Post-treatment with cocaine- and amphetamine-regulated transcript enhances infarct resolution, reinnervation, and angiogenesis in stroke rats – an MRI study

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Recent studies have shown that post-treatment with cocaine- and amphetamine-regulated transcript (CART) has neuroregenerative effects in animal models of stroke. The purpose of this study was to characterize CART-mediated neuronal and vascular reparations using non-invasive MRI techniques. Adult male rats were subjected to a 90 min middle cerebral artery occlusion (MCAo). Animals were separated into two groups with similar infarction sizes, measured by T2-weighted MRI on Day 2 after MCAo, and were treated with CART or vehicle intranasally from Day 3 to Day 12. Diffusion tensor imaging was used to examine changes in plasticity of white matter elements. Susceptibility-weighted imaging (SWI) was used to measure angiogenesis. Post-treatment with CART significantly increased fractional anisotropy (FA) in lesioned cortex on Days 10 and 25 post stroke. A significant correlation between the behavioral recovery in body asymmetry and the change in FA was shown, suggesting that behavioral recovery was associated with reinnervation to the lesioned hemisphere. CART also increased the intensity of SWI and the immunoreactivity of the vascular marker alpha-smooth muscle actin in lesioned cortex. Together, our data support a non-invasive treatment strategy for stroke through angiogenesis and reinnervation by CART. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: MRI; susceptibility-weighted imaging; fractional anisotropy; cocaine- and amphetamine-regulated transcript

INTRODUCTION

Stroke is a major health problem and a leading cause of adult disability worldwide. After the onset of stroke, a series of processes involving neurodegeneration and regeneration are progressively activated. Currently, tissue plasminogen activator (tPA) is the only Food and Drug Administration-approved pharmacological therapy for acute ischemic stroke. tPA dissolves the occluded blood clots at the very early stage of stroke. The effectiveness of tPA is greatly affected by the narrow therapeutic
time window of about 3 h. Less than 3% of stroke patients have received TPA nationwide, mainly because patients did not arrive at hospital early enough for the treatment (1,2) (Stroke Awareness Foundation, http://strokeinfo.org). It is thus important to develop new therapeutic strategies with prolonged therapeutic windows for stroke. Recently several pharmacological therapies have been developed to target the late-onset pathophysiology after stroke (3,4). These treatments enable much longer treatment windows starting from 3 to 6 days after onset of stroke in experimental animals and are potentially useful for stroke patients.

Cocaine- and amphetamine-regulated transcript (CART) is an endogenous peptide found in the brain. It is present in striatum, hippocampus, and cortex (5). The expression of CART is upregulated in cultured cortical neurons after oxygen–glucose deprivation (6) and in the brain after focal cerebral ischemia or electroconvulsive shock in rats (7). Pre-treatment with CART reduces cerebral infarction in mice (6,8). We previously reported that post-stroke treatment with CART, given intranasally from 3 days after middle cerebral artery occlusion (MCAo), upregulated the expression of brain-derived neurotrophic factor in the subventricular zone (SVZ) and growth-associated protein (GAP) 43 in the lesioned cortex, enhanced proliferation of neuroprogenitor cells (NPCs) in the SVZ, increased NPC migration and reinnervation to the lesioned cortex, and improved locomotor activity in stroke rats (4). Similar regenerative action was confirmed by another laboratory using a cellular model of oxygen and glucose deprivation (9). These data suggest that delayed treatment with CART has reparative effects to stroke animals through reinnervation. However, the correlation between reinnervation and functional recovery has not been characterized.

MRI has been widely used to evaluate structural and functional alterations in stroke brains. Fractional anisotropy (FA), an index derived from diffusion tensor imaging (DTI), has been used to quantify the diffusion patterns of water in axonal fibers, reflecting the microstructure and integrity of white matter. Susceptibility-weighted imaging (SWI) has been used in human studies of cerebral vascular malformations due to its high sensitivity in detecting blood oxygen level-dependent phase effects between venous structures and the surrounding brain parenchyma (10). In the present study, we used FA to evaluate the time-dependent plasticity of white matter and SWI to assess cerebral angiogenesis following CART treatment in stroke animals. We demonstrate that post-stroke treatment with CART increases FA and SWI in the ischemic penumbra region. A significant correlation between changes in FA and behavioral recovery is shown in CART-treated animals. Our data support the view that CART-mediated functional recovery is associated with reinnervation and neovascularization in the lesioned cortex in stroke animals.

MATERIALS AND METHODS

The use of animals and experimental protocols were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Surgery

A total of 30 adult male Sprague-Dawley rats were enrolled in this study. Animals were purchased from the Charles River Laboratories (Frederick, MD, USA) and were housed in an enriched environment by providing a toy (nylabone) or crinkle paper in their home cages with a 12 h dark (6 pm to 6 am) and 12 h light (6 am to 6 pm) cycle. Rats were anesthetized with chloral hydrate (0.4 g/kg, intraperitoneally) for stroke surgery as described previously (3). In brief, the right middle cerebral artery was ligated with a 10-O suture and common carotids were clamped bilaterally by non-traumatic arterial clips to generate focal infarction in the cerebral cortex. The ligature and clips were removed after 90 min of ischemia to allow reperfusion. Core body temperature was maintained at 37 °C. The volume of infarction was measured 2 days after MCAo using T2-weighted imaging (T2WI). Animals were separated into two groups with similar lesion sizes, and were treated with CART (n = 15) or saline (n = 15).

Drug administration

CART or saline vehicle was given intranasally daily from Day 3 post-stroke. Rats were anesthetized with isoflurane and were placed in a supine position for the treatment. The CART55–102 (0.1 nmol/10 μL, Peptide Institute, Osaka, Japan) or saline was delivered into the nostrils of each rat at a dose of 40 μL on Day 3 after MCAo and then 20 μL daily for another 6–9 days. For each daily injection, the drug was equally divided into two doses, given 5 min apart, to be delivered via the left and right nostrils. After drug delivery, animals were maintained with isoflurane in a supine position for an additional 5 min to avoid loss of the chemical fluid.

Behavioral measurement

24 of 30 rats (12 CART treated and 12 vehicle treated) underwent a body asymmetry test. Body asymmetry was assessed using a elevated body swing test (11) on Days 2 and 14 after stroke onset. Rats were examined for lateral movements/turning when their bodies were suspended 20 cm above the testing table by lifting their tails. The frequency of initial turning of the head or upper body contra-lateral to the ischemic side was counted in 20 consecutive trials. The frequency of initial turning of the head or upper body contra-lateral to the ischemic side was counted in 20 consecutive trials. The maximum impairment in body asymmetry in stroke animals is 20 contralateral turns/20 trials. In non-stroke rats, the average body asymmetry is 10 contralateral turns/20 trials (i.e., the animals turn in each direction with equal frequency).

MRI scanning

All rats (15 CART treated and 15 vehicle treated) underwent MRI scanning on Day 0 (pre-stroke) and Days 2, 10, and 25 after stroke onset under isoflurane anesthesia (3% for induction and 1.8% for maintenance) in air/O2 (80:20). MRI experiments were performed on a Bruker BioSpin 9.4 T animal MRI scanner (Bruker Medizintechnik, Karlsruhe, Germany) equipped with an actively shielded gradient coil. The inner diameter of the gradient coil was 0.12 m, and the maximum gradient strength was 400 mT/m. A birdcage coil driven in linear mode was used for RF excitation, and a single-turn circular surface coil (2 cm in diameter) was used for signal reception. The animal was secured in a custom-made holder equipped with a nose cone for administration of anesthetic gases and ear bars to minimize head motion. Rectal temperature was maintained at 37.5 ± 0.5 °C with a feedback-controlled, water-circulated heating pad.

Anatomical imaging

T2WI was acquired using a rapid acquisition with relaxation enhancement (RARE) sequence with the following parameters: field
of view (FOV) = 3.2 × 3.2 cm², matrix size = 192 × 192 (zero-filled to 256 × 256), repetition time (T\textsubscript{R}) = 2750 ms, effective echo time (T\textsubscript{E}) = 40 ms, and 23 slices of 1 mm thickness.

**Diffusion tensor imaging**

DTI was carried out using a single-shot echo-planar imaging (EPI) sequence. Scan parameters were the following: 19 slices with 1 mm thickness, FOV = 3.2 × 3.2 cm², matrix size = 96 × 96, T\textsubscript{R} = 9500 ms, and T\textsubscript{E} = 38 ms. Thirty diffusion-weighted images with diffusion gradients along independent orientations (b = 1000 s/mm\(^2\)) and one baseline image (b = 0) were acquired for each slice, and the acquisition was repeated three times to improve signal-to-noise ratio. Geometric distortions in EPI were corrected using the phase labeling for additional coordinate encoding (PLACE) scheme (12). This was done by acquiring an additional set of EPI data with the phase-encoding blip offset by −1 and 0. This dataset was used to derive pixel-wise displacement maps, which were subsequently applied to correct for images acquired with the same EPI parameters.

**Susceptibility-weighted imaging**

Measurements of SWI used a three-dimensional gradient-echo imaging sequence with gradient flow compensation in three directions (10). The acquisition matrix was 256 × 256 × 128 with an FOV of 32 × 32 × 32 mm\(^3\), resulting in a spatial resolution of 0.125 × 0.125 × 0.156 mm\(^3\). The SWI images were acquired with T\textsubscript{R} = 30 ms, T\textsubscript{E} = 10 ms and flip angle = 15°.

**MRI data analyses**

Ischemic injury size was determined from the hyper-intensity region in the T2*-weighted images. The area containing ischemic tissue and peri-lesioned boundaries was first manually traced and then mirrored onto the contralateral side in each slice. Voxels in the lesion area with image intensities higher than the mean ± 2 standard deviations of the intensity in the mirrored contralateral area were defined as the infarct zone (13). The volume of infarction in each animal was obtained from the product of the slice thickness (1 mm) and the sum of lesion areas in all slices. To reduce variability among animals, the change in lesion (relative lesion volume, rLV) on Days 10 and 25 after stroke onset was analyzed by normalizing to the volume of infarction on Day 2 in each animal.

DTI was analyzed according to the technique described in previous studies (14). Diffusion tensors and FAs were calculated using the “dTV II” DTI software (15). For FA analysis, a region of interest (ROI) was selected within the peri-lesioned region in the ipsilateral white matter area (external capsule, coronal slices located approximately from 1.64 to −5.63 mm relative to bregma) based on its relatively clear gray-white matter boundaries. An additional ROI was placed in the area of the contralateral white matter for comparison. To minimize intersubject differences in this DTI-derived parameter caused by factors (such as brain topography, temperature, and iron concentration) that might affect the absolute DTI-derived parameter, the change of FA was expressed as a ratio of lesion to contralateral area (rFA).

For SWI, the phase images were first corrected using a high-pass filter to remove the low-spatial-frequency components of the background field (10). These corrected phase images were then set between zero and unity to obtain the phase mask. The magnitude images were multiplied by the corresponding phase masks five times to enhance the visibility of the venous structures. Venograms were computed using a minimum intensity projection (mIP) technique over 16 slices (2.5 mm). To quantitatively analyze SWI data, an ROI was first manually selected on the fine linear structure within the peri-lesioned boundary of the ipsilateral cortex according to its relatively clear contrast and distinct linear shape of venous structure in each mIP slice containing a lesion. An automatic segmentation method based on the fuzzy c-means clustering (FCM) algorithm was then applied to remove the possible intra-observer variation (16). A six-class FCM algorithm was applied on the selected ROI to identify the first four cluster classes with hypointense signals representative of the venous structure and the other two cluster classes with hyperintense signals representing the non-venous tissues adjacent to the venous structure (17) (Supplementary Fig. 1). An additional ROI of similar size was placed in the area on the contralateral side for comparison. Similar to the FA calculation, change of signal intensity in SWI was expressed as a ratio of lesion to contralateral area (SWI ratio).

**α-SMA immunoreactivity**

Vascular marker alpha-smooth muscle actin (α-SMA) was examined in eight stroke rats (four CART treated and four vehicle treated). Animals were anesthetized and perfused transcardially with saline followed by 4% paraformaldehyde (PFA) in phosphate buffer (PB; 0.1 M; pH 7.4). The brains were dissected, post-fixed in PFA for 16 h, and transferred to 18% sucrose in 0.1 M PB for at least 16 h. Serial sections of the entire brain were cut at 25 μm thickness in a cryostat. One series from every fourth section was stained for α-SMA (a smooth muscle cell marker, mouse monoclonal IgG 1:400, DAKO). Staining was developed with 2.3-diaminobenzidine tetrahydrochloride (0.5 mM in 50 mM Tris-HCl buffer, pH 7.4). Control sections were incubated without primary antibody. Sections were mounted on slides, and cover slipped. Histological images were acquired using an Infinity3 camera and Nikon 80i microscope. Three representative sections from each animal were quantified for the density of arterioles. The surface area for α-SMA positive vessels in the penumbra area in cortex were quantified by Nikon NIS-Elements software and averaged from three sections. All immunohistochemical measurements were made by blinded observers.

**Statistical analyses**

Comparisons of lesion volume, FA or SWI between CART-treated and control animals across all time points were analyzed using two-way analysis of variance (ANOVA) with repeated measures on a one-factor test. Lesion volume, FA, or SWI was treated as the dependent variable and data from different time points were treated as the within-subjects factor. To explore how imaging metrics were related to behavioral recovery, we performed Spearman correlation analyses between imaging metrics and behavior measurements. A p value less than 0.05 was considered statistically significant.

**RESULTS**

The size of the lesion was examined using T2WI on Day 2 post stroke. An intensity increase in the T2WI was found in the cortex ipsilateral to the occlusion side in all animals (Fig. 1A). Animals...
were equally divided into two groups to receive vehicle or CART. No difference in lesion volume was found between these two groups prior to the treatment (204.72 ± 25.39 mm³ versus 203.67 ± 31.83 mm³, \( p = 0.95 \), t test). CART or vehicle was delivered from Day 3 to Day 12 after MCAo. The size of the lesion was examined again by T2WI on Days 10 and 25. rLV was analyzed by comparing with the volume of infarction on Day 2 in each animal to reduce variability among animals (Fig. 1B). Post-stroke treatment with CART significantly reduced lesion volume. A time- (\( p < 0.0001 \)) and treatment- (\( p < 0.0001 \)) dependent reduction in rLV was revealed in the two-way 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 Days 10 and 25 after stroke as compared with saline control animals (\( p = 0.004 \) and \( p = 0.02 \) for Days 10 and 25, respectively, Fig. 1B).

We previously demonstrated that post-stroke CART treatment increased the N-acetylaspartate level (a marker of neuronal viability) as measured by magnetic resonance spectroscopy and retrograde Fluoro-Ruby labeling in the peri-lesioned area, suggesting that CART may increase neuronal activity in the stroke brain (4). To further characterize axonal reinnervation in stroke brain, DTI was used to measure FA in the peri-lesioned area and the corresponding white matter region in the figure.

**Figure 1.** Post-stroke treatment with CART reduced brain infarction area. (A) T2WI was used to measure the size of infarction area on Days 2, 10, and 25 after MCAo. Infarction was found only in the lesioned side cortex in two representative rats before treatment on Day 2. Animals received daily CART (upper panel) or vehicle (lower panel) from Day 3. Post-stroke treatment with CART, compared with vehicle, reduced the size of the lesion area on Days 10 and 25. (B) No difference in volume of infarction area was found on Day 2 before treatment. Post-treatment with CART significantly reduced rLV on Days 10 and 25 after stroke (*\( p < 0.05 \)).
contralateral (or non-lesioned side) hemisphere in 30 stroke rats on Days 2, 10, and 25 post stroke, as previously described (14). We found that absolute FA values in the contralateral (non-stroke side) white matter area were not significantly affected by CART, as no difference was found on Day 2 (prior to CART treatment; \( p = 0.96, t \text{ test} \)), Day 10 (after CART treatment; \( p = 0.97, t \text{ test} \)), or Day 25 (after CART treatment; \( p = 0.95, t \text{ test} \)) after MCAo. Since FA in the contralateral hemisphere was not altered

Figure 2. Post-stroke treatment with CART increased FA in the lesioned cortex. (A) Example of rostral (bregma = −0.36 mm) and caudal (bregma = −2.36 mm) images of FA on Days 2, 10, and 25 (columns, from left to right) after stroke in rats treated with CART or vehicle. The animal treated with CART demonstrated an increase in FA values in peri-lesioned areas (thick arrows) as compared with the vehicle-treated rat (thin arrows). (B) Time course of FA. FA values in the lesioned cortex were normalized to the corresponding value in the contralateral white matter (rFA). CART treatment significantly increased rFA values on Days 10 and 25 after stroke (* \( p < 0.05 \)).
by CART or vehicle, FA in the lesioned hemisphere was normalized to its value in the contralateral hemisphere to reduce variability among animals. rFA was significantly enhanced by CART treatment (p = 0.0005) or time (i.e., days after stroke; p < 0.0001) and there was a significant interaction between treatment and time (p = 0.0006), as revealed by the two-way ANOVA (Fig. 2). CART increased rFA on Days 10 and 25 after stroke (p = 0.004 and p = 0.002 for Days 10 and 25, respectively, post hoc test, Fig. 2B). Typical FA maps from stroke animals receiving CART or vehicle are shown in Figure 2A. Axonal fibers were analyzed by tractography. There was an increase in fiber growth in the CART-treated rats as compared with vehicle-treated rats (Fig. 3) in the peri-lesion areas on Day 25 after MCAo.

An elevated body asymmetry test was used to evaluate neurological deficits in 24 rats (11,18) (n = 12 in each group). All stroke animals developed close to 100% body asymmetry. No difference was found before CART or vehicle treatment on Day 2 (Fig. 4A). Post-stroke CART treatment significantly reduced body asymmetry (CART 13.56 ± 3.29 versus control 18.19 ± 2.37, p < 0.0005, t test) on Day 14 after stroke (Fig. 4A). To further characterize the association between behavioral improvement and reinnervation, the correlation between rFA and body asymmetry was analyzed. We found a significant correlation between the behavioral recovery (difference of body asymmetry between Day 2 and 14) and the change in rFA (rFA difference between Days 2 and 25, ΔrFA) in the CART-treated animals (Fig. 4B, Rho = −0.639, p = 0.025), suggesting that behavioral recovery is associated with reinnervation to the lesioned hemisphere. No correlation of behavioral recovery and rFA was found in the saline-treated control animals.

As angiogenesis is often associated with neuronal reinnervation, we next characterized vascularization using the quantitative SWI (Fig. 5). Stroke rats receiving CART or vehicle treatment showed enhanced vascular activity, indicated by increased local deoxyhemoglobin around the lesion. For rats receiving vehicle, a low-intensity signal with linear shape was identified in the peri-lesion area, indicating an increase in vascularization in this area. A much higher vascular activity with enhanced hypointensity was found in CART-treated rats. Typical SWI images from the two representative CART-treated and two control rats are shown in Figure 5A. The intensity of vascular structure (SWI value) was further quantified (Fig. 5B). CART treatment significantly reduced the intensity of SWI on Day 10 and achieved its minimal value on Day 25 after MCAo. In contrast, in animals receiving vehicle, the SWI value was less reduced on Days 10 and 25 after stroke. A larger reduction in SWI ratio indicates an increase in venous structures in stroke cortex. ANOVA analysis revealed that SWI was significantly affected by time (p < 0.0001) and CART treatment (p = 0.0001). There was also a significant interaction between CART treatment and days after stroke (p = 0.02). The averaged SWI value was significantly reduced by CART treatment on Day 10 (CART 0.64 ± 0.22 versus control 0.85 ± 0.3, p = 0.02, post hoc t test) and Day 25 (CART 0.58 ± 0.21 versus control 0.81 ± 0.19, p = 0.003, post hoc t test) after stroke, suggesting that CART increased vascularization in stroke brain (Fig. 5B).

Using immunostaining, we found that CART enhanced α-SMA expression in the peri-lesion cortex (Fig. 6A). The optical density of α-SMA immunoreactivity in the peri-lesion cortex was quantitatively analyzed by Nikon NIS-Elements software and averaged from three sections with a visualized anterior commissure near bregma from each animal. α-SMA immunoreactivity was significantly enhanced by CART (Fig. 6B, p < 0.01, t test). These data suggest that post-stroke CART treatment increased vascularization in the lesion side cortex.

**DISCUSSION**

In this study, we used non-invasive MRI techniques to characterize structural and functional changes in a rat model of stroke up to 25 days following the stroke onset. We found that post-stroke
treatment with CART, given intranasally from Day 3 after MCAo, reduced brain infarct volume while increasing FA, measured by DTI. Stroke rats receiving CART also demonstrated behavioral recovery, as their body asymmetry was significantly reduced compared with those receiving vehicles. Collectively, our data support the view that post-treatment with CART provides beneficial outcomes after the onset of stroke. The \( T_2 \)-weighted image in acute stroke reflects infarct volume – with edema and inflammatory processes. The greater reduction over time with CART versus saline suggests an augmented resolution of the acute edema associated with neuronal and glial pathology.

Cerebral ischemia can increase endogenous neurorepair. De novo neurogenesis in the SVZ (19) and sprouting from the surviving neurons (20,21) were found in adult mammalian brain after stroke. FA has been used to monitor the maturation of axonal fibers or white matter (22) and degree of myelination (23). We demonstrated that ischemic brain injury increased FA in the peri-lesioned area on Days 10 and 25, compared with Day 2 in control rats, indicating that an endogenous reparative mechanism has been activated in stroke brain. We and others have reported that endogenous neurorepair can be modulated by pharmacological intervention (3,4,14). Post-stroke treatment with CART promoted proliferation of NPCs in the SVZ, facilitated the migration of NPCs to the lesioned cortex, and increased the neuronal signal N-acetyl aspartate in the lesioned side hemisphere (4). In this study, we used non-invasive MRI techniques to measure time-dependent reinnervation by FA in stroke animals. Treatment with CART significantly increased FA in the lesioned cortex on Days 10 and 25. Together with previous immunohistological findings that CART increased GAP43 expression in the lesioned cortex (4), our data suggest that CART post-treatment enhanced neural reinnervation in the lesioned cortex. To further characterize the association of the behavior function with reinnervation to lesioned cortex, the correlation of FA and body asymmetry was analyzed in each animal. We found a significant linear correlation between changes in FA and behavioral recovery in the CART-treated animals. Taken together, these data support that post-treatment with CART enhances neuronal reorganization in the peri-lesioned region, which may contribute to the recovery of neurological function in stroke brain.

Post-stroke angiogenesis is an endogenous reparative process to restore oxygen and nutrient supply to the impaired brain tissue. Angiogenesis and neurogenesis are closely related in response to injury in adult brain (24). Hypoxia or ischemia can activate angiogenic factors, such as vascular endothelial growth factor (25), which modifies angiogenesis and neurogenesis, mainly in the penumbra area (26–28). Clinical studies have shown that higher blood vessel counts in stroke patients correlated with longer survival (29). Proliferation of endothelial cells occurred as early as 12–24 h and a significant increase in vessel density was found 3 days after stroke in experimental animals (30). Pharmacological agents (such as the phosphodiesterase 5 inhibitor sildenafil) or proteins (such as erythropoietin, Ntrk1, or stromal cell-derived factor) that enhance angiogenesis can induce functional recovery in stroke animals (31–34). These data suggest that angiogenesis promotes neurorepair after stroke (see Review (35)).

CART has been shown to alter vascular functions. CART immunoreactivity was found in peripheral blood vessels (36) or nerve fibers that innervate peripheral blood vessels (37). CART has a direct acute effect on cerebral vessels (38,39). In this study, SWI was used to examine cerebral vascular action angiogenesis in stroke brain as described previously (27,40). As SWI also detects cerebral hemorrhage, angiogenesis was further determined by its unique SWI properties. (1) Hemorrhage mainly presents in the ischemic core, while angiogenesis primarily occurs in the peri-lesioned areas (27,28). (2) Hemorrhage induces a round-shaped SWI image while angiogenesis produces enhanced signals with linear structure around the peri-lesioned boundary (27,28,41). In our study, enhanced signals with linear structure in the SWI image were found in the peri-lesioned boundary after treatment with CART, compared with vehicle on Days 10 and 25 after MCAo, suggesting that CART promoted angiogenesis in stroke brain. This MRI finding was further supported by immunohistochemistry. CART increased cerebral vascular

**Figure 4.** CART treatment reduced body asymmetry. (A) Body asymmetry was measured on Days 2 and 14 after stroke onset. Post-stroke treatment with CART significantly reduced body asymmetry on Day 14 (*) \( P < 0.05 \). (B) Correlation of the functional outcomes with the change in rFA. There is a linear correlation between changes in rFA (difference of rFA between Days 2 and 25, \( \Delta rFA \)) and behavioral recovery (difference of body asymmetry between Day 2 and 14, \( \Delta \) body asymmetry) in CART-treated animals (Rho = –0.639, \( P = 0.025 \)). Saline-treated control animals did not show any significant correlation.

density in the ischemic brain, as the α-SMA immunoreactivity in the peri-lesioned areas was significantly enhanced by CART treatment.

In conclusion, we have used quantitative SWI and FA techniques to measure angiogenesis and white matter reinnervation non-invasively in stroke brain. We found that CART treatment,
CONFLICT OF INTEREST

The National Institute on Drug Abuse. This work was supported by the Intramural Research Program of the National Institute on Drug Abuse. Given 3 days after MCAo, increased innervation to the lesioned area, which correlated with the behavioral recovery. Our data support a non-invasive treatment strategy for stroke through angiogenesis and reinnervation by CART.

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CONFLICT OF INTEREST

There are no conflicts of interest to declare.

REFERENCES


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