Bull Yamaguchi Med Sch 44(3-4) : 59-61, 1997

Effect of Local Administration of Basic Fibroblast Growth Factor against Neuronal Damage Caused by Transient Intracerebral Mass Lesion in Rats

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(Received August 20, 1997, revised March 8, 1998)

Keywords : Angiogenesis, Basic fibroblast growth factor ; CA1 ; Intracerebral mass lesion ; Ischemia.

An acute intracerebral mass lesion such as intracerebral hemorrhage (ICH) carries a high rate of mortality and morbidity, and there has been some controversy regarding the timing and techniques of surgical evacuation of the hematoma. In animal models of experimental ICH, Nehls et al. reported that early removal of a spontaneous mass lesion improved local cerebral blood flow and late outcome[8]. Some experimental studies have demonstrated the ischemic area surrounding the intracerebral mass[2,8]. Basic fibroblast growth factor (b-FGF) has been shown to exert neuroprotective effects on hippocampal CA1 neurons in animal models of ischemia[1,7]. The purpose of this study was to examine the protective effect of locally administered b-FGF against neuronal damage caused by a transient intracerebral mass lesion.

Adult male Sprague-Dawley rats were used in these experiments. Anesthesia was induced in a transparent Perspex container with 3% halothane in a mixture of 30% oxygen and 70 % nitrous oxide. Anesthesia was then maintained by spontaneous respiration of 0.5 -1.0% halothane in the same oxygen and nitrous oxide mixture. The animals' body temperature was maintained at 37℃. In all groups, the mean arterial blood pressure was maintained above 70mmHg. PaCO2 was maintained at 43-47mmHg through adjustments of the halothane concentration, and PaO2 was kept above 90mmHg. In this study, we used the model of experimental intracerebral mass[3]. Each animal was placed in a stereotactic head frame, the skull was exposed through a 10-mm midsagittal incision, and a single burr hole was made above the right caudate nucleus. The dura mater was opened, and a microballoon mounted on a 25-gauge needle was inserted stereotactically into the center of the caudate nucleus. After a 3-min stabilization period, the balloon was inflated for 30 sec. with 50μl of normal saline delivered from a microsyringe. The animals were then assigned to four groups of six each. In a sham-treated group (Group 1), the balloon was inserted but not inflated. In other groups (Groups 2-4), the balloon was inflated for 10min, and then deflated. The needle and the balloon were then withdrawn quickly. Bovine b-FGF was dissolved in 5 μl of artificial cerebrospinal fluid (ACSF) containing bovine serum albumin (BSA). In the b-FGF-treated group, after withdrawal of the microballoon, a 25-

gauge needle was inserted into the right caudate nucleus. Then b-FGF was administered into the cavity left by the intracerebral mass using a 10-μl microsyringe pump over 30 s at a dose of 500ng (Group 3) or 1000ng (Group 4). In the vehicle-treated group (Group 2), 5 μl of ACSF containing BSA was administered. At 7days after balloon insertion, the animals in all groups underwent anesthetic induction as described above, and were sacrified. The brains were removed and post-fixed overnight in 10% paraformaldehyde. Coronal sections, at the level of 1.5mm posterior to the caudal end of the inflated microballoon and at the level of balloon insertion, from each animal were cut, stained with hematoxylin and eosin, and examined histologically using a light microscope. The number of living neurons in the right CA1 subfield was counted on one histological coronal section and expressed as neuronal cell density per 1 mm linear length. Neurons with a normal appearance of the nucleus and cell body were counted as intact neurons. Those with pyknotic nuclei or shrunken somata were not counted as injured neurons. To quantify the degree of angiogenesis in the evacuated cavity, the maximal thickness of the capillary layer was measured in the upper and lower parts of the cavity in a coronal section at the level of microballoon insertion. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Scheffé's multiple comparison for physiological parameters and CA1 neuronal damage. Differences at $p<0.01$ were considered significant. A Wilcoxon paired test was used to compare the thickness of the capillary layer between the vehicle-treated group and the b-FGF-treated groups.

There were no significant differences among the groups in physiological parameters. In the sham-treated group, pyramidal neurons in the hippocampal CA1 subfield were intac . In the vehicle-treated group, some of the neurons in the CA1 subfield showed shrunken somatoma and pyknotic nuclei, and intact neurons were more decreased than those in the sham-treated group. In the b-FGF-treated groups, intact neurons were well preserved in the right CA1 compared with those in the vehicle-treated group. In the b-FGF-treated groups, the numbers of intact neurons were significantly higher than those in the vehicle-treated group. There were no differences between Groups 3 and 4. In the sham-treated group, an increase of glial cells was demonstrated along the needle tract, but no other neurological change was recognized. In the vehicle-treated group, the lesions around the cavity were characterized by neuronal ischemic change. In b-FGF-treated -groups, the number of capillaries was increased in the wall of the evacuated cavity. The maximal thickness of the capillary layer tended to be greater in the lower part of the cavity wall in the b-FGF-treated groups. In the lower part of the cavity, there was a significant difference ($p<0.05$) in the thickness of the capillary layer between Groups 2 and 4.

In this study, we demonstrated that local administration of b-FGF into the evacuated cavity in the caudate nucleus had protective effect against the secondary neuronal damage in CA1 pyramidal cells, and that it increased the angiogenesis in the tissue adjacent to the evacuated cavity.

Previous studies have demonstrated that a localized area of ischemia surrounds an experimental mass lesion[2,9] and in this study it was reasonable to attribute the neuronal death observed in the CA1 subfield of the hippocampus to ischemia. The CA1 subfield was located 1.5 mm behind the lesion cavity where it was not subjected to direct tissue destruction. Although we did not measure the local cerebral blood flow, substantial ischemia was expected to occur in the hippocampus. In fact, the number of intact pyramidal neurons was decreased significantly in the vehicle-treated group in comparison with the sham-treated group. This reduction was prevented in the b-FGF-treated groups. Basic-FGF is known to have neurotrophic effects on neurons and it has protective effect against ischemic neuronal death in vivo[1] . It increases neuronal survival and neurite outgrowth and antagonizes the excitotoxicity of glutamate in vitro hippocampal neurons[5,6]. It has also been demonstrated that chronic intraventricular infusion of b-FGF prevents delayed neuronal death of hippocampal CA1 neurons in experimental models of

ischemia[7]. Rosenblatt et al reported that the neuroprotective effects of b-FGF may also be due to direct effects on cerebral vessels and cerebral blood flow in vivo[10]. Another intersting observation in our study was that b-FGF increased the amount of angiogenesis in the cavity wall to a greater extent than in the vehicle group. This angiogenesis was more prominent in the lower part of the cavity, where a higher concentration of b-FGF was expected. An angiogenic effect of b-FGF has been reported by many researchers[4,9], and it is reasonable to attribute the increase of capillaries in the cavity wall to the effect of locally injected b-FGF.

In conclusion, this study has shown a beneficial effect of locally administered b-FGF into the evacuated cavity in terms of a substantial neuroprotective effect against CA1 neuronal damage and facilitation of local repair. These observations indicate the possibility of a new approach for the management of intracerebral hematoma to achieve better neurological recovery.

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