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Nanoparticle-based delivery system of therapeutic nucleic acids and peptides for the treatment of ischemic stroke

Abstract

Receptor for advanced glycation end products (RAGE) is a multi-ligand receptor that is involved in ischemia/reperfusion (I/R) injury. In this study, RAGE binding peptide (RBP) was produced by recombinant DNA technology for blocking the RAGE signaling. RBP was originated from the RAGE binding domain of high mobility group box-1. Cytokine assays and immunohistochemistry results showed that RBP was an effective antagonist of RAGE, reducing inflammatory cytokines and RAGE. Along with RBP, the heme oxygenase-1 gene (pHO-1) was used as a therapeutic gene for protection of brain cells in ischemic stroke animal models. For combination delivery of RBP and pHO-1, deoxycholic acid-conjugated low molecular weight polyethylenimine (DA-PEI) was synthesized and used as a gene delivery carrier. A ternary-complex was prepared with pHO-1, DA-PEI and RBP by charge interaction. The size of the ternary-complex was approximately 130 nm. In vitro transfection assay to hypoxic neuron cells showed that the ternary-complex had higher gene delivery efficiency than pDNA/DA-PEI, pDNA/PEI, pDNA/lipofectamine binary-complexes. Furthermore, the toxicity of the ternary-complex was lower than those of the binary-complexes. In addition, the cyto-protective effect of the ternary-complex was confirmed by Annexin V and TUNEL assay. The animal models of ischemic stroke were produced by middle cerebral artery occlusion (MCAO) and reperfusion. The ternary-complex was injected into the animal model by stereotaxic injection. The results showed that the ternary-complex reduced the infarct volume effectively in the stroke animal models. The results suggest that the pHO-1/DA-PEI/RBP ternary-complex has anti-inflammatory and cyto-protective effects in the cells under hypoxia. Therefore, the ternary-complex, composed of pHO-1, DA-PEI, and RBP may be useful for the treatment of ischemic stroke.

Figures

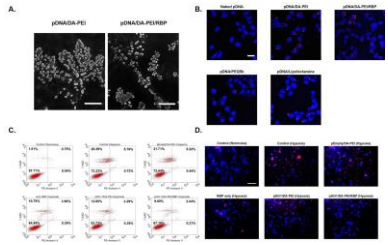

Figure 1: Characterization of ternary-complex and cyto-protective effect under hypoxia. (A) Morphology of ternary-complex measured by SEM (scale bar = 1 μm). (B) Cellular uptake of ternary-complex using Cy5 labeled-DNA in Neuro2a cell (scale bar = 20 μm). (C) and (D) Cyto-protective effect of ternary-complex under hypoxia. (C) Annexin V assay. (D) TUNEL staining (scale bar = 100 μm).

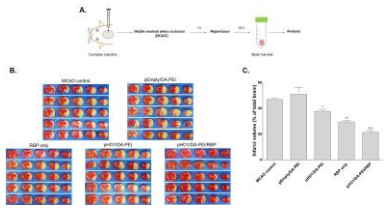

Figure 2: Reduction of infarct volume by ternary-complex in MCAO. (A) Scheme of *in vivo* experimental procedure. (B) TTC staining. (C) Quantification of infarct volume. *p<0.05 compared with MCAO control and pEmpty/DA-PEI. **p<0.05 compared with MCAO control, pEmpty/DA-PEI, and pHO1/DA-PEI. ***<0.05 compared with other groups.

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