F-01

Cilostazol inhibits leukocyte-endothelial cell interactions in murine microvessels after transient bilateral common carotid artery occlusion

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Leukocyte behavior in the cerebral microvasculature following vessel occlusion has not been fully elucidated. The purpose of this study was to investigate the effects of cilostazol on leukocyte behavior (rolling and adhesion) in murine cerebral microvessels following transient bilateral carotid artery occlusion using intravital fluorescence microscopy. Four groups of mice were assigned: a sham group (n=16); an ischemia (induced by 15-min occlusion of bilateral common carotid arteries) and reperfusion (I/R) group (n=13); I/R+cilostazol (I/R+CZ3mg/kg) group (I/R after oral administration of cilostazol at 3mg/kg) (n=8) and I/R+cilostazol (I/R+CZ30mg/kg) group (I/R after oral administration of cilostazol at 30mg/kg) (n=12). Leukocytes labeled with 0.05% acridine orange were administered intravenously and their behavior was investigated at 3 and 6 h after reperfusion. Numbers of rolling or adherent leukocytes were expressed as the count per square millimeter per 30s. Numbers of rolling and adherent leukocytes at 3 and 6h after reperfusion were significantly higher in the I/R group than in the sham or I/R+CZ30mg/kg groups in both pial veins ($P<0.05$) and pial arteries ($P<0.05$). Cilostazol (30mg/kg) inhibited leukocyte-endothelial interactions following cerebral ischemia and reperfusion.

F-02

Impairment of CO₂ reactivity in RBC velocity and CBF after cortical spreading depression in anesthetized mice

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Background: We previously reported that cortical spreading depression (CSD) drastically suppresses red blood cell (RBC) velocity and alters cerebral blood flow (CBF) and vessel diameter in cortical arteriole. It has been reported that CSD induces disruption of neurovascular and neurometabolic coupling.

Objective: To further understand mechanisms involved in the disturbance of microcirculation, reactivity to carbon dioxide (CO₂) in RBC velocity flowing in intraparenchymal capillaries and CBF was measured before and after CSD passage.

Methods: To visualize blood vessels, we used Tie2-GFP transgenic mice (N=10), in which specifically vascular endothelial cells emit fluorescence. Under urethane anesthesia and artificial ventilation, RBC velocity was measured using a confocal laser-scanning microscope with high-speed camera (125 fps) and an original image analyzing system of KEIO-IS2 working on MATLAB through a cranial window installed on the temporo-parietal region of the cerebral cortex, along with CBF by laser Doppler flowmeter. CO₂ reactivity was measured with 5% CO₂ inhalation for 1 min. CSD was induced by microapplication of 1M KCl through a tiny cranial hole posterior to the cranial window.

Results: RBC velocity was measured in 4 to 21 capillaries in each mouse. CO₂ inhalation increased partial pressure of arterial CO₂ by 14.1 ± 3.9 mmHg. During hypercapnia, CBF and RBC velocity averaged in each mouse increased by $14.1 \pm 11.3\%$ and $17.7 \pm 19.0\%$ with significant correlation between the increases ($r=0.79$, $n=8$). After CSD passage, increase in CBF and RBC velocity were reduced to $7.3 \pm 21.8\%$ and $11.6 \pm 22.9\%$, respectively, and the correlation was lost ($r=-0.15$, $n=11$).

Conclusion: CSD attenuated CO₂ reactivity in CBF and RBC velocity by different mechanism, probably due to impairment of neurovascular and neurometabolic coupling.