Late stimulation of the Sphenopalatine Ganglion in ischemic rats improves NAA levels and DWI characteristics

A. Bar-Shir1, N. Shemesh1, R. Nossin-Manor2, and Y. Cohen1

1School of Chemistry, Tel Aviv University, Tel Aviv, Israel; 2Diagnostic Imaging, The Hospital for Sick Children, Toronto, ON, Canada

Introduction
Stroke is a major cause for disability, death and health care expenditure. To date, the most common treatment for ischemic stroke is tissue plasminogen activator (tPA). However, despite its widespread use, this treatment has some limitations, especially its short therapeutic window. Therefore other therapeutic materials and devices should be developed. Electrical stimulation of the parasympathetic nerve fibers derived from the sphenopalatine ganglion (SPG) and the ethmoidal branch of the nasociliary nerve increases cerebral blood flow (CBF). It was previously demonstrated that SPG-stimulation preserves the perfusion/diffusion mismatch and reduces infarct size in the permanent MCAO rat suture model. In this study we examined the effect of SPG-stimulation on normalized N-Acetyl Aspartate (NAA) levels, DWI characteristics and behavioral performance of healthy control rats. We compared treated and untreated rats when treatment was started 18±2h after the stroke onset.

Methods

Electrode implementation: A hook stimulating electrode that was used to generate the electrical stimulation of the SPG was hooked onto the exposed posticular SPG (SPG) fibers of all rats. Ischemic procedure: A day after the electrode implementation, 2h of transient MCAO (t-MCAO) procedure was applied for all rats using the suture model. MR experiments were performed using a 7T/30cm BioSpec system (Bruker, Germany). Control (N=7) and treated (N=6) rats were examined by MRS and MRI under isoflurane anaesthesia. Each rat was examined at three time points: 16±2h (before starting treatment), 8 days (after one week of treatment) and 28 days (at the chronic stage) after the t-MCAO. (i) MRI: T2WIs were collected using the RARE sequence (RARE factor=8) with the following parameters: FOV of 2.56x2.56cm2 and 256x128 digital resolution reconstructed to 256x256. Eight continuous 2mm slices were collected, using TR/TE of 3000/75ms. ADC maps were calculated from two spin-echo four-shot EPI data sets, collected with and without diffusion sensitizing gradient pulses and with the following parameters: Δ=4.5ms, Δ=40ms and G=173mTm, resulting in a b of 15000s/mm2. The same slices and FOV used in T2WI was used in the DWI protocol. For diffusion imaging, the matrix was 96x96 reconstructed to 128x128 with TR/TE=2000/50ms. (ii) 1H-MRSI: 2mm slice-selected two-dimensional (2D) 1H-MRSI was performed with the following parameters: FOV of 2.56x2.56cm2 with VAPOR water suppression, a matrix of 8x8 reconstructed to 16x16, resulting in 256 voxels of 1.6x1.6x2.0mm3. TR/TE=2000/135ms were used. Behavioral Tests: A neurological modified Neuro Severity Score (mNSS) test, scale 0-18, was performed at the three experimental time points. SPG Stimulation: After the completion of the first MR protocol (18±2h post-t-MCAO) the SPG-stimulated rats (treated group) were moved to a dedicated RF activation cage (BrainsGate, Israel) which enables wireless stimulation. SPG-stimulation started 18±2h after t-MCAO surgery and was applied for 3h, for seven consecutive days.

Results
Figure 1 depicts the changes in the normalized NAA values for the three experimental time points after the t-MCAO. These values represent the ratio of the ipsi-lateral to contra-lateral NAA values (NAA ipsi/NAA contra). Figure 1A sows the changes in the total normalized NAA. This Figure clearly shows that there was a significant difference between the total normalized NAA values of the two groups at the end of the study (28 days post occlusion) whereas such a difference was not observed 16±2h after the t-MCAO. The stimulated and control groups started from very similar averaged values of total normalized NAA values of 0.52±0.03 and 0.54±0.03, respectively (P=0.7), and reached different averaged normalized NAA values of 0.60±0.04 and 0.50±0.04, respectively, 28 days after the t-MCAO (P=0.05). To obtain more specific information from the MRS data we classified the normalized NAA values in the ischemic hemisphere at 16±2h post t-MCAO into three categories: voxels with normalized NAA values greater than 0.7, voxels with normalized-NAA values between 0.4 and 0.7, and voxels with normalized NAA values smaller than 0.4. We found no significant differences between the groups at all three time points for voxels with normalized NAA values greater than 0.7. Figure 1B displays the changes in normalized NAA values of the two groups at the end of the study (28 days post occlusion) whereas such a difference was not observed 16±2h after the t-MCAO. The stimulated and control groups started from similar averaged values of total normalized NAA values of 0.20±0.04 and 0.20±0.04, respectively (P=0.97), and reached different averaged normalized NAA values of 0.20±0.04 and 0.20±0.04, respectively, 28 days post occlusion (P=0.97). 28 days after the t-MCAO, the difference in the mNSS between the groups (P=0.04) was found. 28 days after the t-MCAO, the difference in the mNSS between the groups was maintained (4.3±0.9 versus 2.3±0.5) but was was much less significant (P=0.08).

Conclusion
This study shows that SPG-stimulation treatment (3h a day for 7 consecutive days) of rats after 2h of t-MCAO that was started 18±2h after the induction of stroke, improves NAA levels in the ischemic hemisphere of the treated rats and also prevents DI values deterioration computed from DWI data, 28 days after t-MCAO. The results from both parameters, i.e. NAA levels and DI values were corroborated by behavioral examinations. The fact that treatment was started 18±2h after the ischemic event shows that this unique treatment of the electrical stimulation of the SPG can potentially extend the therapeutic window for the treatment of ischemic stroke.

References