Neuroscience Letters 603 (2015) 31–36

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research paper

Fluoxetine enhanced neurogenesis is not translated to functional outcome in stroke rats

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HIGHLIGHTS

- Fluoxetine encouraged more neuroblasts toward the damaged striatum after stroke.
- Fluoxetine increased the dendritic complexity of newborn dentate granule cells.
- Fuoxetine treatment did not improve sensorimotor recovery following ischemia.

ARTICLE INFO

Article history:
Received 20 March 2015
Received in revised form 2 June 2015
Accepted 28 June 2015
Available online 18 July 2015

Keywords:
Stroke
Selective serotonin reuptake inhibitors
Subventricular zone
Neurogenesis
Functional recovery

ABSTRACT

Fluoxetine is widely used in clinical practice. It regulates hippocampal neurogenesis, however, the effect of fluoxetine on neurogenesis in the subventricular zone (SVZ) remains controversial. We aimed to study the effect of fluoxetine on neurogenesis in the SVZ and subgranular zone (SGZ) of dentate gyrus (DG) in relation to behavioral recovery after stroke in rats.

Adult male Wistar rats were randomly assigned to four groups: sham-operated rats, sham-operated rats treated with fluoxetine, rats subjected to cerebral ischemia, and rats with ischemia treated with fluoxetine. Fluoxetine was orally administrated starting 1 week after ischemia, with a dose of 16 mg/kg/day for 3 weeks. Focal cerebral ischemia was induced by intracranial injection of vasoconstrictive peptide endothelin-1(ET-1). Behavioral recovery was evaluated on post-stroke days 29–31 after which the survival rate and fate of proliferating cells in the SVZ and DG were measured by immunohistochemistry.

The production of neuroblasts in both the SVZ and DG was significantly increased after stroke. Chronic post-stroke fluoxetine treatment increased the dendritic complexity of newborn dentate granule cells. However, fluoxetine treatment did not influence the survival or differentiation of newly generated cells. Neither fluoxetine treatment improved sensorimotor recovery following focal cerebral ischemia.

1. Introduction

Despite extensive research, current effective treatments for stroke are limited. For most stroke patients, functional impairments are inevitable. However, it is interesting that many stroke patients show some degree of functional recovery even without any treatment. Thus, the brain is considered to be highly plastic after stroke. Augmenting the self-repair may be an alternative approach to restore the impaired functions after stroke.

Fluoxetine, one of the selective serotonin reuptake inhibitors (SSRIs), is commonly prescribed for patients to treat post-stroke depression (PSD). There are studies showing that chronic or subchronic treatment with fluoxetine regulates hippocampal neurogenesis, associated with improved the cognitive functions [15]. However, the effect of fluoxetine on neurogenesis in the subventricular zone (SVZ) of the adult brain remains controversial. Majority of the studies have revealed no significant influences of fluoxetine on neurogenesis in the SVZ [19]. One study showed that chronic administration of fluoxetine for more than 6 weeks even decreased

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http://dx.doi.org/10.1016/j.neulet.2015.06.061
0304-3940/

neurogenesis in the SVZ of adult mice [13].This study aimed to compare the effects of fluoxetine on the neurogenesis in the SVZ and dentate gyrus (DG) after experimental focal stroke in relation to behavioral recovery. We provided a new finding that fluoxetine had a significant influence on neurogenesis in the SVZ after focal stroke, but enhanced sensorimotor recovery was not seen.

2. Materials and methods

2.1. Animals

We used adult male Wistar rats (200–250 g) provided by China Medical University (Shenyang, China). The rats were randomly assigned to four groups: sham-operated rats ($n = 8$, SHAM), sham-operated rats treated with fluoxetine ($n = 8$, SHAM + FLUO), rats subjected to cerebral ischemia ($n = 8$, ISCH), and rats with ischemia treated with fluoxetine ($n = 8$, ISCH + FLUO). All rats were housed in a temperature- and humidity-controlled room with a 12 h light/dark cycle and the rats had free access to food and water.

Anesthesia was induced by a mixture of 3% isoflurane in 30% oxygen and 70% nitrous oxide and animals were maintained with 1.5% isoflurane through the surgery. The study protocol was approved by the Institutional Animal Care and Use Committee of China Medical University [permit No.: SCXK (Liao) 2008-0005].

2.2. Endothelin-1 (ET-1) stroke model

To induce focal cerebral ischemia, the vasoconstrictive peptide endothelin-1 (ET-1) (Sigma, USA) was injected at the following coordinates: (1) AP +0.7 mm, ML +2.2 mm, DV −2.0 mm; (2) AP +2.3 mm, ML +2.5 mm, DV −2.3 mm; and (3) AP +0.7 mm, ML +3.8 mm, DV −5.8 mm according to the rat brain atlas by Paxinos and Watson [18]. ET-1 was injected at 0.5 μl/min by an infusion pump, and the needle left in situ for 3 min post-injection before being slowly removed to minimize backflow. The volume of each injection was 2 μl (0.5 μg/μl). Sham-operated animals were received the same surgery except saline was injected instead of ET-1.

2.3. Drug treatment and 5-bromo-2-deoxyuridine labeling

Rats consumed an average of 16 mg/kg/day with drinking water containing 10 mg/ml of fluoxetine for 3 weeks, the dosage was selected based on previous reports [9], which was shown to lead to serum levels of fluoxetine that were equivalent to therapeutic doses used in patients receiving fluoxetine as antidepressant medication.

To label newly generated cells, all rats received twice daily intraperitoneal injections of 5-bromo-2-deoxyuridine (BrdU; 100 mg/kg, Sigma–Aldrich) during postoperative days 5-6 (Supplementary Fig. 1A).

2.4. Tissue preparation and immunohistochemistry

Rats were anesthetized and perfused with 4% paraformaldehyde. The brains were removed and postfixed, and embedded in OCT medium. Brain tissue was cut into 40-μm-thick sections on a cryotome (Thermo Electron, Waltham, MA, USA). Sections (40 μm) were processed for immunohistochemistry as previously described [10]. BrdU staining was preceded by DNA denaturation and incorporated BrdU was detected using sheep anti-BrdU (1:500, Abcam, USA). The following antibodies for phenotyping were applied in combination with anti-BrdU: guinea pig anti-DCX (1:800, Millipore, USA), mouse anti-NeuN Alexa Fluor®488 conjugated (1:500, Chemicon, USA), rabbit anti-Iba-1 (1:500, Abcam, USA) or rabbit anti-GFAP (1:1000, Abcam, USA). After rinsing, sections were incubated with appropriate secondary antibodies Alexa Fluor 594 or Alexa Fluor 488 (1:500, Invitrogen, USA) for immunofluorescent labeling. Sections were mounted, and then cover slipped (Invitrogen, USA).

2.5. Morphological analyses

The immunofluorescence images of $BrdU^+/DCX^+$ cells in the SVZ ipsilateral to the infarct were acquired with a 20× objective (every sixth section between bregma levels +0.96 mm and −0.24 mm, 5 sections per rat). Results were presented as the average areas of $BrdU^+/DCX^+$ cells per section. The number of $BrdU^+/NeuN^+$ cells, $BrdU^+/GFAP^+$ cells and $BrdU^+/Iba\text{-}1^+$cells in the peri-infarct striatum were counted with a 100× objective (every sixth sections between bregma levels +0.96 mm and –0.12 mm, 5 sections per rat). Immunopositive cells were counted in each section by an experimenter blinded to the treatment conditions. DCX-positive cells counted in the DG were obtained using a microscope (Olympus, BS51, Japan) with a 10×objective (every sixth section between bregma levels −3.0 mm and −3.96 mm, 4 sections per rat). Images of DCX-positive cells in the DG were selected on a random basis using a confocal system (Leica SP2, FV-1000, Germany) on a multitrack configuration. Z-series stacks of 1024 × 1024 pixel images were taken using a 40× objective, three-dimensional reconstructions of entire dendritic arbors were made and the total length of the dendrites was traced using NIH Image [5]. A mean cell count was obtained.

2.6. Measurement of infarct volume

For assessment of the infarct volume, sections were collected between +4.5 mm and −7.5 mm from the bregma at 1 mm intervals for measurement of the infarct volume with cresyl violet (Sigma, USA). The contralateral and ipsilateral hemisphere areas were measured by a blinded observer using NIH Image J. Infarct volumes were calculated by subtracting the area of the injured hemisphere from the area of the contralateral hemisphere in each section and areas were multiplied by the distance between sections to obtain the respective volumes [14].

2.7. Tapered/ledged beam-walking test

Rats were pre-trained for 3 days to traverse the beam before ischemia induction and were then tested at 29 days after ischemia. Performance in the beam walking test was videotaped and later analyzed by calculating the slip ratio of the impaired (contralateral to lesion) forelimb or hindlimb (number of slips/number of total steps). Steps onto the ledge were scored as a full slip and a half slip was given if the limb touched the side of the beam [25].

2.8. Cylinder test

Rats were placed individually in a clear plexiglas cylinder (20 cm in diameter, 45 cm high) and videotaped from the below for 3 min via an angled mirror. A blind observer viewed the videotapes and counted the contacts by both forelimbs and by either left or right forelimb to the walls of the cylinder. The percentage of impaired (contralateral to lesion) forelimb use was calculated according to the following formula: impaired forelimb contacts/(impaired + unimpaired + both limb contacts) × 100% [24].

2.9. Statistics

Statistical analyses were conducted using SPSS software (version 19). The data of the behavior tests were analyzed using one-way analysis of variance (ANOVA). Statistical differences between groups were analyzed using the least significant difference