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

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Introduction

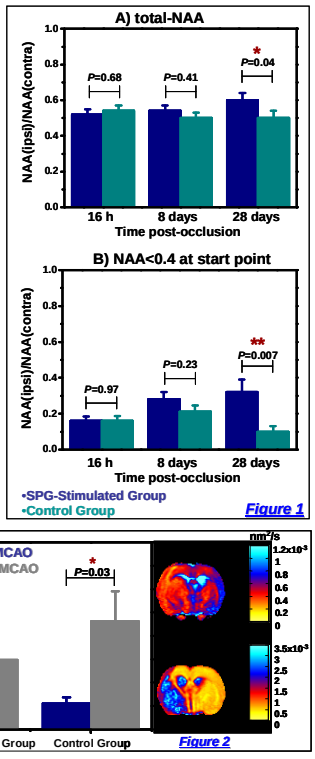
Stroke is a major cause for disability, death and health care expenditure. To date, the most common treatment for ischemic stroke is tissue plasminogen activator (tPA).[1] However, despite its widespread use, this treatment has some limitations, especially its short therapeutic window. Therefore other 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).[2] It was previously demonstrated that SPG-stimulation preserves the perfusion/diffusion mismatch and reduces infarct size in the permanent MCAO rat suture model.[3] In this study we examined the effect of SPG-stimulation on normalized N-Acetyl Aspartate (NAA) levels, DWI characteristics and behavioral performance of ischemic rats. We compared treated and un-treated 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 post ganglion (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 anesthesia. 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:*** T_2WIs were collected using the RARE sequence (RARE factor=8) with the following parameters: FOV of 2.56×2.56cm^2 and 256×128 digital resolution reconstructed to 256×256. 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=173mT/m, resulting in a b_{max} of 1500s/mm^2. The same slices and FOV used in T_2WI was used in the DWI protocol. For diffusion images, the matrix was 96×96 reconstructed to 128×128 with TR/TE=2000/53ms. ***(ii)^{1}H-MRSI:*** 2mm slice-selected two-dimensional (2D) ^{1}H-MRSI was performed with the following parameters: FOV of 2.56×2.56cm^2 with VAPOR water suppression, a matrix of 8×8 reconstructed to 16×16, resulting in 256 voxels of 1.6×1.6×2.0mm^3. 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±2 h 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 at starting point. For voxels with initial normalized NAA values greater than 0.4 and smaller than 0.7, we found an increase in normalized NAA values in the SPG-stimulated group compared to the control group. For these voxels, the normalized NAA values of the SPG-stimulated group increased from 0.54±0.02 (16±2h) to 0.64±0.05 (day 8) and then to 0.69±0.04 (day 28), while those of the controls did not change significantly (from 0.57±0.04 at 16±2h to 0.59±0.05 28 days post t-MCAO). The most interesting results were found for voxels in which normalized NAA values were smaller than 0.4 at 16±2h post-occlusion, as shown in Figure 1B. These voxels, which showed the most dramatic reduction in NAA levels 16±2h post occlusion, also exhibited the most dramatic response to treatment. For these voxels, the control group showed a decrease in the normalized NAA levels with time, from 0.16±0.03 at 16±2h to 0.10±0.03 28 days post-occlusion whereas the treated group showed an opposite trend. In the SPG-stimulated animals, NAA levels from these voxels (Figure 1B) significantly improved from 0.16±0.03 at 16±2h to 0.32±0.03 28 days post MCAO (P=0.01). For these voxels we found that although the control and SPG-stimulated groups had had the same initial NAA values at 16±2h post-occlusion (0.16±0.03, P=0.97), 28 days post occlusion a dramatic difference in the normalized NAA values of the SPG-stimulated animals (0.32±0.07) and the controls (0.10±0.03, P=0.007) was found (Figure 1B). In addition, a damage index (*DI*), an index that expresses the diffusion characteristics of the ipsi-lateral hemisphere as compared to the contra-lateral hemisphere and which was calculated from the DWI data of the first (16±2h post-occlusion) and last (28 days post occlusion) time points of the two groups is presented in Figure 2. From this figure we found a significant deterioration of the *DI* values for the controls which was not observed for the stimulated animals. Both groups started with the same *DI* values (28±18 for the SPG-stimulated animals and 28±15 for the control rats, P=0.98). The DWI data show that the deterioration in the *DI* values are smaller in the SPG-stimulated group compared to the controls. For the SPG-treated animals, the calculated *DI*s did not change significantly at day 28 post-occlusion (P=0.15). However, the deterioration in the *DI* of the untreated animals was statistically significant (P=0.03). From the mNSS examination we also found that although both groups started from the same mNSS value (P=0.9), eight days after the t-MCAO a significant difference in the mNSS of the two groups (5.6±0.8 for the control rats and 3.8±0.4 for the SPG-treated animals, 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 somewhat 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

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