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Apparent diffusion coefficient in the aging mouse brain: A magnetic resonance imaging study

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Abstract

Novel magnetic resonance imaging sequences have and still continue to play an increasing role in neuroimaging and neuroscience. Among these techniques, diffusion-weighted imaging (DWI) has revolutionized the diagnosis and management of diseases such as stroke, neoplastic disease and inflammation. However, the effects of aging on diffusion are yet to be determined. To establish reference values for future experimental mouse studies we tested the hypothesis that absolute apparent diffusion coefficients (ADC) of the normal brain change with age. A total of 41 healthy mice were examined by T2-weighted imaging and DWI. For each animal ADC frequency histograms (i) of the whole brain were calculated on a voxel-by-voxel basis and region-of-interest (ROI) measurements (ii) performed and related to the animals' age. The mean entire brain ADC of mice <3 months was $0.715(\pm 0.016) \times 10^{-3}$ mm^2/s, no significant difference to mice aged 4 to 5 months ($0.736(\pm 0.040) \times 10^{-3}$ mm^2/s) or animals older than 9 months $0.736(\pm 0.020) \times 10^{-3}$ mm^2/s. Mean whole brain ADCs showed a trend towards lower values with aging but both methods (i+ii) did not reveal a significant correlation with age. ROI measurements in predefined areas: $0.723(\pm 0.057) \times 10^{-3}$ mm^2/s in the parietal lobe, $0.659(\pm 0.037) \times 10^{-3}$ mm^2/s in the striatum and $0.679(\pm 0.056) \times 10^{-3}$ mm^2/s in the temporal lobe. With advancing age, we observed minimal diffusion changes in the whole mouse brain as well as in three ROIs by determination of ADCs. According to our data ADCs remain nearly constant during the aging process of the brain with a small but statistically non-significant trend towards a decreased diffusion in older animals.

Keywords: Magnetic resonance imaging; Diffusion-weighted MRI; Apparent diffusion coefficient; Aging; Animal experiment; Mouse; Brain

Introduction

Magnetic resonance imaging (MRI) is a widely used technique to examine brain pathology such as cerebrovascular disease, neuroinflammatory disorders and neoplasms (Warach et al., 1992; Meyding-Lamade et al., 1998, 1999; Schaefer et al., 2000; Schellinger et al., 2000; Sellner et al., 2004). Since its introduction in the 1980s, MRI techniques have constantly developed and improved granting an increasingly broad application in research as well as in clinical management of neurological disease. One of the recently developed techniques–diffusion-weighted imaging (DWI)–is sensitive to the Brownian motion of molecular free water and is quantified by the calculation of the apparent diffusion coefficient (ADC). The ADC is therefore directly linked to physiologic and pathologic mechanisms at microscopic tissue level (Le Bihan, 1995).

Changes of diffusion occur at an early stage of disease and affect DWI. Normal aging also leads to changes in brain structure and tissue morphology such as brain atrophy and reduced tissue water contents which may alter ADCs. Physiologic aging and distinction from pathologic phenomena will be a challenge in diagnostic imaging due to the ever increasing percentage of the elderly in western populations. Many of the pathologic conditions above mentioned are

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studied in animal models among which the murine model plays an important role. Therefore this study aimed to determine and evaluate comparative absolute ADC values of healthy mice at different ages.

Materials and methods

Female SJL mice were purchased from Bomholtgard Breeding and Research Center (Ry 8680, Denmark) and had an average age of 45 days and a weight range of 19.0 to 23.0 g upon arrival. The animals were held under standardized conditions with unrestricted access to food and water in the animal lab of the University of Heidelberg during the whole experiment.

The study was approved by our institutional review board. All experiments were conducted according to the European Communities Council Directive of 24 November 1986 (86/609/EEC).

For the MRI scanning procedure mice received an intraperitoneal anaesthesia to minimize pain or discomfort. A combination of 10 mg/kg body weight of xylazine and 80 mg/kg body weight of ketamine were applied 5 to 10 min prior to starting the scanning protocol. During the examination, animal body temperature was maintained constant by using a heat fan. MRI was performed using a 2.4 Tesla Bruker Biospec 24/40 (Ettlingen, Germany) scanner. After acquisition of the survey scan, DWI was achieved by spin-echo echo planar imaging (SE-EPI). Parameters were as follows: TR/TE/averages=3000/63/4, diffusion gradient duration (δ)=5 ms, diffusion time (Δ)=45 ms, b-values=200, 300, 400, 500, 600, 700 s/mm^2, slice thickness=2.0 mm, interslice gap=2.0 mm, field of view=4×4 cm^2, number of slices=6 coronal slices covering the entire brain, matrix=128×64. Furthermore T2-weighted images (T2-WI) were acquired using a multi-echo sequence (TR=3 s, 12 echoes with echo times from 8 to 96 ms, 1 average; all other parameters identical to DWI).

Image data were transferred to a separate workstation (Sparc 10; Sun Microsystems, USA). Using a standard mouse atlas (Franklin and Paxinos, 2001) regions-of-interest (ROI) were first chosen on T2-WI before calculating ADCs on DWI. ROIs were placed bilaterally in the parietal and temporal lobes and in the thalamus. To examine the overall change of ADCs in the aging mouse brain, the brain was manually segmented on all slices. Then ADCs were calculated within these regions on a voxel-by-voxel basis. From these ADC maps, a frequency histogram was created showing the number of voxels with ADCs in certain ranges (Figs. 3 and 4). All above mentioned calculations were achieved using a previously developed procedure in Matlab (version 5.3.1, The MathWorks, Inc., Natick, MA, USA). After proof of normality for all data sets and subsets using Kolmogorov–Smirnov and Shapiro–Wilk statistics, we performed an analysis of variance (ANOVA) with Tukey's post hoc testing followed by the calculation of Pearson correlation coefficients and regression analysis (SPSS 11.0, Chicago, USA). Data are presented as mean±standard deviation. Statistical significance was assumed at $p<0.05$.

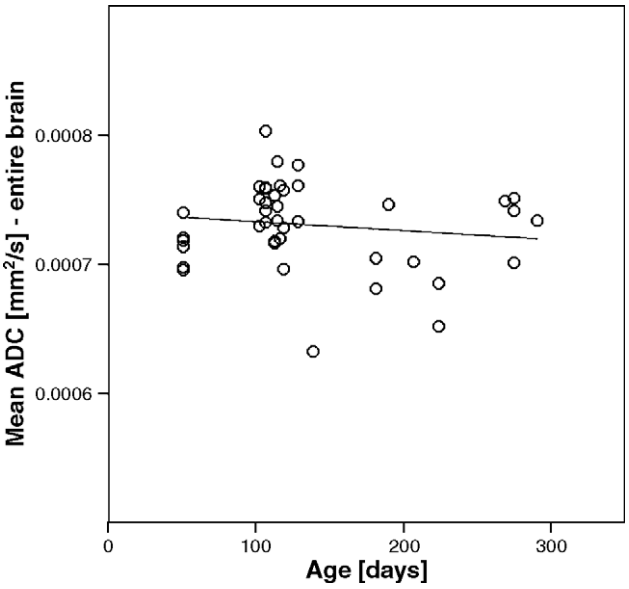

Fig. 1. ADC-scatterplot. Scatter diagram of whole brain mean ADCs. The diagram shows a non-significant trend to lower ADC values with advancing age ($p=0.38$).

Results

A total of 41 mice were studied at different ages ranging from less than 3 months to over 9 months. Animals were

Table 1
Mean ADC values in 3 regions-of-interest as compared to mean ADC for the whole brain

Region	Age group 1, <3 months	Age group 2, 3 months	Age group 3, 4–5 months	Age group 4, 6–9 months	Age group 5, >9 months	All
	(n=6)	(n=12)	(n=12)	(n=6)	(n=5)	(n=41)
Parietal lobe	0.706±0.029	0.743±0.030	0.742±0.082	0.681±0.053	0.704±0.032	0.723±0.057
	(0.678–0.755)	(0.698–0.793)	(0.613–0.969)	(0.604–0.728)	(0.649–0.728)	(0.604–0.969)
Thalamus	0.643±0.040	0.680±0.040	0.662±0.031	0.639±0.033	0.644±0.024	0.659±0.037
	(0.588–0.695)	(0.629–0.762)	(0.605–0.714)	(0.598–0.673)	(0.624–0.677)	(0.588–0.762)
Temporal lobe	0.679±0.052	0.696±0.032	0.683±0.053	0.643±0.027	0.674±0.116	0.679±0.056
	(0.606–0.747)	(0.653–0.773)	(0.591–0.785)	(0.607–0.690)	(0.568–0.839)	(0.568–0.839)
Whole brain	0.715±0.016	0.748±0.023	0.736±0.040	0.696±0.031	0.736±0.020	0.731±0.033
	(0.696–0.741)	(0.717–0.804)	(0.633–0.780)	(0.652–0.747)	(0.702–0.751)	(0.633–0.804)

Apparent diffusion coefficient (ADC) values are shown as mean±standard deviation×10^{-3} mm^2/s and the range in parentheses. Table depicts ADC data for different regions-of-interest as well as for the whole brain plotted against animal age. Group 1: animals under 3 months of age, group 2 aged 3 months, group 3: 4 and 5 months old, group 4: 6 to 9 months and group 5 over 9 months.