Direct neuronal protection by the protease-activated receptor PAR4 antagonist ML354 after experimental stroke in mice

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Abstract

Background and Purpose Thrombo-inflammation is a key feature of stroke pathophysiology and provides multiple candidate drug targets. Thrombin exerts coagulation-independent actions via protease-activated receptors (PAR), of which PAR1 has been implicated in stroke-associated neuro-inflammation. The role of PAR4 in this context is less clear. This study examined if the selective PAR4 antagonist ML354 provides neuroprotection in experimental stroke and explored the underlying mechanisms. Experimental Approach Mouse primary cortical neurons were exposed to oxygen-glucose deprivation (OGD) and simulated reperfusion ± ML354. For comparison, functional Ca2+-imaging was performed upon acute stimulation with a PAR4 activating peptide or glutamate. Male mice underwent sham operation or transient middle cerebral artery occlusion (tMCAO), with ML354 or vehicle treatment beginning at recanalization. A subset of mice received a platelet-depleting antibody. Stroke size and functional outcome were assessed. Abundance of target genes, proteins and cell markers was determined in cultured cells and tissues by qPCR, immunoblotting and immunofluorescence. Key Results Stroke upregulates PAR4 expression in cortical neurons in vitro and in vivo. OGD augments spontaneous and PAR4-mediated neuronal activity; ML354 suppresses OGD-induced neuronal excitotoxicity and apoptosis. ML354 applied in vivo after tMCAO reduces infarct size, apoptotic markers, macrophage accumulation and interleukin-1β expression. Platelet depletion did not affect infarct size in mice with tMCAO ± ML354. Conclusions and Implications Selective PAR4 inhibition during reperfusion improves infarct size and neurological function after experimental stroke by blunting neuronal excitability, apoptosis and local inflammation. PAR4 antagonists may provide additional neuroprotective benefits in patients with acute stroke beyond their canonical antiplatelet action.

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Figure 1