30PE-17

CHARACTERIZATION OF L-PHENYLALANINE TRANSPORT AT THE BLOOD-BRAIN BARRIER IN THE ISCHEMIA-REPERFUSION MODEL OF MOUSE BRAIN

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Cerebral endothelial cells are characterized by the formation of tight junctions, the presence of several transport mechanisms, which together comprise the blood-brain barrier (BBB). During the pathophysiologic conditions such as ischemia-reperfusion (I/R), damage to cerebrovascular endothelial cells causes alterations of BBB function which can exacerbate neuronal cell injury and death. To clarify changes of BBB transport at early phase of cerebral I/R is important for understanding the BBB function on therapy after cerebral ischemia. The purpose of the present study was to characterize the transport of L-phenylalanine (Phe), as a substrate of LAT1 (L-type amino acid transporter 1), at the BBB during I/R. I/R model was produced by blood recirculation following the occlusion of the middle cerebral artery in mice. The effect of the ischemia on the opening of tight junction at the BBB was investigated using sodium fluorescein (SF), as a marker of paracellular route. The integrity of the BBB by the reperfusion following the ischemia was measured by [14C]sucrose. The permeability of the BBB to [3H]Phe was assessed during I/R using the in situ brain perfusion technique. A quantitative fluorescence method indicated that the brain penetration of SF did not occur during 0.75 h of ischemia. Furthermore, the intravascular space did not change during 2 h after reperfusion following the ischemia. In mice treated with I/R (0.75 h/0.5 h), [3H]Phe was found to be taken up into the brain in a saturated manner, and kinetic evaluation revealed that the Michaelis constant (Km) and the maximum uptake velocity (Vmax) were 5.7 μM and 3.7 nmol/min/g brain, respectively. Km and Vmax values were reduced similarly by 42% compared with those of the control. The present study suggests that the intrinsic transport clearance (Vmax/Km ratio) of amino acid is maintained to identical level to the normal at the early phase of I/R without the BBB disruption.

30PE-18

EFFECT OF DISEASE STAGE ON GENE EXPRESSION BY POLYPLEX AND LIPOPLEX IN MURINE HEPATITIS INDUCED BY CARBON TETRACHLORIDE

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In order to elucidate the effect of hepatic disease-stage on polyplex- and lipoplex-mediated gene delivery, we investigated the gene expression of polyplexes and lipoplexes in various tissues of mice with time after subcutaneous injection of CCl4. Liver injury after injection of CCl4 was confirmed by determination of serum AST and ALT activities. The plasmid DNA (pDNA) encoding firefly luciferase was used as a model reporter gene. pDNA was incubated with cationic polymers such as branched and linear polyethylenimine (B-PEI, L-PEI) to formulate polyplexes. Two kinds of liposomes constructed with N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammoniumchloride and dioleylphosphatidylethanolamine (DOTMA-DOPE) or DOTMA and cholesterol (DOTMA-CHOL) were used to prepare lipoplexes. We determined luciferase activities in various organs after intravenous administration of the polyplexes and lipoplexes in control and CCl4-treated mice. B-PEI polyplexes, L-PEI polyplexes, and DOTMA-DOPE lipoplexes showed a significant decrease of transgene expression in the liver and spleen of CCl4-treated mice at 18 h after CCl4 injection (the stage of severe hepatitis) comparing to control mice. On the other hand, B-PEI polyplexes, L-PEI polyplexes, and DOTMA-CHOL lipoplexes showed a significant increase of transgene expression in the liver of CCl4-treated mice at 48 h (the stage of liver regeneration). Significant differences in gene expressions between CCl4-treated mice and control mice vanished in most organs at 168 h (the stage of hepatitis subsidence). These results indicate the necessity of considering the timing, dose, and vector for gene therapy according to the disease stage.