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Research article

The anti-apoptotic effect of fluid mechanics preconditioning by cells membrane and mitochondria in rats brain microvascular endothelial cells

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ABSTRACT

Exercise preconditioning is a simple and effective way to prevent ischemia. This paper further provided the mechanism in hemodynamic aspects at the cellular level. To study the anti-apoptotic effects of fluid mechanics preconditioning, Cultured rats brain microvascular endothelial cells were given fluid intervention in a parallel plate flow chamber before oxygen glucose deprivation. It showed that fluid mechanics preconditioning could inhibit the apoptosis of endothelial cells, and this process might be mediated by the shear stress activation of Tie-2 on cells membrane surface and Bcl-2 on the mitochondria surface.

1. Introduction

Ischemic stroke is one of the leading causes of movement, speech, cognition, and swallowing dysfunction [1]. Exercise preconditioning, as a safe intervention method with less adverse effects, is suggested for ischemic prevention [2]. Exercise before the occurrence of cerebral vascular events can reduce cerebral inflammation, improve cerebral vascular function, inhibit excessive release of excitatory amino acid neurotransmitter and reduce blood brain barrier dysfunction [3–5]. However, little attention has been paid to mechanical aspects. In this study, we would make a preliminary study on hemodynamic effects of the exercise preconditioning.

Exercise is a systemic movement, which leads blood supply re-arrangement [6]. For brain, metabolic reduction, vascular function enhancement and bloodstream adding is significant in this process [6,7]. As the component of blood vessels, brain microvascular endothelial cells (BMECs) cannot be avoided under the mechanical effects of blood flow [8]. Studies showed that endothelial membrane could directly feel the flow alternation, and this mechanical signals transformed into biological signals to affect cells structure, function and survival [9,10]. On these basis, We studied whether blood stress preconditioning was effective for endothelial survival in ischemia.

This study was performed at the cellular level for reduction and avoidance of interference. Laminar shear stress (LS) is a parallel force to the direction of flow and closely related with blood characteristics and vascular morphology. When other conditions remain unchanged, its size is proportional to the flow velocity [11,12]. In this paper, LS, as an indicator of the flow changes, could be measured by the instrument of parallel plate flow chamber. This chamber [13,14] provided adherent cells fluid intervention, just like the blood with the form of laminar flow on vessels. Rat BMECs (rBMECs) cultured in this chamber were treated with flow intervention(Laminar shear stress about 1 ± 0.05 dyn/cm^2) [15–17] before ischemic oxygen/glucose deprivation (OGD) [18]. The apoptotic changes mediated by cells membrane and mitochondria were observed with PE Annexin V/7-AAD [19] and JC-1 [20]. Tie-2 and Bcl-2 related with apoptosis were also detected by western blot. Tie-2 was a tyrosine kinase receptor on cell membrane, which was able to accept the shear mechanical signal to reduce apoptosis [21]. Bcl-2, located on or transferred to the mitochondrial outer membrane after stimulus, could able to regulate the mitochondrial membrane potential and play a role in inhibiting apoptosis [22]. We wished fluid mechanics preconditioning provided anti-apoptotic effect in cells membrane and mitochondria mechanism.

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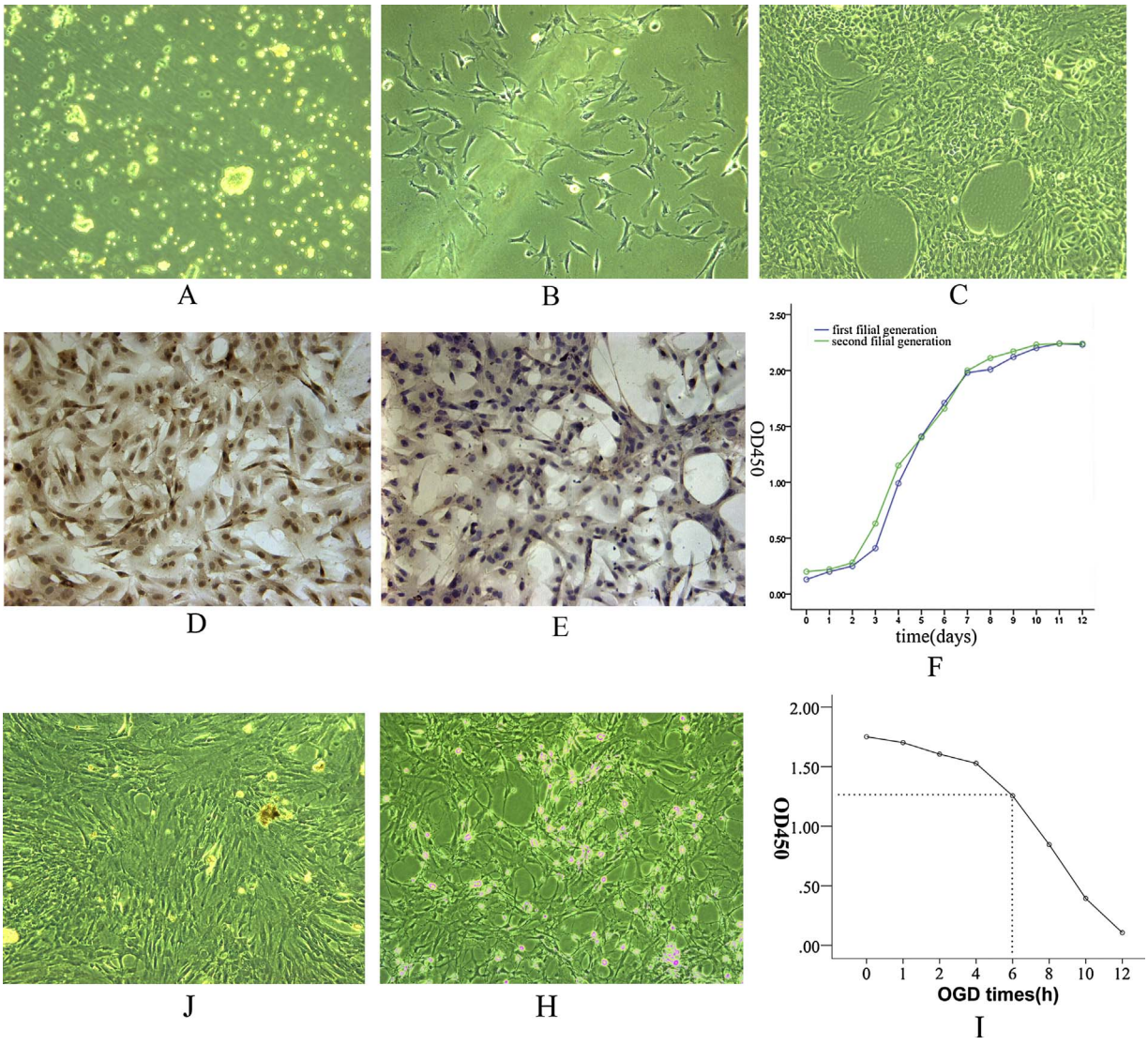

Fig 1. rBMECs for identification and ischemic OGD intervention. (A-C): Morphology of cells in the early(A), medium(B) and late(C) stages (100x). The cells of fusiform and polygon began to adhere and stretch out the parapodium; (D,E): vWF staining(+) with brown yellow cytoplasm if vWF antibodies added(D) while vWF staining(−) without brown yellow cytoplasm if no antibodies added(E); (F):Diagram showed the growth trends of first and second generations of cells, almost no difference. Passage did not destroy the cells growth trend; (J,H): Normal cells morphology with full fusiform (J) and apoptotic cells with shrunken form under inverted microscope(H)(100x); (I): Cells were respectively given OGD for 0, 1, 2, 4, 6, 8, 12 h. Cells viability was detected with OD450 by WST-8 assay. OD450 was directly proportional to the number of viable cells. OGD within 6 h was the compensatory phase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Material and methods

All experiments were performed in cultured rBMECs from male Sprague–Dawley (SD) rats (50–60 g). Each experiment required 10 rats and cells growth density was 0.1×10^6 in 12-well plates. All the experiments were three replicates in each group and repeated three times.

2.1. Culture of rBMECs

Referring to literature [13,14], cerebral cortex was extracted from rats and cutted for about 1 mm^3 in size. The capillary segments were preserved on the screen when the tissues filtered through mesh metal screen, and then digested with collagenase at 37 °C for rBMECs. Cells resuspended in high glucose DMEM medium with 20% FBS(DMEM and FBS purchased from Invitrogen,USA).

2.2. Immunohistochemical detection of Von Willebrand factor(vWF)

vWF was endothelial specific factor [14]. rBMECs crawled on the slides were incubated with vWF antibody at 4 °C overnight and then with second antibody for 20 min in room temperature. Finally, after stainning with DAB and hematoxylin, they were photographed under microscope.

2.3. WST-8 detection of cell proliferation and toxicity [23]

WST-8 assay was used to detect the amount of cells at different days in growth stage. Cells in 96-well plates cultured in 37 °C with WST-8 intervention liquid. OD value was measured with microplate reader.

2.4. OGD and LS intervention

OGD model was adopted for cell ischemia in vitro [18]. The parallel-plate flow chamber had a medium liquid circulation system to provide LS intervention and a gas circulation system to provide continuous gas [17]. In this study, 1.0 ± 0.05 dyn/cm^2 and 5% CO_2 + 95% O_2 was first given to provide fluid mechanics preconditioning for 2 h. Then sugar-free DMEM static medium (purchased