The role of the hippocampus and the function of calcitonin gene-related peptide in the mechanism of traumatic brain injury accelerating fracture-healing


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Abstract. – OBJECTIVE: This research attempts to identify the part the hippocampus plays in accelerated fracture-healing after traumatic brain injury as well as to test functions of calcitonin gene-related peptide (CGRP) during this process.

MATERIALS AND METHODS: Experiments were carried out on Male Sprague-Dawley rats that were split into four groups at random: TBI-fracture group, fracture-only group, TBI-only group, and control group. In the first week, blood specimen would be drawn from rats among the groups except those of the control group at three-time points (24, 72 and 168 hours) post-damage. These rats would be assessed from the neurological perspective based on their grades of performance in a sequence of tests 24 hours before and 12 hours after brain injury. Blood samples were also taken from the control group 24 hours before the injury, and whole brain tissues in the injured groups were harvested at 72 and 168 hours post-injury. We compared the serum CGRP concentration, the distribution of CGRP, the CGRP expression, and the expression of CGRP in the hippocampus, the expression of CGRP in the hippocampus, the expression of CGRP in the hippocampus, and the expression of CGRP in the brain by immunohistochemistry, Western blotting, RT-Of CGRP RNA expression levels.

RESULTS: Neurological examinations suggested that the functions of the cerebral cortex, cerebellum, and brain stem showed significant differences pre- and post-injury (p < 0.001). ELISA analysis indicated a great density of CGRP in TBI-fracture group at different time points. Furthermore, in the TBI-fracture group, CGRP in both hippocampus and the whole brain showed a noticeable augment in RT-PCR and western blot analysis at 72 and 168 h post-injury, and only in this group, immunohistochemistry analysis indicated that CGRP was present in the hippocampus at 168 hours post-injury.

CONCLUSIONS: We observed that the hippocampus and CGRP were responsible for quick bone-healing mechanisms. We suggest a role for the hippocampus in accelerated fracture healing. CGRP expression, as determined by IHC, cannot be observed in other groups, indicating that the hippocampus may be the specific component of the brain that responds to “big stress”.

Key Words: Hippocampus, Fracture-healing, Traumatic brain injury, Calcitonin gene-related peptide.

Introduction

Traumatic brain injury (TBI) combined with a fracture is common in road accidents, which is a dominant reason for death as well as disability. However, fracture-healing of patients with TBI is often accelerated in clinical practice. According to Perkins and Skirving in 1987, speeded-up callus formation along with sooner union existed in patients who had long-bone fractures and TBI. As a result, various researches have been conducted with the intention of identifying mechanisms causing this phenomenon over past several years, which, however, remains unclear today. The recovery for fracture united with peripheral nerve injury is likely to be tardy. On the contrary, fracture with damage to the central nervous system (CNS) accelerates the healing process. Thus, the CNS should be the focal point of current research. The hippocampus is sensitive to outside injury information, which is a primary part of the human brain, reposining on its medial temporal lobe. Despite the importance
Calcitonin gene-related peptide (CGRP) is a neuropeptide produced by cells of the central and peripheral nervous system. The function of CGRP in bone metabolism has been well demonstrated. Osteoblasts possess receptors for CGRP which can stimulate osteoblast proliferation, growth factor and cytokine synthesis, collagen synthesis and bone formation. Following a subarachnoid hemorrhage, CGRP is also released. In addition, the survival of tissues is correlated with the greater density of CGRP immunoreactivity, and endogenous CGRP exerts a regulatory impact on ischemia. Thus, CGRP is a candidate for connecting the CNS to the fracture site. The study attempts to identify the function of the hippocampus in accelerated fracture-healing as well as to test functions of CGRP in this process.

Materials and Methods

Animals
Experiments were carried out on 300-350g Male Sprague-Dawley rats (Laboratory Animal Center of the Fourth Military Medical University, Xi’an, China). The animals were split into four groups at random: TBI-fracture group, fracture-only group, TBI-only group, and control group. Experiments on the rats were done in accordance with related Chinese regulations on Animal Welfare, and had acquired the permission from the Ethical Committee of the Fourth Military Medical University, Xi’an, China.

Surgical Technique
Closed transverse femoral shaft fractures were created as stated by Bonnarens and Einhorn. In short, after weighed by the balance, the rats would be anesthetized by injected 20% urethane solution intraperitoneally. Their left rear legs were de-haired, sterilized and draped. A median parapatellar incision had been conducted before the same operation into the joint capsule (stretching from the midline throughout the vastus medialis muscle until the patellar tendon insertion). Tardy flexion of the knee while moving the patella resulted in lateral dislocation. An intercondylar entry point was made with a handheld drill so that a 1.2 mm diameter Kirschner wire could be inserted into the medullary space, which would stick in till the greater trochanter was met. Next, the wire was mildly retracted, cut, reinserted, and buried under the knee cartilage surface. Surgical spots and the skin would be sutured. At the next step, an obtuse guillotine would be used to create the fracture, and radiographs would be made to locate the pin placement and the fracture configuration. Comminuted fractures or fractures not happening in the middle position of the diaphysis would not be included.

Traumatic Brain Injury
TBI was produced by a soundly-built strike acceleration system. This model was proven to reproducibly impart a diffuse axonal injury with a resemblance to what had been discovered in patients with TBI. In case the rat exhibited traces of a normal healing of the closed femoral shaft fracture, a nickel-plated cap would be fixed above the rat’s skull. After that, the rat’s head would be placed on a foam board under stainless steel tubing with the smooth inner wall, the end of which was around the nickel plate straightly over the rat’s head. Then, a 250 g weight was fallen onto the rat’s head from as high as exactly 1 m above. Upon the contact, the foam board, as well as the rat, was taken away to avert a rebound hit so as to restrict the damage to a one-time strike. Rats were taken back to their cages and were permitted to heal in observation.

Neurological Examination
The rats have been assessed from the neurological perspective based on their scores of behavior in a sequence of tests 24 hours before and 12 hours after brain injury according to the established protocols of Arrieta-Cruz et al and Ziporen et al. See Table I for additional details.

Specimen Collection
Blood specimen have been drawn from animals in the TBI-fracture, fracture-only and TBI-only groups at three-time points in the first week (24, 72 and 168 hours) post-injury. Blood samples were also taken from the control group 24 hours before damage. All specimens were centrifuged at 10000 g for 15 min in 30 min after gathering, and the consequent supernatant was reserved at -80°C for ELISA analysis. Whole brain tissues in the injured groups were gathered at 72 and 168 hours post-damage and were formalin-fixed and climbed for Hematoxylin and Eosin (H&E) staining and immunohistochemistry (IHC). The hippocampus and the whole brain were also used for Western blot (at 72 hours) and reverse transcription-polymerase chain reaction (RT-PCR)
Brain and blood specimens were also acquired from rats in the control group to serve as controls. All samples had been acquired at around 08:00 a.m. for the purpose of keeping resembling situation for all groups.

### ELISA Analysis

Employing a CGRP ELISA immunoassay kit (Multi Science, Hangzhou, China), an ELISA analysis was performed for the quantitative determination of CGRP existing in the rat serum.

**Table I.** Neurological tests applied to brain-injured rats.

<table>
<thead>
<tr>
<th>NO</th>
<th>Tests</th>
<th>Evaluating method</th>
<th>Method</th>
<th>CNS localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Skin color</td>
<td>0=blanched, 0.3=blue or violet, 0.6=bright, deep red flush, 1=pink</td>
<td>Noticed for consciousness, breeding, curiosity, grooming, and autonomous motions.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Urination and defecation</td>
<td>0=absent, 1=present</td>
<td>Grasping the dorsum of the front paws near a desk edge leads to laying of palms on the desk.</td>
<td>Cerebral cortex</td>
</tr>
<tr>
<td>3</td>
<td>Body position</td>
<td>0=incapacity, 0.5=limited movement, 1=normal</td>
<td>Letting one hind leg down from the table (along with other three legs on the table) causes uplifting of the leg to the table.</td>
<td>Cerebral cortex</td>
</tr>
<tr>
<td>4</td>
<td>Front leg placing</td>
<td>0=absent, 1=present</td>
<td>The rat is captured at the tail above a table and is lowered till the beard is close to the table. The paws are then placed on the table.</td>
<td>Cerebral cortex</td>
</tr>
<tr>
<td>5</td>
<td>Hind leg placing</td>
<td>0=absent, 1=present</td>
<td>Incapability of walking on a 6-cm wide beam or walking in straight line</td>
<td>Cerebellum</td>
</tr>
<tr>
<td>6</td>
<td>Placing reflex</td>
<td>0=absent, 1=present</td>
<td>Uplifting the rat by its tail results in posterior legs abduction, unfolding of toes, arching of the back, and uplifting of the head.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Gait</td>
<td>0=Inability to walk straight, 0.5=Inability to walk on 6-cm-wide beam, 1=walk on 6-cm-wide beam</td>
<td>The rat is put head down on a platform tilted at 30°, and upward climbing is observed.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Equilibrium</td>
<td>0=absent, 1=present</td>
<td>Uplifting the rat by its tail results in posterior legs abduction, unfolding of toes, arching of the back, and uplifting of the head.</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Postural reflex</td>
<td>0=absent, 1=present</td>
<td>Incapability of walking on a 6-cm wide beam or walking in straight line</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Righting reflex</td>
<td>0=no response, 0.5=slow response, 1=active over its feet and impossible to evaluate</td>
<td>When the rat is placed on its back, it turns over immediately.</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Flexion reflex</td>
<td>0=slight withdrawal or none, 0.5=moderate withdrawal, 1=brisk rapid withdrawal</td>
<td>The rat is picked up, and the toes are pinched with forceps, leading to (not brisk), foot withdrawal.</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Grip</td>
<td>0=less than 14 seconds, 0.5=from 15 to 30 seconds, 1=more than 30 seconds</td>
<td>A neurologically normal rat should be able to hang suspended on a stationary bar for at least 30 seconds.</td>
<td></td>
</tr>
</tbody>
</table>
The CGRP assay was conducted based on the producer’s specifications. The optical density was surveyed at a wavelength of 450 nm by a microplate reader (Multiskan Ascent, Thermo Labsystems, Shanghai, China). Concentrations were worked out and presented in pg/ml.

**Immunohistochemistry Assay**

A 4 µm-thick tissue section was set on a slide. Having been deparaffinized and dehydrated by xylene and ethanol, respectively, the slide was prepared for antigen retrieval by subjecting to microwave pretreatment for 10 min. Incubation with 3% H2O2 was adopted to prevent endogenous peroxidase in the tissue. After washes with phosphate-buffered saline, the slide was incubated for the whole night with a rabbit anti-rat CGRP primary polyclonal antibody (Abcam, Cambridge, MA, USA) at 4°C. In the next step, the slide would be rinsed and treated for 15 min with a goat anti-rabbit secondary antibody as well as the Avidin-Biotin reagent for 30 min in succession. The slide was then incubated in diaminobenzidine for 10 min, counterstained with hematoxylin, dehydrated in a graded series of alcohols, cleared in xylene and mounted. Sections from the normal brain of the control group were included as controls.

**Western Blot Analysis**

Western blotting was used in order to detect CGRP expression in the hippocampus. The samples were homogenized in 10 mmol/L Tris homogenization buffer (pH 7.4) with protease inhibitors (1 tablet in 50 m, Sigma-Aldrich, St. Louis, MO, USA) and centrifuged at 12,000 rpm for 20 min, after which the supernatant was gathered. Samples (50 µg protein) were filled into an 8% SDS gel; the proteins were divided by electrophoresis before moved to nitrocellulose membranes, which were then incubated with a rabbit anti-rat CGRP primary polyclonal antibody (Abcam, Cambridge, MA, USA). The membranes were rinsed 3 times for 10 min and incubated with goat anti-rabbit IgG-HRP (1:2000, Santa Cruz Biotecnology, Santa Cruz, CA, USA). An ECL western blotting detection kit visualized the immunoreactive bands (Pierce, Rockford, IL, USA) on light-sensitive film. β-actin was used as an endogenous reference, and average light density analysis was employed to work out the ratio of CGRP expression to β-actin.

**Reverse Transcription of mRNA and RT-PCR Analysis**

Total RNA was separated from the whole brain by the Trizol reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the producer’s instructions. The primary sequences applied to amplify CGRP were 5’-AAGTTCTCCCTTT-TCCGTG-3’ as well as 5’-GCCTCTTCTTCTCTC- CTC-3’. The PCR protocol contained 1 cycle at 94°C for 30 seconds, 30 cycles at 52°C for 30 seconds, 1 cycle at 72°C for 30 seconds, and 1 cycle at 72°C for 8 minutes. β-actin was enlarged and used as an internal standard; the primary sequences were 5’-TGCCGCATCCTTTCTCT-3 and 5’-GGATGTAAACGTACACTTC-3’. For the purpose of deciding the expression levels of CGRP RNA in the brain, average light density analysis worked out the ratio of CGRP mRNA to β-actin mRNA.

**Statistical Analysis**

The data were noted down as the mean±SD and subjected to a normality test to check the normal distribution. In the case of the failure of the normality test, a non-parametric test would be used for the analysis of the data. In the case of the success of the normality test, one-way ANOVA and the succedent Student-Newman-Keuls test were employed for the assessment of statistical analysis with intention of comparing the variations between different groups. All statistical analyses were carried out by the employment of the statistical program for social sciences (SPSS Inc., Chicago, IL, USA) 17.0. p <0.05 was treated of statistical significance.

**Results**

**General Observation**

After the operation, two rats died in the TBI-fracture group as a result of head trauma, which led to a massive growth in morbidity. One animal in the TBI-fracture group, as well as one in the fracture-only group, were excluded because of a comminuted fracture. Infections were not detected among all groups; neither was nonunion in either the TBI-fracture or fracture-only groups. Nearly all brain-damaged rats appear apnea at once following the damage with a decrease of the respiratory rate by approximately 22% for 20-30 min, and then their respiration slowly returned to normal with no remarkable discrepancy from rats in the control group. Blood pressure increased
approximately 17% compared to the pre-injury level at 15 seconds since impact before gradually dropping back to normal in 30 minutes of it. Heart rate reduced from a pre-injury level of 341±28 to 261±51 beats/min at 1 min post-injury before slowly returned to normal in 10 min post-injury. Generalized convulsions developed immediately after injury and lasted approximately 15-30 seconds. Most animals suffered decortication flexion deformity of the forelimbs. No one group showed differences in capability of walking one day after the injury.

Pathological Changes

**Gross pathological observation.** Subarachnoid, intraventricular and brain stem petechial hemorrhages were discovered among brain-injured rats; however, the brains appeared otherwise normal without any contusions or additional focal lesions. Skull fractures did not develop. **Microscopic changes.** In the control group, the neurons distributed regularly and the structures were intact. In the brain-injured rats, the neurons in the cortex and hippocampus exhibited a disordered distribution with a concomitant reduction in normally distributed neurons. Shrunken neurons integrated with perineuronal vacuolation were detected in these fields, and so were dark, contracted neurons with corkscrew-like processes (Figure 1).

**Neurological Function Analysis**

There was a significant difference in the total neurological scores among brain-injured animals before and after injury ($p < 0.001$). The cerebral cortex, cerebellum, and brain stem functions showed significant differences pre- and post-injury ($p < 0.001$); there were not prominent changes in spinal cords ($p > 0.05$). Rats in the control group also presented no difference in function pre- and post-injury ($p > 0.05$; Table II).

**ELISA Analysis**

At 24 hours after the injury, no prominent changes in CGRP density were discovered in the fracture-only group or the TBI-only one in comparison with the control group ($p > 0.05$); however, the TBI-fracture group presented more CGRP compared with the control group ($p < 0.05$). Then, at 72 hours post-fracture, all experimental groups expressed greater density of CGRP compared with the control one, while the TBI-fracture group had more CGRP compared with the fracture-only group ($p < 0.05$). At 168 hours post-fracture, CGRP expression in the TBI-fracture group along with the fracture-only group kept elevated ($p > 0.05$), and the TBI-fracture group had higher levels of CGRP compared with the fracture-only group ($p < 0.05$; Table III).

**Immunohistochemistry Assay**

CGRP was discovered in the hippocampus in the TBI-fracture group only at 168 hours post-injury; CGRP was not detected at 72 hours post-injury in either the TBI-fracture or the fracture-only group, nor was it discovered in the fracture-only group at 168 hours post-injury or the control group (Figure 2).

![Figure 1. Photomicrographs of the hippocampus 3 days after head injury in rats. Neurons were distributed regularly, and structures were intact in Control group. The neurons were distributed in a disorderly manner, and thus the number of normally distributed neurons was fewer. Dark, contracted neurons were observed in Brain-injured group (Magnification×200).](image-url)

<table>
<thead>
<tr>
<th>Injury time</th>
<th>Total scores (TBI group)</th>
<th>Cerebral cortex (TBI group)</th>
<th>Cerebellum (TBI group)</th>
<th>Brain stem (TBI group)</th>
<th>Spinal cord (TBI group)</th>
<th>Total scores (control group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-injury</td>
<td>11.70±0.56</td>
<td>2.80±0.56</td>
<td>2.00±0.00</td>
<td>3.00±0.00</td>
<td>0.97±0.20</td>
<td>11.90±0.32</td>
</tr>
<tr>
<td>12 h post-injury</td>
<td>6.20±0.99*</td>
<td>1.60±0.63*</td>
<td>0.26±0.50*</td>
<td>0.97±0.55*</td>
<td>0.80±0.32#</td>
<td>11.50±0.71 #</td>
</tr>
</tbody>
</table>

*p < 0.001 vs. scores before injury; *p > 0.05 vs. scores before injury
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**Western Blot Analysis**

CGRP expression was higher in the TBI-fracture group compared with the fracture-only and control ones at 72 hours post-fracture ($p < 0.05$ and 0.001, respectively) and was increased further at 168 hours ($p < 0.001$). The TBI-fracture group expressed more CGRP contrasted with the fracture-only group ($p < 0.01$; Figure 3).

**RT-PCR Analysis**

RT-PCR showed that the mRNA expression of CGRP rose in the TBI-fracture group at 72 hours after brain injury ($p < 0.05$, contrasted to the control group). At 168 hours after the fracture, CGRP expression increased in the TBI-fracture and fracture-only groups compared with expression at 72 h ($p < 0.001$ and 0.05, respectively; Figure 4).

**Discussion**

Clinical observations of long-bone fractures in patients who also had TBI have shown an increase in fracture healing speed. In 1987, patients with long-bone fractures and TBI manifested added callus formation and sooner union, according to Perkins and Skirving. Over past few years, a number of studies have been carried out to reveal the mechanisms responsible for this phenomenon. However, until now, mechanisms behind the situation were unclear. Many orthopedic resear-

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**Table III.** ELISA analysis of serum CGRP.

<table>
<thead>
<tr>
<th>Group</th>
<th>CGRP (pg/ml)</th>
<th>0 h</th>
<th>24 h</th>
<th>72 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBI-fracture</td>
<td>27.22±10.69</td>
<td>35.33±4.81</td>
<td>34.45±6.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fracture-only</td>
<td>21.22±2.36</td>
<td>27.89±5.83</td>
<td>27.33±4.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBI-only</td>
<td>22.33±5.71</td>
<td>29.44±6.17</td>
<td>24.00±3.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>19.22±4.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2.** Expression of CGRP, as determined by immunohistochemistry, 168 h post-damage. CGRP was detected in the hippocampus in the TBI-fracture group, instead of in the fracture-only group (Magnification: ×16 for left images; ×100 for right images).

**Figure 3.** Expression of CGRP in the hippocampus at different time points post-injury (0, 72 and 168 h). Data were calculated as the ratio of expression to β-actin expression. (A) Control (0.0259±0.0253). (B) TBI-fracture group at 72 h (0.2851±0.0868). (C) Fracture-only group at 72 h (0.1625±0.0631). (D) TBI-fracture group at 168 h (0.5851±0.0995). (E) Fracture-only group at 168 h (0.3282±0.0732). *$p < 0.001$ vs. control, $p < 0.05$ vs. fracture-only group at 72 h, **$p<0.001$ vs. TBI-fracture group at 72 h.
chers focused directly on humoral factors or local fracture sites, and many cytokines and growth factors have been shown to participate in the mechanisms. Nevertheless, our studies mainly focused in another direction. When a fracture occurs at the same time as peripheral nerve injury, the recovery may be tardy. On the contrary, fractures associated with spinal injury and cerebral cortex injury speed up the recovery course. Differences mentioned above imply that the CNS should be the focus of current research, and humoral factors may be used to carry out such research.

Using the neurological tests of Arrieta-Cruz et al and Ziporen et al as a guide, we developed a new assay in which the difficult-to-implement and strong subjective items had been removed. In this assay, the neurological state of animals was assessed based on a sequence of neurological tests that were based on neuroanatomy and neurophysiology. This assay is a gross technique for revealing main neurological dysfunction, resembling a clinical neurological examination. The assay is not perfect, and not all of the measures are statistically independent of each other. However, in the present experimental TBI model, the assay was able to reveal deficits. In our work, changes in neurological scores indicated that functions of the cerebral cortex, cerebellum, and brain stem were altered.

As a 37-amino-acid neuropeptide, CGRP is encoded by the Calca gene and is expressed and alternatively spliced in cells of central and peripheral nervous system. The role of CGRP in bone metabolism has been well demonstrated. Osteoblastic cells possess receptors for CGRP, which can stimulate osteoblast proliferation, growth factor and cytokine synthesis, collagen synthesis as well as bone formation. CGRP also adds the quantity of bone colonies formed by bone marrow stromal cells in vitro and inhibits bone resorption in vitro. In our previous report, CGRP was indicated to play a large part in accelerating bone healing. CGRP exists in a large amount in the central and peripheral nervous system. CGRP-receptor component protein (RCP) is a coupling protein needed for the CGRP-receptor signaling. RCP-immunoreactive perikarya extensively and alternatively exist in the cerebral cortex, hippocampus and other parts of the brain. CGRP significantly ameliorates vasospasm, improves cerebral blood flow, and reduces cortical and endothelial cell death. Moreover, CGRP can stimulate angiogenesis and increase the formation of insulin-like growth factor-I in the brain, which imposes advantageous influence on the cognitive function through improving synaptic transmission and through stimulating neurogenesis in the hippocampus. The greater density of CGRP is correlated with tissue survival, while endogenous CGRP exerts a regulatory impact on ischemia. Following subarachnoid hemorrhage, CGRP is released. In our study, serum CGRP increased 24 hours post-injury in the TBI-fracture group and kept raising with time. However, in the fracture-only group, the values did not exceed those in the TBI-fracture group at any time point. This finding indicated that the increased CGRP in the TBI-fracture group can only be attributed to TBI. Furthermore, the accelerated healing indirectly suggested that CGRP has a marked effect on both TBI and fracture healing.

The blood-brain barrier (BBB) contains non-fenestrated endothelial cells associated with tight junctions and conforms to a lasting basement membrane, which is a structural and functional barrier between cerebral capillaries and brain parenchyma, and restricts the free pass of large molecules from blood into the CNS. The BBB reflects an important barrier to various CNS-active agents. The novel approach of re-
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CGRP expression in the hippocampus was not in accordance with those of the whole brain. Thus, the increase in CGRP mRNA in the brain in the TBI-fracture group may also be released from another part of the brain. Determining the specific area of the hippocampus that is responsible for these mechanisms and whether other parts of the brain also will be the topic of a future study. As the mechanism of rapid fracture-healing becomes further understood, patients will hopefully soon benefit from faster healing after the fracture.

Conclusions

Taken above, dynamic changes in CGRP expression in the hippocampus were not in accordance with those of the whole brain. Thus, the increase in CGRP mRNA in the brain in the TBI-fracture group may also be released from another part of the brain. Determining the specific area of the hippocampus that is responsible for these mechanisms and whether other parts of the brain also will be the topic of a future study. As the mechanism of rapid fracture-healing becomes further understood, patients will hopefully soon benefit from faster healing after the fracture.

Funding

This study was supported by the National Natural Science Research Foundation of China (No: 81670467), Beijing Scientific & Technologic Supernova Supportive Project (No: 1511000030000/XXJH2015B100), Youth Project of National Natural fund (No: 81406048), the Beijing Natural Science Fund (No: 7152136), Youth Culture Project of Chinese PLA (No: 13QNP171), Surface Project of Hainan Province Natural Fund (No: 20158315), and Clinical Science Fund of PLA General Hospital (No: 2015FC-ZHCG-2007, 2012FC-TSYS-4028).
Acknowledgments
We thank Rong Lv, Jie Wu and Jing Li for help with the photomicrographs. We also thank Xin Bu for help with the data analysis and Shusen Wang and Xiaorui Cao for their thoughtful discussions.

Authors’ contributions
Song Y carried out all experiment in this study, Zhu HY participated in the design of the study and performed the statistical analysis. Han GX, Chen L, Zhai YZ, Dong J, Chen W and Li TS conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Conflict of interest
The authors declare no conflicts of interest.

References
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