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BRAIN RESEARCH

Research Report

The impact of paracrine signaling in brain microvascular endothelial cells on the survival of neurons

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ABSTRACT

Neurons depend for survival on local neurotrophic factors which act in an autocrine/paracrine manner. However, the effect of paracrine signaling of brain microvascular endothelial cells (BMECs) under pathological conditions on neuron survival is not fully understood. In this study we cultured rat BMECs and cortical neurons. BMECs were cultured in oxygen-glucose-deprived (OGD) conditions to mimic cerebral ischemia in vitro. The conditioned media of normal BMECs or OGD-injured BMECs were used to culture normal or injured neurons. Neuron activity, free Ca^{2+} concentration, NMDA receptor status, mitochondrial membrane potential and cytochrome C release level were determined. The results showed: mitochondrial activity of injured neurons was significantly increased and lactate dehydrogenase (LDH) leakage was decreased ($P<0.05$) by grown in conditioned medium of normal BMECs. Inversely, mitochondrial activity of normal or injured neurons was decreased and LDH leakage was significantly increased ($P<0.05$) by grown in conditioned medium of injured BMECs. The changes in free Ca^{2+} concentration, NMDA receptor status, mitochondrial membrane potential and cytochrome C release level were consistent with the changes in neuronal activity. These findings suggest that the conditioned medium of normal BMECs has a neuroprotective effect. However, this protective effect was lost after BMECs injury; in fact, the conditioned medium became neurotoxic. Therefore, it appears that early recovery of BMECs might be helpful for neuron survival.

1. Introduction

The last twenty-five years have seen remarkable progress in the understanding of the pathophysiology of ischemic stroke, to which members of this consortium have significantly contributed. More and more researchers have agreed that injury or death of neurons in cerebral ischemia should be studied in terms of the whole histological background, and that thinking should be gradually converted from a neuron-centered view to a comprehensive concept of injury. The "neurovascular unit" composed of brain microvascular endothelium cells (BMECs), astrocytes, neurons and extracellular matrix (ECM) provides a conceptual model for a variety of nervous system diseases. The integrity of the brain tissue unit emphasizes the interaction and dynamic equilibrium of cells to cells and of cells to matrix (Lo et al., 2003). In this functional unit, vascular endothelial cells (VECs), astrocytes and ECM constitute the micro-environment for the survival of neurons. The maintenance and changes of the micro-environment play an important role on the survival and functions

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of neurons. Previous studies have shown that cerebral ischemia or hypoxia could lead to brain injury directly. The influence of the cellular network cascade on brain function triggered by injury was greater than the injury itself and could decide the fate of injured but not dead neurons. VECs, glial cells and other cells were involved in the cascade (Tacconi, 1998). Therefore, in recent years, the concept of the BBB has evolved from one of a discrete barrier to include a more integrative view whereby the BBB is best viewed as a modulatory interface between blood and brain. In this context, regulation of this interface occurs via paracrine interactions between the capillary endothelium and its neighboring cells (Cohen et al., 1996). However, these interactions among cells have not been fully understood. Traditionally it was considered that BMECs not only regulate hemodynamics and maintain normal BBB during the normal state but also mediate inflammatory reaction and BBB injury during pathological processes. We speculated that the paracrine signaling of BMECs could affect the survival of peripheral neurons directly during physiological and pathological process. Thus, in this study, we observed the effects of the conditioned medium of BMECs on neurons ex vivo. We cultured rat BMECs and cortical neurons, simulated the pathological process of ischemic stroke by glucose and oxygen deprivation, and collected the conditioned medium of BMECs in both the biological and pathological states. In order to prove that the observed effects are BMEC-specific, 3T3-swiss cells, one of non-endothelial cells, were used as negative control. The conditioned media of 3T3-swiss cells were collected as BMECs. Normal neurons and ischemically injured neurons were cultured in various rBMEC- and 3T3-cultured media. We also detected neuron activity, free Ca^{2+} concentration, and the status of N-methyl-D-aspartate receptor (NMDAR) and mitochondria. Our results were significant in that the conditioned medium from normal endothelial cell cultures (EC-N-CM[1]) had a neuroprotective effect. However, once ECs were injured, the neuroprotective effect disappeared; on the contrary, a neurotoxic effect was apparent. In the process of imitating ischemic injury, the neurotoxic effect could further deteriorate the condition of the injured neurons and promote their death. The conditioned medium from 3T3-swiss cells didn't show analogical effect. The final study confirmed our speculation that paracrine signaling of BMECs had a direct impact on the survival of peripheral neurons.

2. Results

2.1. *Ischemically-injured BMECs under OGD condition*

The rBMECs were cultured in OGD conditions for various times to mimic cerebral ischemia in vitro, as previously described (Zhang et al., 1999). The change of mitochondrial activity was measured by MTT assay. As shown in Fig. 1, the mitochondrial activity of rBMECs was significantly changed with time under OGD conditions. Compared with the normal control group, optical density (OD) values in injured-rBMEC group at both 1 h and 2 h were significantly increased ($P<0.01$), indicating that there was a stress period with increased activity in the initial period of injury. OD values began to decrease at 4 h, but there was no statistically significant difference among the groups at that time. OD values further decreased at 6 h, and the difference was significant between injured cells and normal controls ($P<0.001$), indicating that the cells were injured. OD values were much lower at 8 h. Thus we used the injured rBMEC at 6 h after OGD.

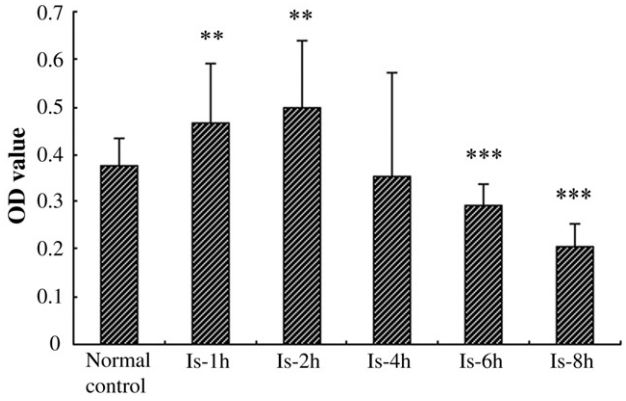

Fig. 1 – Ischemically-injured BMECs under OGD conditions. The change of rBMECs mitochondrial activity, which was measured by MTT assay, was significantly changed with time under OGD conditions. Compared with the normal control group, OD values in injured-rBMEC group at both 1 h and 2 h were significantly increased. At 4 h OD values began to decrease, but there was no statistically significant difference among the groups. OD values further decreased at 6 h, indicating that the cells were injured. OD values were much lower at 8 h. $^{**}P<0.01$, $^{***}P<0.001$ versus normal control group.

2.2. *Change in neuronal mitochondrial activity in different BMEC-culture conditioned media*

Mitochondrial activity of neurons was determined by MTT assay. As shown in Fig. 2A, for normal neurons, the OD value of the MTT assay in EC-N-CM[1] (the conditioned medium from the normal rBMEC cultures) had no significant change compared with normal neuron culture medium (Fig. 2A), but the OD value was significantly decreased in EC-I-CM[2] (the conditioned medium from the injured-rBMEC cultures) ($P<0.05$), which indicates that the neuronal mitochondrial activity decreased significantly. The OD values in 3T3-N-CM (the conditioned medium from the normal 3T3-swiss cells cultures) and 3T3-I-CM (the conditioned medium from the injured 3T3-swiss cells cultures) had no significant change compared with normal control, which indicates that 3T3-N-CM and 3T3-I-CM had no significant effect on mitochondrial activity of normal neurons. For injured neurons, the OD value was significantly increased in EC-N-CM compared with normal neuron culture medium ($P<0.05$) (Fig. 2B), indicating that neuronal mitochondrial activity increased. The OD value

[1] The conditioned medium from normal rBMECs cultures (EC-N-CM).

[2] The condition medium from injured rBMECs cultures (EC-I-CM).