Investigation of Angiogenesis following CART peptide Treatment in Transient Ischemic Rat Stroke Model Using Susceptibility-Weighted Imaging

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Introduction:
Cocaine- and amphetamine-regulated transcript (CART) is a peptide that was found in various brain regions, including striatum, hippocampus, and cortex. It is involved in several important physiological responses in energy metabolism and neural injury [1]. The expression of CART is upregulated in the brain after focal cerebral ischemia in rats. Although enhanced neuroregeneration and behavioral recovery following CART therapy in rat stroke models have been shown recently [1], studies using a noninvasive imaging method to evaluate the cerebral angiogenesis after CART treatment are limited. Susceptibility-imaging (SWI) has been increasingly used in human studies of cerebral vascular malformations with improved visualization due to its high sensitivity in detecting blood oxygen level-dependent phase effects between venous and the surrounding brain parenchyma [2]. In the present study, we applied the SWI technique in a rat stroke model to quantitatively investigate cerebral angiogenesis after stroke with or without CART treatment.

Materials and methods:
Animal preparations. A total of 30 male Sprague-Dawley rats (250-350 g) were included in this study. Bilateral common carotids were ligated with nontraumatic arterial clips first and the right middle cerebral artery was ligated with a 10-O suture to generate focal infarction in the cerebral cortex. The ligature and clips were then removed after 90-min ischemia to allow reperfusion. All animals received either CART (CART5–102, 0.1nmol/10ml) or saline delivered into the nostrils of each rat at a dose of 40 ml on day 3 after stroke and then 20 ml daily for another 6 to 9 days.

MRI scanning. Rats were divided into the CART-treated and control groups according to similar lesion size measured from T2WI on day 2. Lesion volumes on day 10 and 25 were quantified relative to that on day 2 (rLV). For the DTI data, fractional anisotropy (FA) was derived using the dtv software (University of Tokyo Hospital, Tokyo, Japan). On FA maps, a ROI was drawn within the perilesional hyperintense region on the lesion-containing slice [3]. For comparison, an additional ROI was placed in contralateral white matter. After normalizing signal from the perilesional region to the contralateral white matter, the FA ratio was compared between the CART and control groups. SWI maps were acquired using the in-house developed Matlab script based on the previous study [4]. A ROI was first manually placed on the linear perilesional boundary of the ipsilateral cortex. An automatic segmentation method based on the fuzzy c-means clustering (FCM) algorithm was then applied to remove the possible intra-observer variation [5]. A six-class FCM algorithm was applied to the selected ROI to identify the first four cluster classes of T2 hypointensities representative of the venous structure and the other two cluster classes with hyperintensities that represent normal white matter. An ROI was placed in the ipsilateral cortex in a contralateral area for comparison. Change of SWI signal intensity was expressed as a ratio of lesion-to-contralateral area. Comparisons of rLV, FA, or SWI ratios between CART and control animals across all stages were analyzed using a two-way ANOVA with repeated measures. A p value < 0.05 was considered statistically significant.

Histology. To evaluate the vascular activity, α-smooth-muscle actin (αSMA) immunohistochemistry was applied in the sacrificed animals and the number of αSMA-positive vessels were counted in the penumbra of lesional cortex.

Results:
The lesion volume on day 2 after stroke determined from T2WI for the CART-treated and control groups were 204.72±25.39 mm³ and 203.67±31.83 mm³, respectively. There was a time (p<0.0001) and a treatment (p<0.0001) dependent reduction in rLV as revealed in the ANOVA analysis. There was a significant interaction between treatment and follow-up time points (p=0.003). Post-hoc analysis showed that CART significantly reduced rLV on 10 and 25 days after stroke as compared with control animals (p=0.004 and p=0.02 for 10 and 25 days, respectively, Fig. 1). Absolute FA values in the contralateral white matter area were not significantly different between the two groups (CART: 0.74±0.18 vs. control: 0.74±0.20) as well as after CART treatment on day 10 (CART: 0.74±0.12 vs. control: 0.75±0.08) and day 25 (CART: 0.74±0.18 vs. control: 0.74±0.09). FA ratio in the perilesional cortex was significantly enhanced by CART treatment (p=0.0005) or follow-up time (p=0.0001, two-way ANOVA). There was a significant interaction between treatment and follow-up time (p=0.0006). CART significantly increased FA on days 10 and 25 days and post-hoc effect for 25 days, respectively, post-hoc test, Fig. 2. Quantitative analysis of FA ratio using SWI characterization after stroke is presented in Figure 3. Ten days after stroke onset, the linear hypointensity identified in CART-treated animals rapidly decreased and achieved the minimal value at 25th day. In contrast, the value in control animals showed a relatively gradual decrease within 25th day after stroke. ANOVA revealed a time effect (p<0.0001) and treatment (p<0.0001) effects in the SWI data. There was also a significant interaction between treatment and follow-up time points (p=0.02). The averaged SWI signal intensity values related to angiogenesis exhibited significant differences between treated and control rats on 10 days (CART: 0.64±0.22 vs. control: 0.85±0.3, p=0.02, Post-hoc) to 25 days (CART: 0.58±0.21 vs. control: 0.81±0.19, p=0.003, Post-hoc) after stroke. Representative SWI data from the two CART-treated and two control rats are shown in Fig. 4 (left). The low intensity linear structure representing the perilesional boundary in SWI images were clearly present for CART-treated rats. Control rats also showed linear low intensity structure around the perilesional cortex, the signal intensity is relatively lower as compared with the CART-treated animals. Supportive data with enhanced density of α-SMA immunoreactivity are presented in Fig. 4 (right).

Discussion and conclusions:
In this study we used SWI to evaluate changes of vascular activity following CART treatment in a rat stroke model. The CART-treated animals showed enhanced SWI values in areas surrounding the infarction, compared with the control groups. Our data demonstrated that SWI identified cerebral angiogenesis after stroke in rats, and CART treatment enhanced angiogenesis compared to the saline treated rats. The further showed that animals treated with CART showed reduced rLV and higher FA values in areas surrounding the infarction. These imaging findings are consistent with previous report that intranasal CART treatment facilitates neuroregeneration in stroke brain [1]. Our previous studies have demonstrated that CART administration increased synaptic connections and showed improvements in a skilled reaching task [1]. Together, these studies demonstrate the feasibility of quantitative SWI as a noninvasive maker to reflect the level of angiogenesis and possibly to monitor the progression of stroke recovery.

References: