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A self-assembly peptide nanofibrous scaffold reduces inflammatory response and promotes functional recovery in a mouse model of intracerebral hemorrhage

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Abstract

Self-assembly peptide nanofibrous scaffold (SAPNS), such as RADA16-I, has been shown to reduce acute brain injury and enhance functional recovery in rat intracerebral hemorrhage (ICH) models. The acidic property of RADA16-I, however, limits its application in patients. In the present study, by using a modified neutral SAPNS (the RADA16mix) in collagenase IV induced ICH mice, we detected there were less microglial and apoptotic cells in mice injected with RADA16mix, meanwhile, more cells survived in this group. In addition, behavioral tests indicated that mice treated with RADA16mix showed better functional recovery than RADA16-I. Local delivery of RADA16mix reduces acute brain injury by lowering the number of apoptotic cells, decreasing glial reaction, reducing inflammatory response and, therefore promotes functional recovery. Moreover, new nerve fibers have grown into this new SAPNS, which indicates RADA16mix is able to serve as a bridge for nerve fibers to grow through.

From the Clinical Editor: Acute brain injury, such as intracerebral hemorrhage is a serious problem. In this work, self-assembly peptide nanofibrous scaffold (SAPNS) were tested in a rat model to aid functional recovery. Several items have been considered, such as histology, brain water content, hematoma volume, cell death and survival, inflammatory response, and nerve fiber growth. The positive data generated should pave the way towards better treatment options.

Key words: Intracerebral hemorrhage; Self-assembly peptide nanofibrous scaffold; Inflammatory response; Functional recovery

Intracerebral hemorrhage (ICH) is defined as non-vascular rupture hemorrhage caused by traumatic brain parenchyma, accounting for 10-15% of all strokes and affecting 10-30 cases per 100,000 adults.[1-4] Early mortality of ICH is high and most survivors leave sequela such as movement disorders, cognitive deficits and speech impediment.[5] Intracerebral hemorrhage occurs due to destructive changes in brain arteries like segmental lipohyalinosis, pseudo-aneurysm or rupture,[6] these changes cause pain to patients and impose great pressure on family and the society. Therefore, being fully aware of the severity of ICH and finding effective therapy become extremely urgent. Studying the use of self-assembly peptide nanofibrous scaffolds (SAPNSs) for tissue repair has become a really hot area in recent years. It is very promising for using functionally designed SAPNSs in regenerative medicine.

There was no prior or upcoming presentation of abstracts at meetings regarding the research.

Conflict of interest: There was no conflict of interest.

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Traditional SAPNS, BD PuarMatrix Peptide Hydrogel solution (BD Bioscience, Cat: 354250, USA) which was commonly called RADA16-I (AC-RADARADARADARADA-$CONH_2$ R, arginine; A, alanine; D, aspartate) was frequently used in tissue engineering. Untreated 1% RADA16-I has a very low pH (about 3.5). In cell culture process, it has been reported that the acidic property of RADA16-I could be neutralized by changing the medium frequently, which could reduce damage to cultured cells and enhance the survival rate after being grafted into injured spinal cord.[7] A previous study has devised an ICH model incorporating with stereo tactic minimally invasive hematoma aspiration plus intralesional injection, which removed hematoma immediately and provided space for local delivery of drugs or nano-materials.[8] With this ICH model, it has been found that RADA16-I could reduce acute brain injury and decrease inflammatory response, however, injection of RADA16-I suggested very fine functional recovery and even no nerve fibers were detected inside the material.[9] These results may be due to its poor biocompatibility caused by its acidity. To overcome these disadvantages, we have modified the original SAPNS. In the present study, we use a newly synthesized neutral SAPNS and its effects on mice ICH model have been studied. Structures of this new SAPNS are as follows: RADA16-RGD (Ac-RADARADARADARADA-DGDRGDS, R, arginine; A, alanine; D, aspartate; G, glycine) and RADA16-IKVAV (Ac-RADARADARADARADA-ARIKVAV, I, Isoleucine; K, lysine; V, valine; A, alanine; V, valine), which was obtained by directly conjugating bioactive short peptide motifs to RADA16-I peptide, each of them bearing a different biological signal. Previous studies have shown that RADA16-RGD could promote cell adhesion and provide cells a 3-D environment.[10] IKVAV is known to promote and guide neurite outgrowth.[11,12] Studies showed that IKVAV sapeptide scaffolds also promoted greater and faster differentiation of the progenitor cells into neurons.[13-15] However, IKVAV sapeptide scaffold alone was unable to self-assemble into a hydrogel, so we conjugated IKVAV to RADA16-I peptide. When used, we mix RADA16-RGD and RADA16-IKVAV at 1:1 ratio. For narrative simplicity, we name the new self-assembly peptide nanofibrous scaffold as RADA16mix.

In this study, the effect of this new SAPNS, RADA16mix on ICH was studied compared with the traditional SAPNS, the RADA16-I. We measured histology, hematoma volume, brain water content, apoptosis, cell survival and inflammatory response during the acute phase of ICH. Following that, we assessed functional recovery in weekly interval and finally we detected fiber growth, cell type inside the material in long survival time.

Methods

All surgical procedures done in mice were approved by Laboratory Animal Ethics Committee at Jinan University. Adult C57BL/6 mice (purchased from Guangdong Medical Laboratory Animal Center), 2-3 months old and weighting 22-28 g, were used in this study. All surgery was performed under 2.5% avertin anesthesia, and all efforts were made to minimize the suffering and number of animals used. Animals were kept on a 12/12 light/dark cycle with *ad libitum* access to food and water. The animal holding areas were under constant monitoring, the temperature was kept at 21 ± 2 °C, and the humidity was kept at 44 ± 2 pw.

The mice were randomly assigned to one of three groups: the RADA16mix group, the RADA16-I group and the saline group. Each group was disposed with induction of ICH, hematoma aspiration, and intra-lesional injection of the RADA16mix solution, RADA16-I solution and saline solution respectively.

Mice ICH model was caused by intracranial injection of type IV collagenase (Sigma-Aldrich, St. Louis, USA). Corpus striatum was the target region. Briefly, a burr hole of 0.15 mm diameter was drilled along the right coronal suture at 2.0 mm lateral to the bregma. A 30-gauge (G) needle was inserted into the right striatum with its tip at 0.26 mm anterior to the bregma, 2 mm lateral to the midline and 3.75 mm underneath the dural surface. ICH was induced by a slow injection of 0.25 U collagenase IV in 1 μL saline into the right striatum over 10 min. Three and a half hours after collagenase IV injection, aspiration was carried out by gentle suction through a 1 ml syringe attached to a 23-G needle. The needle was placed at the same stereotactic coordinates as the collagenase injection. Four aspirations were performed over 15 min. Following aspiration, 10 μL RADA16mix solution, 10 μL RADA16-I solution or 10 μL saline was injected into the lesion over 10 min respectively. Finally, the burr hole was sealed with bone wax, and the incision was sutured.

The experiment procedure was illustrated in Figure 1. Before surgery, 24 mice were pre-trained for consecutive three days to adapt to rotarod system in order to assess the regular level of function. Then, three behavioral tests (rotarod test, grip strength test and catwalk analysis) were done in week interval until six weeks post-injury. The second batch of mice was sacrificed three days after injury for brain edema, brain water content and immunohistochemical analysis including apoptosis, cell survival, and inflammatory response. Histology examination was done at three days after injury in order to view whether SAPNS injection succeeded or not and whether SAPNS could perfect fit with the host. The third batch of mice was sacrificed 6 weeks after surgery for the assessment of cell type, nerve fiber growth and electron microscope.

Tissue preparation

Mice under deep anesthesia with 2.5% avertin were perfused with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer saline (PBS, pH = 7.4) for 20 min. For Nissl staining, the brain tissues were collected and post-fixed in Bouie's fixative for 48 h at room temperature. Then the brain between 2 mm anterior to the bregma and 4 mm