged ADCs for the transgenic group ($P < 0.028$).

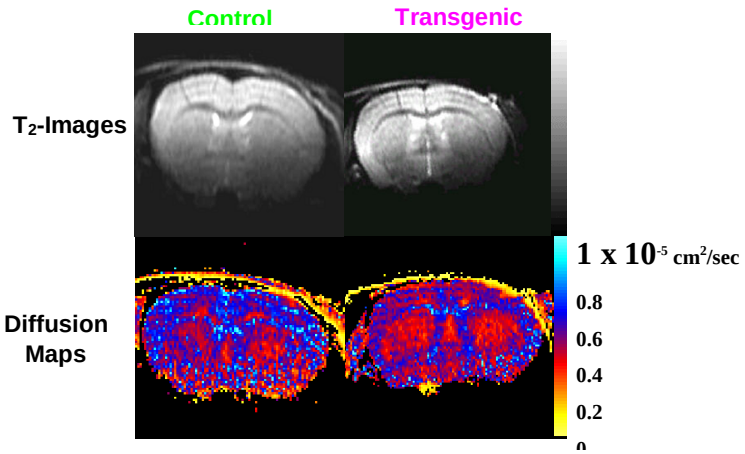

Figure 1

All control mice showed similar perfusion signal decay time course following Gd-DTPA injection (Fig. 2). A much more pronounced variability in the perfusion time course was observed in the transgenic mice. Figure 2 shows that signal intensity of the control mice reduced to 57 ± 5% of the initial signal intensity, after the Gd-DTPA injection, while the signal intensity of the transgenic mice reduced to a maximum of 48 ± 7 % of the initial signal intensity. The recovery of the signal intensity in the brain after the Gd-DTPA injection was also found to be different for the two mice groups. The recovery of the signal intensity of the transgenic mice group was found to be slower than the recovery for the control mice over the period of up to 100 s following the injection of the contrast agent. The differences between the T_1-weighted images pre- and post Gd-DTPA injection of the control and the transgenic mice imply that more contrast agent molecules are present in the brain of the transgenic mice which in turn might suggest a more leaky BBB in the transgenic mice as compared with the control mice. A comparison between the wt control and the hAChE-Tg PFC revealed over 200 genes with greater than 2-fold difference between the two groups most of which are ion channels, transports and adhesion proteins. The body temperature was found to be higher in the AChE-ty mice, which were found to have a lower ADC as compared with wt controls.

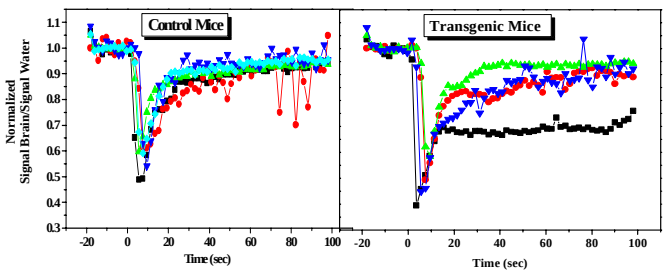

Figure 2

Discussion

Surprisingly, hAChE-Tg animals displayed lower baseline ADC values than their age-matched controls. ADC is a common parameter for assessing cerebral ischemia, cytotoxic edema as well as water dynamics and other pathologies in the CNS, but baseline differences in ADC between control and transgenic animals has not yet been reported. Here, we report a reduction of ADC as well as BBB leakage in the brains of hAChE-Tg mice as compared with control mice, correlated with profound changes in gene expression – mostly of brain channels and adhesion proteins.