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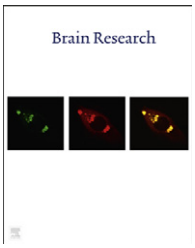

Research Report

Delayed administration of the nucleic acid analog 2Cl-C.OXT-A attenuates brain damage and enhances functional recovery after ischemic stroke

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ABSTRACT

2Cl-C.OXT-A (COA-Cl) is a novel nucleic acid analog that enhances angiogenesis through extracellular signal-regulated kinase 1 or 2 (ERK1/2) activation. ERK1/2 is a well-known kinase that regulates cell survival, proliferation and differentiation in the central nervous system. We performed in vitro and in vivo experiments to investigate whether COA-Cl can attenuate neuronal damage and enhance recovery after brain ischemia. In primary cortical neuron cultures, COA-Cl prevented neuronal injury after 2 h of oxygen-glucose deprivation. COA-Cl increased phospho-ERK levels in a dose-dependent manner and COA-Cl-induced neuroprotection and ERK1/2 activation was inhibited by suramin or PD98059. The effect of COA-Cl was evaluated in vivo with 60 min of middle cerebral artery occlusion combined with bilateral common carotid artery occlusion. COA-Cl or saline was injected intracerebroventricularly 5 min after reperfusion. COA-Cl significantly reduced infarct volume and improved neurological deficits upon injection of 15 or 30 μg/kg COA-Cl. Moreover, COA-Cl reduced the number of TUNEL positive cells in ischemic boundary, while rCBF was not significantly changed by COA-Cl administration. We also evaluated the effect of delayed COA-Cl administration on recovery from brain ischemia by continuous administration of COA-Cl from 1 to 8 days after reperfusion. Delayed continuous COA-Cl administration also reduced infarct volume. Furthermore, COA-Cl enhanced peri-infarct angiogenesis and synaptogenesis, resulting in improved motor function recovery. Our findings demonstrate that COA-Cl exerts both neuroprotective and neurorestorative effects over a broad therapeutic time window, suggesting COA-Cl might be a novel and potent therapeutic agent for ischemic stroke.

Abbreviations: COA-Cl, 2Cl-C.OXT-A; ERK, extracellular signal-regulated kinase; LDH, lactate dehydrogenase; MAPK, mitogen-activated protein kinase; MCAO, middle cerebral artery occlusion; MEK, MAP kinase kinase; OGD, oxygen-glucose deprivation; rCBF, regional cerebral blood flow; TUNEL, terminal deoxynucleotidyl transferase-dUTP nick end labeling

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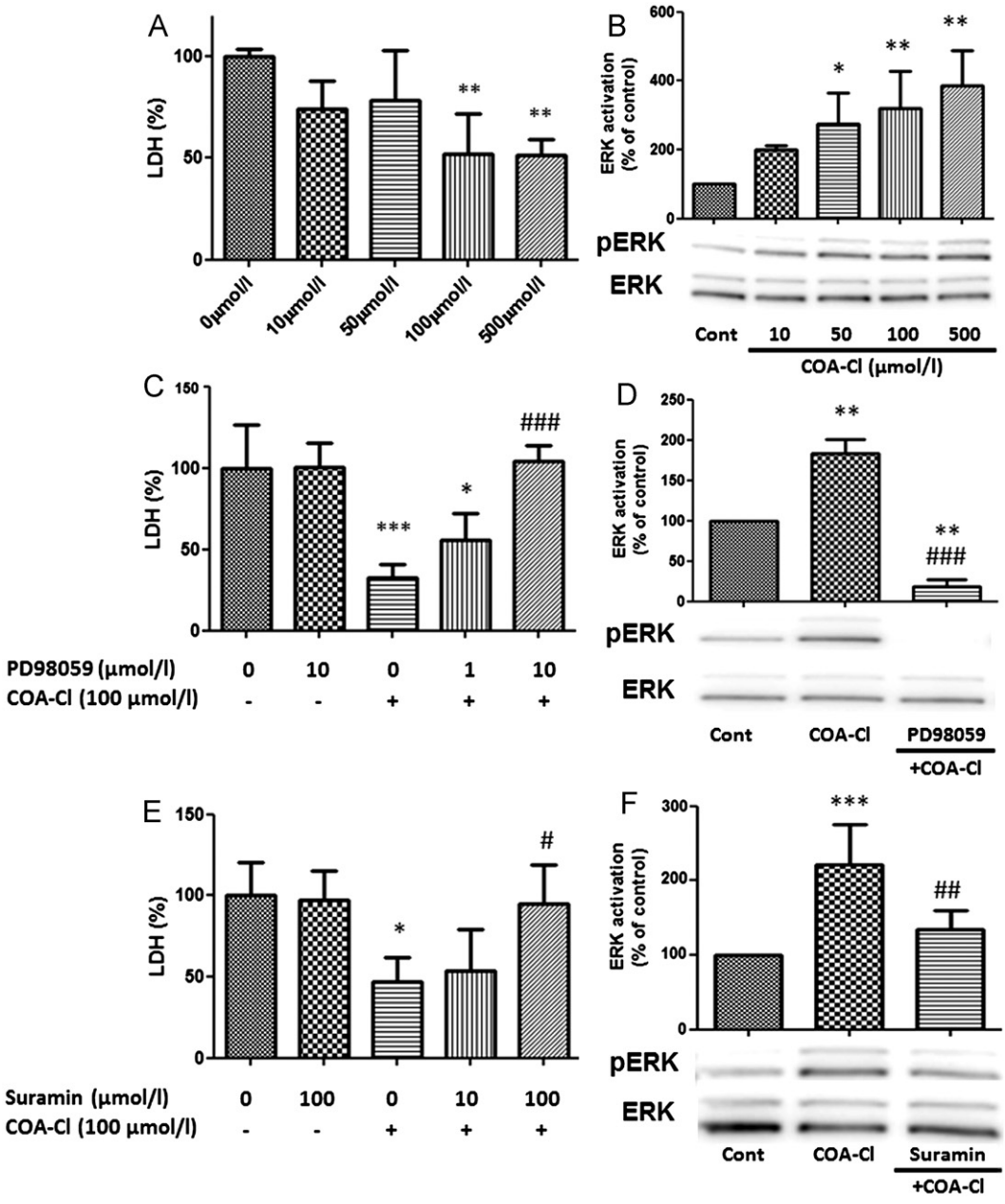

Fig. 1 – COA-Cl protects neurons from OGD-induced neuronal injury in an ERK1/2 activation-dependent manner: (A) concentration response of COA-Cl-induced neuroprotection. After 2 h of OGD, primary cortical neurons were incubated with COA-Cl for 24 h. $**P<0.01$ vs. vehicle-treated OGD control. Data are normalized to the amount of LDH released from vehicle-treated cells after OGD (100%) and are corrected for baseline LDH release (0%) measured in control cell cultures for each experiment. (B) Concentration response of COA-Cl-induced ERK1/2 activation. After 2 h of OGD, primary cortical neurons were treated with COA-Cl for 15 min. Representative western blots and semi-quantitative data of p-ERK and total ERK1/2 activation are shown. $*P<0.05$, $**P<0.01$ vs. vehicle control. (C) Co-exposure of the neurons to COA-Cl (100 μM) and PD98059 abolished the protective effect of COA-Cl. $^{*}P<0.05$, $^{***}P<0.001$ vs. vehicle-treated OGD control. $^{\#\#\#}P<0.001$ vs. COA-Cl without antagonists. (D) PD98059 (10 μM) abolished COA-Cl induced ERK1/2 activation. $^{**}P<0.01$ vs. vehicle control. $^{\#\#\#}P<0.001$ vs. COA-Cl without antagonists. (E) Co-exposure of the cells to COA-Cl and suramin abolished the protective effect of COA-Cl. $^{*}P<0.05$ vs. vehicle control; $^{\#}P<0.05$ vs. COA-Cl without antagonists. (F) Suramin (100 μM) abolished COA-Cl-induced ERK1/2 activation. $^{***}P<0.001$ vs. vehicle control. $^{\#\#}P<0.01$ vs. COA-Cl without antagonists. Data in such graphs show the mean ± SD derived from at least 4 independent experiments.

1. Introduction

Recent investigations into the pathophysiological events that follow acute ischemic stroke suggest an important role for angiogenesis, which results in improved collateral circulation (Wei et al., 2001; Gu et al., 2001) and may impact medium-to-long term recovery (Krupinski et al., 1994). Several substances that promote angiogenesis, such as fibroblast growth factors, platelet-derived growth factors, and vascular endothelial growth factors, are known. However, all these growth factors