MR elastography in a murine stroke model reveals correlation between macroscopic viscoelastic properties of the brain and neuronal density

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Target audience: Physicians and biologists interested in the mechanical properties of the brain as a new biomarker for tissue repair after stroke.

Objective: Blood flow interruption in a cerebral artery causes brain ischemia with dramatic impact to metabolism and function of the brain. It was recently shown by ultrasonic shear wave imaging that the elasticity of ischemic brain tissue continuously decreases up to day 7 after middle cerebral artery occlusion (MCAO) in a rats [1]. We use magnetic resonance elastography (MRE) [2] to reveal the alteration of elasticity and viscosity in the murine brain within 28 days after MCAO and compared the results to histological markers.

Methods: 20 C57/ B6 mice (20-25 g body weight) were investigated on days 3, 7, 14 and 28 (n = 5 per group) after exposure to 60 minutes transient focal ischemia following the commonly applied left hemispheric MCAO . Immunohistochemical stainings were carried out in order to assess damage size and progress for NeuN, GFAP and ApopTag. Cell counts were carried out in 0.5-mm brain slices dorsal from Bregma within the region analyzed by MRE. A total of 5 representative ROIs for each hemisphere were counted. MRE was performed on a 7 T scanner (Bruker Pharma Scan, Ettlingen, Germany). A FLASH sequence was customized for MRE by sinusoidal motion sensitizing gradients (MSG) in through-plane direction and 900 Hz frequency matched to the mechanical vibration induced by air-cooled Lorentz coils [3]. Further imaging parameters were: 128x128 matrix, 25 mm FoV, 14.3 ms TE, 116.2 ms TR, 285 mT/m MSG strength, eight dynamic scans over a vibration period. The complex modulus G^* was calculated by direct Helmholtz inversion of complex wave images in a central transversal slice. Mechanical constants were averaged within the left hemisphere representing the region of stroke and within the right hemisphere for internal reference. The following representations of the complex shear modulus were tabulated: G'=Re(G^*) (storage modulus, synonym to elasticity), G''=Im(G^*) (loss modulus related to viscostiy), abs(G^*) and $\phi = \arctan(G''/G')$ (which relates to the geometry of the viscoelastic network).

Results: Two-way ANOVA revealed that MRE constants within each? hemisphere were not significantly altered from day 3 to day 28 after onset of stroke. Mean values of G' and abs(G^*) averaged over time prove that the brain elasticity is reduced in the stroke region compared to the control hemisphere (G' = 6.2±1.8 vs. 6.9±1.8 kPa, P = 0.03 [Wilcoxon signed rank test]). Regarding solely day 7, a stroke-related loss is discernable in G', G'' and abs(G^*) (P < 0.01, 0.05, 0.01, respectively). The time course of G' and G'' of both hemispheres is shown in **Fig.1**. The typical appearance of neuronal cell bodies with immunhistochemical staining is shown in **Fig.2**. The number of neurons provided by the micrographs was significantly reduced in the stroke region (NeuN_left) compared to the contralateral side (NeuN_right, 825±88 vs. 1506±29, P < 0.001) without influence of time. The correlation between the complex shear modulus and the number of neurons in the stroke area is illustrated in **Fig.3**. For G', G'' and abs(G^*) Pearson's correlation coefficients were R = 0.65, 0.76, and 0.67, respectively (all P < 0.005) while there was no correlation between ϕ and NeuN_left. The variation of NeuN_right among all mice was only 6% compared to 17% found for the stroke region. Nevertheless, G' and abs(G^*) were also sensitive to the small variation of NeuN_right as revealed by correlation coefficients R = 0.62 (P = 0.003) and R = 0.55 (0.013), respectively.

Fig.1

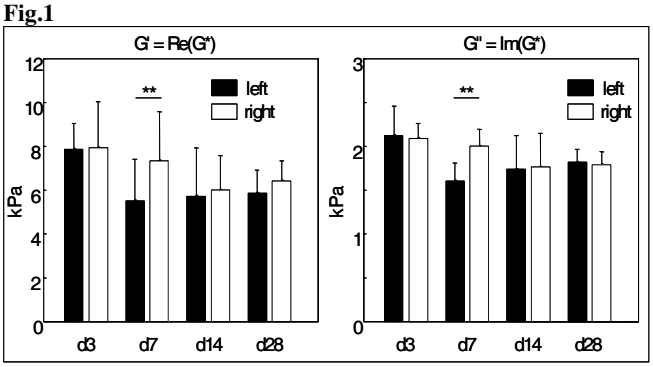


Fig.2

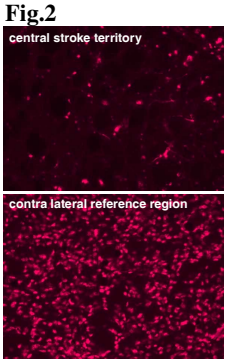
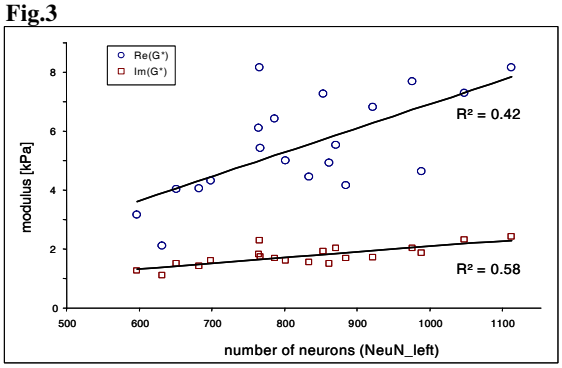


Fig.3



Discussion Our results provide strong evidence that neurons contribute to the macroscopic mechanical properties of brain tissue. Reduction of the neuronal density yields a loss of brain stiffness similarly observed during multiple pathophysiological processes in humans [4,5]. Specifically, MRE-parameters G', G'' and abs(G^*) were significantly correlated with the number of neurons and displayed a stroke-related reduction at day 7 after stroke. In contrast, ϕ was not sensitive to the number of Neurons suggesting a constant ratio G''/G' despite alteration of the neuronal network. Overall, the observed alteration of MRE parameters between hemispheres was smaller than expected [1] which is possibly due inversion-related biases of time-harmonic wave fields limiting the spatial resolution of MRE parameter maps and thus yielding an interregional correlation of viscoelastic constants.

Conclusion: MRE has been proven sensitive to stroke-related neuronal degeneration observed in mice after MCAO.

Literature: [1] Martin A, Mace E, Boisgard R, Montaldo G, Theze B, Tanter M, Tavitian B. Imaging of perfusion, angiogenesis, and tissue elasticity after stroke. J Cereb Blood Flow Metab 2012;32(8):1496-1507. [2] Muthupillai R, Ehman RL. Magnetic resonance elastography. Nature Med 1996;2(5):601-603. [3] Riek K, Klatt D, Nuzha H, Mueller S, Neumann U, Sack I, Braun J. Wide-range dynamic magnetic resonance elastography. J Biomech 2011;44(7):1380-1386. [4] Wuerfel J, Paul F, Beierbach B, Hamhaber U, Klatt D, Papazoglou S, Zipp F, Martus P, Braun J, Sack I. MR-elastography reveals degradation of tissue integrity in multiple sclerosis. Neuroimage 2010;49(3):2520-2525. [5] Freimann FB, Streitberger KJ, Klatt D, Lin K, McLaughlin J, Braun J, Sprung C, Sack I. Alteration of brain viscoelasticity after shunt treatment in normal pressure hydrocephalus. Neuroradiology 2012;54(3):189-196.