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CITALOPRAM ENHANCES NEUROVASCULAR REGENERATION AND SENSORIMOTOR FUNCTIONAL RECOVERY AFTER ISCHEMIC STROKE IN MICE

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Abstract—Recent clinical trials have demonstrated that treatment with selective serotonin reuptake inhibitors after stroke enhances motor functional recovery; however, the underlying mechanisms remain to be further elucidated. We hypothesized that daily administration of the clinical drug citalopram would produce these functional benefits via enhancing neurovascular repair in the ischemic peri-infarct region. To test this hypothesis, focal ischemic stroke was induced in male C57/B6 mice by permanent ligation of distal branches of the middle cerebral artery to the barrel cortex and 7-min occlusion of the bilateral common carotid arteries. Citalopram (10 mg/kg, i.p.) was injected 24 h after stroke and daily thereafter. To label proliferating cells, bromo-deoxyuridine was injected daily beginning 3 days after stroke. Immunohistochemical and functional assays were performed to elucidate citalopram-mediated cellular and sensorimotor changes after stroke. Citalopram treatment had no significant effect on infarct formation or edema 3 days after stroke; however, citalopram-treated mice had better functional recovery than saline-treated controls 3 and 14 days after stroke in the adhesive removal test. Increased expression of brain-derived neurotrophic factor was detected in the peri-infarct region 7 days after stroke in citalopram-treated animals. The number of proliferating neural progenitor cells and the distance of neuroblast migration from the sub-ventricular zone toward the ischemic cortex were significantly greater in citalopram-treated mice at 7 days after stroke. Immunohistochemical staining and co-localization analysis showed that citalopram-treated animals generated more new neurons and microvessels in the peri-infarct region 21 and 28 days after stroke. Taken together, these results suggest that citalopram promotes post-stroke sensorimotor recovery likely via enhancing neurogenesis, neural cell migration and the microvessel support in the peri-infarct region of the ischemic brain.

Key words: ischemic stroke, SSRI, citalopram, neurogenesis, angiogenesis.

INTRODUCTION

Stroke is the fourth-leading cause of death and the primary cause of long-term disability in the United States; however, there are limited effective treatments for stroke patients (Towfighi and Saver, 2011). Stroke patients often suffer from post-stroke depression, which can be treated with anti-depressant drugs to alleviate depressive symptoms (Anderson et al., 1994; Wiart et al., 2000; Bilge et al., 2008). Enhanced functional recovery observed in post-stroke depression patients treated with selective serotonin reuptake inhibitors (SSRI) prompted a number of clinical trials looking for further therapeutic benefits of anti-depressant administration after stroke in non-depressed as well as depressed patients (Acler et al., 2009; Chollet et al., 2011; Mikami et al., 2011).

Clinical data suggest that anti-depressant treatments after stroke enhance motor functional recovery independent of their anti-depressant activity. Three months of treatment with the SSRI fluoxetine after stroke significantly improved patient scores in the Fugl-Meyer motor scale (FMMS) and the motor portion of the National Institutes of Health stroke scale (NIHSS) (Chollet et al., 2011). Similarly, in another clinical study, administration of the SSRI citalopram for 4 months after stroke improved NIHSS performance, unrelated to mood (Acler et al., 2009). Fluoxetine and the tricyclic anti-depressant nortriptyline increased scores in the modified Rankin scale, which measures independence in activities of daily life (Chollet et al., 2011; Mikami et al., 2011). These studies provide a foundation supporting the use of anti-depressants for improving stroke recovery. However, the mechanisms behind these therapeutic benefits need to be further elucidated in order to improve treatment and develop more targeted and specific therapeutics.

SSRIs have been noted for their effect on neural progenitor proliferation in the hippocampus, dentate gyrus, and sub-ventricular zone (SVZ). Neuronal progenitor proliferation and migration after stroke to the

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Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BrdU, bromodeoxyuridine; DCX, doublecortin; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; NeuN, neuronal nuclei; NIHSS, National Institutes of Health stroke scale; OCT, optimal cutting temperature; PBS, phosphate-buffered saline; SSRI, selective serotonin reuptake inhibitor; SD, standard deviation; SVZ, sub-ventricular zone; TrkB, tyrosine kinase receptor; TTC, 2,3,5-triphenyltetrazolium chloride.

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damaged area may contribute to long-term recovery (Lindmark and Hamrin, 1988; Kreuzberg et al., 2010). Brain-derived neurotrophic factor (BDNF) is physiologically and pathologically important in the control of survival, proliferation and migration of neural progenitor cells in the SVZ. Chronic SSRI treatment increases the expression of neurotrophic factors in the brain, particularly BDNF mRNA and protein (Balu et al., 2008). BDNF activates the tyrosine kinase receptor (TrkB) that mediates down-stream signaling cascades involved in growth and survival including phosphot idylinositol-3 kinase/Akt pathways, the c-AMP response element (CREB) and the Ras/Mitogen-activated protein kinase pathways (Russo-Neustadt and Chen, 2005). Enhanced BDNF expression is believed to modulate neuronal plasticity, survival, and cell-signaling pathways during development, and contribute to axonal plasticity, learning, memory, and sensorimotor recovery (Mizuno et al., 2000; Schabitz et al., 2004).

Repair of the neurovasculature after stroke is an important step in restoring brain function (Ohab et al., 2006). Vascular regeneration after stroke is also a potential target for SSRI-mediated therapies. BDNF–TrkB receptor activation and downstream Akt has been shown to promote endothelial cell survival (Kermani and Hempstead, 2007). Therefore, SSRI treatments may contribute to new vessel formation and promote the survival of existing vessels after ischemic insult (Greene et al., 2009). The present investigation tested whether administration of the SSRI drug citalopram after stroke could enhance sensorimotor functional recovery in parallel with neurovascular repair in the ischemic peri-infarct region. Citalopram was selected in the investigation based on its clinical applications and functional benefits after stroke evaluated by the NIH stroke score (Acler et al., 2009).

EXPERIMENTAL PROCEDURES

Middle cerebral artery occlusion (MCAO)

The Institutional Animal Care and Use Committee at the Emory University approved all *in vivo* experimental procedures. The focal ischemic stroke targeted to the right barrel cortex was induced as previously described (Li et al., 2007; Ogle et al., 2012) with some modifications. Briefly, adult male C57 mice (Charles River Labs; Wilmington, MA) weighing 20–25 g were anesthetized with 4% chloral hydrate. A distal branch of right middle cerebral artery (MCA) supplying the barrel cortex was permanently ligated by 10–0 suture and the bilateral common carotid arteries (CCA) were occluded for 7-min and then reperfused. Animal body temperature was maintained at 37 ± 0.5 °C using a heating pad controlled by the temperature control unit (Thermocare; Incline Village, NV, USA) during the surgery and in an environmentally controlled incubator after surgery until they recovered from the anesthesia. The mortality rate due to surgery and anesthesia was equal to or less than 10% in this investigation. Fully recovered animals were then returned to their home cages with free access to food and water.

Drug administration

All animals were subjected to the same MCAO procedure and were randomized to saline or citalopram treatment groups after stroke. Researchers were blinded to experimental groups. Citalopram (10 mg/kg) was diluted in sterile saline and injected intra-peritoneally (i.p.) 24 h after stroke and then daily for 7, 14, 21, or 28 days. This chronic drug administration paradigm was chosen due to previous research suggesting that SSRI's effect on depression was due to delayed neurochemical mechanisms and potentially by increasing BDNF levels (Stahl, 1998; Balu et al., 2008). In addition, the 24-h treatment window after stroke provides a clinically relevant paradigm for stroke therapy. In neuroprotection experiments, Citalopram was administered 30 min after stroke and then daily for 3 days until sacrifice at day 3 (n = 20, 10 per group). Bromo-deoxyuridine (BrdU) was diluted in sterile saline (5 mg/ml) and was injected i.p. (10 mg/kg) beginning 72 h after stroke and then daily until sacrifice unless otherwise indicated.

Infarct volume of the ischemic brain

Infarct volume was assessed with a sample size of ten animals per group. Animals were randomly assigned (10 and 10) to citalopram and saline groups and injected i.p. with the appropriate solution 30 min, 24 and 48 h after MCAO. The mortality rate of 10% due to anesthesia and/or surgery resulted in the animal number of nine in each group for analysis. The animals were sacrificed 72 h post-stroke for ischemic infarct size assessment as previously described (Ogle et al., 2012). Briefly, animals were sacrificed under anesthesia; brains were removed and then sliced into 1-mm thick coronal sections. Brain sections were then stained with 2% 2,3,5-triphenyl tetrazolium chloride (TTC) solution at 37 °C for 10 min and were then placed into 10% buffered formalin. After 24 h, brain sections were scanned and images imported into Image J software (NIH, Bethesda, MD, USA), where the stroke infarct, ipsilateral, contralateral, and total area were measured by a blinded researcher. The infarct volume (mm^3) and indirect infarct volume ratio were calculated. Student's t test was used to detect differences between the saline control and citalopram groups.

Edema measurement

Edema or water content of the brain was assessed 72 h after MCAO. Animals were randomly assigned (n = 6 per group) to citalopram or saline treatment groups and injected i.p. with the appropriate solution 30 min, 24 and 48 h after MCAO. After 72 h, the brains were removed and sectioned into three 2-mm thick sections. The contralateral and ipsilateral hemispheres were separated and each was weighed on a piece of pre-weighed tin foil to determine the wet weight. This procedure took less than 30 s to complete. According to other studies, the water loss during this 30 s accounts for less than 0.3% of the wet weight of the hemisphere (Ito et al., 1979). Therefore, the water loss during the procedure was considered negligible. Sections were then dehydrated at