

论文

阳离子膜融合脂质体介导反义寡核苷酸在Hela细胞中的转染实验研究

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摘要:

目的研究阳离子膜融合脂质体(CFL)介导反义寡核苷酸(ASON)的细胞转染效率及影响因素。方法 逆相蒸发法制备3种不同阳离子含量的脂质体(CL),在CL上引入仙台病毒形成CFL,将制得的阳离子膜融合脂质体与反义寡核苷酸混合得到复合物,考察形态学及载药量,用MTT法考察该载体的细胞毒性,流式细胞仪测定阳性细胞百分率和平均荧光强度。结果制得的CFL形态均匀,粒径为(168±65) nm。载药量随着磷脂/ASON(+/-)电荷比增加而增加。CFL细胞毒性明显低于相同电荷比的CL,细胞转染效率是随阳离子含量、磷脂/ASON(+/-)电荷比增加而增加,血清和低温均对CFL的细胞转染有影响。结论阳离子膜融合脂质体作为载体在低电荷比条件下可降低细胞毒性并可提高细胞转染效率,可作为该ASON的给药系统而进一步研究。

关键词: 阳离子膜融合脂质体 反义寡核苷酸 仙台病毒 转染效率

STUDY ON THE TRANSFECTION EFFICIENCY OF ANTISENSE OLIGODEOXYNUCLEOTIDE AGAINST TELOMERASE BY CATIONIC FUSOGENIC LIPOSOME

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Abstract:

AIMTo investigate the factors affecting transfection efficiency of antisense oligodeoxynucleotides (ASON) by cationic fusogenic liposome (CFL). METHODSThree types of cationic liposomes (CL) were prepared by reverse-phase evaporation vesicles and cationic fusogenic liposomes (CFL) were obtained through fusion with Sendai virus. The CFL/ASON complexes were formed by physical absorption. Transmission electron microscope was used to observe its morphology. Drug loading capacity was examined by Nanosep centrifuge tube. The cellular damage of two vectors was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Transfection efficiency was estimated with the use of fluorescein phosphoramidite (FAM)-antisense oligodeoxynucleotides by flow cytometric analysis. RESULTSThe mean diameter of CL and CFL was (124±19) nm and (168±65) nm, respectively. The drug loading capacity was depended on the charge of the ratio of lipid/ASON (+/-) and the cationic charge density on the lipid membrane. The cellular damage of CFL was obviously inferior to the same lipid/ASON (+/-) charge ratio of CL. The fluorescence intensity was shown to enhance the content of 3β-[N-(N',N'-dimethylaminoethane) carbamoyl] cholesterol (DC-Chol) and lipid/ASON (+/-) charge ratio. However, the transfection efficiency mediated by CFL at low lipid/ASON (+/-) charge ratio increased about two fold than that by CL. Later, both serum and temperature affect the capacity of cellular uptake by CL or CFL. The effect of the transfection efficiency using CFL was much weaker. CONCLUSIONCationic fusogenic liposome (CFL) improved transfection efficiency and decreased cellular damage at the condition of low lipid/ASON (+/-) charge ratio. So, the vector need further study as ASON delivery system.

Keywords: antisense oligodeoxynucleotides Sendai virus transfection efficiency cationic fusogenic liposome

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