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超声靶向微泡破裂联合PEI增强小鼠EGFP基因心肌转染

Enhancement of EGFP gene transfection to rat myocardium: ultrasound-targeted microbubble destruction combined with PEI

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中文摘要:

目的 探讨超声靶向微泡破裂(UTMD)联合聚乙烯亚胺(PEI)增强BALB/c小鼠心肌绿色荧光蛋白基因(EGFP)转染的可行性和应用价值。方法 实验分为7组:PBS组、裸质粒组、质粒+超声辐照组(P+US)、质粒+SonoVue+超声辐照组(P+UTMD)、质粒+PEI组(P+PEI)、质粒+PEI+超声辐照组(P+PEI+US)、质粒+PEI+SonoVue+超声辐照组(P+PEI+UTMD)。由BALB/c小鼠尾静脉注入EGFP质粒和SonoVue微泡或PEI的复合物,处理4 d后检测心肌基因表达效率及HE染色,并对超声辐照后的质粒完整性进行分析。结果 电泳显示超声辐照不会损坏DNA或PEI/DNA复合物。非超声辐照时,EGFP只在心内膜下层表达;而超声辐照时,表达最强的位置为靠近探头的左室前壁;超声联合PEI时,EGFP的分布差异不明显。P+PEI+UTMD组的转染率最高,荧光强度最强。结论 UTMD联合PEI可高效、靶向地将质粒DNA输送至心肌,这种非侵入性的技术在心脏基因治疗上很有前景,有望应用于迅速发展的心脏病基因疗法。

英文摘要:

Objective To determine whether ultrasound-targeted microbubble destruction (UTMD) combined with polyethylenimine (PEI) enhances gene transfection in vivo to BALB/c mice heart. **Methods** BALB/c mice were divided into 7 groups: PBS group, naked plasmid group, plasmid plus US irradiation group (P+US), plasmid plus US irradiation and SonoVue group (P+UTMD), plasmid plus PEI group (P+PEI), plasmid plus US irradiation and PEI group (P+PEI+US), plasmid plus PEI and US irradiation and SonoVue group (P+PEI+UTMD). Plasmid DNA encoding enhanced green fluorescent protein (EGFP) which was mixed with SonoVue or PEI was injected by tail vein to BALB/c mice, and the hearts were exposed to transthoracic US. Gene expression and HE stain in myocardium were evaluated four days after treatment. In addition, gel electrophoresis analysis was performed to determine the structural integrity of plasmid DNA or PEI/DNA after US exposure. **Results** There was no damage to DNA or PEI/DNA complexes after sonication in electrophoresis gel assay. The EGFP was significantly expressed in the subendocardial layer when the heart was not exposed to US. The strongest expression was detected at the anterior wall of the left ventricle that had faced the US probe. Distributional difference of EGFP was not obvious when US combined with PEI. The fluorescence intensity and transfection efficiency were the highest in the P+PEI+UTMD group. **Conclusion** The present study shows that this method of UTMD combined with PEI can be used to deliver plasmid DNA to the myocardium selectively and effectively. This noninvasive technique is a promising method for cardiac gene therapy and could be applied in the rapidly developing gene therapy for heart diseases.

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