Gliosis in the Amygdala Following Myocardial Infarction in the Rat

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ABSTRACT. We observed gliosis with cell death in the rat amygdala 3 and 14 days after myocardial infarction (MI). Cresyl violet-positive neurons had condensed cytoplasm, and Fluoro-Jade B-positive cells were detected in the amygdala 14 days, not 3 days, after MI. Only a few glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes and ionized calcium-binding adapter molecule 1 (Iba-1)-immunoreactive microglia showed activated forms; hypertrophied cytoplasm, and highly ramified and retracted processes of astrocytes and microglia in the amygdala at 3 days after MI, respectively. At 14 days after MI, many astrocytes and most of microglia showed activated forms. These results suggest that MI may induce neuronal death and reactive gliosis in the amygdala.

KEY WORDS: amygdala, gliosis, myocardial infarction, rat.

Myocardial infarction (MI), also known as a heart attack, occurs when the blood supply to the heart is interrupted and finally causes damage to the myocardium. Recent studies have demonstrated that depression is frequently observed after MI, suggesting a link between heart disease and brain function [6, 9, 22]. A previous study reported that neuronal death occurred in the amygdala 72 hr after a 40-min coronary artery occlusion [15, 23]. The amygdala is a central component of the limbic system, and it plays a crucial role in behavioral responses to emotional stress [7, 12, 17].

Microglia and astrocytes are dynamic cells within the brain that alter their structural and physiologic capacities in response to the functional state of neural tissue. Although some researchers have shown neuronal death in the amygdala after MI [22, 23], there are no studies concerning gliosis in the rat amygdala after MI. Therefore, in this study, we examined morphological changes in astrocytes and microglia in the amygdala 3 and 14 days after MI.

MATERIALS AND METHODS

Experimental animals: Male Sprague-Dawley rats were obtained from Orient Bio (Gapyeong, Korea) and were used at 8 weeks (B.W. 300 g, n=15) of age. The animals were housed in a conventional state under adequate temperature (23°C) and humidity (60%) control with a 12-hr light/12-hr dark cycle, and provided with free access to water and food. The procedures for handling animals and their care conformed to the guidelines that are in compliance with current international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985, revised 1996). All of the experiments were conducted to minimize the number of animals used and suffering caused by such procedures.

Induction of MI: The animals were anesthetized by intraperitoneal injection of the mixture of urethane (30 mg/kg) and xylazine (2 mg/kg). After adequate anesthesia, all animals were intubated in a supine position and ventilated on room air with the small animal ventilator (Model SAR-830/P, CWE Inc., Ardmore, PA, U.S.A.). MI was induced by previous mentioned method [13]. Left thoracotomy was performed at the third intercostal space, and the pericardium was opened. The left coronary artery was ligated permanently beneath the left atrial appendage by use of a 6-0 sterile silk. The effective ligation was confirmed by changing the color from red to white in the left ventricle below the ligation site and by the enlargement of the left atrium just after ligating the coronary artery. After completing ligation, the thorax was closed. Sham-operated animals were subjected to the same surgical procedures except that the left coronary artery was not ligated.

Tissue processing for histology: For histology, animals (n=5 in each group) were anesthetized with urethane (2 g/kg) on the days 3 and 14 after ischemia/reperfusion, and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate-buffer (PB, pH 7.4). The brains and hearts were removed and the infarct size was measured as a ratio of the infarcted area to the mean lateral ventricle circumference in sections (thickness=2 mm) of the hearts using NIH ImageJ software. The brain tissues were cryoprotected by infiltration with 30% sucrose overnight. Thereafter, frozen tissues were serially sectioned on a cryostat (Leica, Wetzlar, Ger-
many) into 30 μm coronal sections and then the sections were collected into six-well plates containing PBS. To elucidate the neuronal death and reactive gliosis, 5 sections with 200 μm interval from each animal according to anatomical landmarks corresponding to Bregma –3.00 ~ –4.08 mm of rat brain atlas [18].

Histochemistry for neurons and cell death: To elucidate the neuronal damage induced by MI, sections were stained with cresyl violet 3 and 14 days after the surgery as mentioned previous report [12]. In addition, Fluoro-Jade B (F-J B, a high affinity fluorescent marker for the localization of neuronal degeneration) histofluorescence staining procedures were conducted according to the method by Candelario-Jalil et al. [5].

Immunohistochemistry for glial markers: In order to examine the accurate degree of reactive gliosis after MI, immunohistochemistry was processed under the same conditions 3 and 14 days after the surgery as mentioned previous study [16]. Briefly, sections were incubated with rabbit anti-glial fibrillary acidic protein (GFAP) (diluted 1:1,000, Chemicon International, Temecula, CA, U.S.A.) for astrocytes and rabbit anti-ionized calcium-binding adapter molecule 1 (Iba-1) (diluted 1:500, Wako, Osaka, Japan) for microglia, and subsequently exposed to biotinylated goat anti-rabbit IgG (diluted 1:200, Vector, Burlingame, CA, U.S.A.) and avidin-biotin complex kit. They were then visualized with reaction to 3,3’-diaminobenzidine tetrachloride (Sigma, St. Louis, MO, U.S.A.) in 0.1 M Tris-HCl buffer (pH 7.2) and mounted on gelatin-coated slides. After dehydration the sections were mounted with Canada Balsam (Kanto Chemical, Tokyo, Japan).

Negative controls for all immunohistochemical experiments were generated by incubation in rabbit IgG at the same concentration as the primary antibodies. The negative control resulted in the absence of immunoreactivity in all structures.

RESULTS

Infarct size of heart after MI: The infarct size of the hearts of MI-operated rats was 46.34 ± 3.28 and 44.43 ± 2.67% at 3 and 14 days after MI (n=5), respectively, whereas no abnormalities in infarct size were observed in the sham-operated rats (data not shown).

Neuronal death in the amygdala after MI: Neurons in the amygdala of rats in the sham-operated group were well stained with cresyl violet (Fig. 1A). In the MI-operated group, we could not find distinct neuronal death in the amygdala 3 days after MI (data not shown). However, 14 days after MI, many neurons showed cytoplasmic condensation in the amygdala (Fig. 1B). F-J B-positive cells were not detected in the amygdala of the sham-operated group (Fig. 1C). Also, we could not find distinct F-J B-positive cells death in the amygdala 3 days after MI (data not shown). However, F-J B-positive cells were detected in the amygdala of rats in the 14 days post-MI group (Fig. 1D).

Fig. 1. Cresyl violet (A and B) and F-J B (C and D) staining of the amygdala in sham- (A and C) and MI-operated (B and D) rats 14 days after the induction of MI. Many cresyl violet-positive neurons in the amygdala in the MI-operated group show cytoplasmic condensation (black arrows). F-J B-positive cells are detected in the amygdala in the MI-operated group (white arrows), but not in the sham-operated group. Scale bar =50 μm.
Reactive gliosis in amygdala after MI: In the sham-operated group, GFAP-immunoreactive astrocytes were detected in the amygdala, and they had thread-like processes, indicating that they were in the resting form (Fig. 2A and 2B). In the 3 days post-MI group, GFAP-immunoreactive astrocytes were generally similar to those in the sham-operated group (Fig. 2C). Only a few GFAP-immunoreactive astrocytes showed hypertrophied cytoplasm (Fig. 2C and 2D). However, in the 14 days post-MI group, many GFAP-immunoreactive astrocytes showed morphological changes: The cytoplasm of astrocytes was hypertrophied, and their processes were highly ramified (Fig. 2E and 2F).

In the sham-operated group, Iba-1-immunoreactive microglia were detected in the amygdala, and they had small cytoplasms and thin processes, indicating that they were in the resting form (Fig. 3A and 3B). In the 3 days post-MI group, most of Iba-1-immunoreactive microglia were resting forms and a few Iba-1-immunoreactive microglia showed hypertrophied cytoplasm and retracted processes in the amygdala (Fig. 3C and 3D).

However, in the 14 days post-MI group, most of Iba-1-immunoreactive microglia showed hypertrophy of the cytoplasm and retracted processes in the amygdala (Fig. 3E and 3F).

DISCUSSION

The neuronal death induced by MI could change the size of the amygdala, which is one of the main causes of major depression [1, 3, 10, 20]. In this study, we observed cytoplasmic condensation in neurons in the amygdala at 14 days, not 3 days, after MI, and we detected cells positive to F-J B.
a high affinity fluorescent marker for the localization of neuronal degeneration, in this region. This result suggests that the neuronal death dose not occur at early time (3 days) after MI, but occurs at late time (14 days) after MI. This result is supported by the findings of a previous study showing that the amygdala is most vulnerable to MI because the amygdala is involved in the regulation of cardiovascular functions, mainly by modulation of neuronal activity, including the autonomic parasympathetic system and the baroreceptor reflex arc [21]. Recently, it was reported that, in an ischemia/reperfusion (a 40-min coronary artery occlusion) rat model, neuronal death using caspase-3 activity occurred early in the amygdala after ischemia/reperfusion: Caspase-3 activity was significantly increased in the medial amygdala within 1 day and in the lateral amygdala 2 days after ischemia/reperfusion [15]. This discrepancy may be associated with the type of MI. We occluded the coronary artery permanently and this induced late neuronal death, whereas the transient occlusion made fast and severe neuronal death [2].

In this study, we found reactive gliosis in the amygdala after MI because astrocytes and microglia were implicated in CNS damage. GFAP-immunoreactive astrocytes and Iba-1-immunoreactive microglia showed activated morphology in the amygdala at 14 days after MI although only a few astrocytes and microglia changed their morphology with activated form at 3 days after MI. This result means that the reactive gliosis at 3 days after MI is precedent to neuronal damage and the reactive gliosis at 14 days after MI may be associated with neuronal damage in the amygdala induced by MI. It has been reported that astrocytes change their appearance and undergo characteristic hypertrophy of their cellular processes in neurologic disease [8] and that reactive astrocytes play a role in post-traumatic healing [19]. In addition, morphological changes in microglia under pathological conditions progress from the delicately rami-

Fig. 3. Iba-1 immunohistochemistry in the amygdala in sham- (A and B), MI-operated groups at day 3 (C and D) and 14 (E and F) after the induction of MI. Many Iba-1-immunoreactive microglia in the 14 days post-MI group show hypertrophy of the cytoplasm and retracted processes compared to those in the sham-operated group. Scale bar =100 μm.
fied phenotype to cells with larger somata, displaying shorter and coarser cytoplasmic processes [11]. This result is supported by a previous study using spreading depression model that OX–42 immunoreactive microglia and GFAP immunoreactive astrocytes were significantly increased in the neocortex compared to that in the control neocortex [4].

In conclusion, light reactive gliosis is precedent to MI-induced neuronal death in the amygdala at early time (3 days post-MI) after MI, and neuronal death and distinct reactive gliosis are prominent at 14 days after MI.

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