酒精增加非離子性共聚合物微膠體吸入劑型之基因傳遞作用機制探討

The Mechanisms Studies of Ethanol Enhanced In Vivo Gene Delivery with Non-Ionic Polymeric Micelles Inhalation

中文摘要

選擇適當的載體及降低物質穿透的屏障能更有效將基因以吸入劑型傳遞到肺部組織，所以我們使用一種具有生物相容性及生物降解性的非離子性嵌段式聚合物poly(ethyleneoxide)- poly(propyleneoxide)- poly(ethyleneoxide) (PEO-PPO-PEO)來形成共聚合物微膠體作爲基因吸入劑型的載體，並且併用乙醇(10%-40%)來評估增加質體 DNA 的穿透與基因表現作用。結果顯示只有併用 10%乙醇下，以吸入劑型在兩天內 8 小時的間隔給予 6 個劑量(100 μg)的 pCMV-Lac Z 與共聚合物微膠體到裸鼠時，可以發現其基因表現β-Gal 在肺組織內細支氣管周圍會增加 38%的表現，並且在氣管(32%)、胃(22%)及腸(36%)組織也有明顯表現。同時以mannitol 及 estradiol 分別作爲細胞間和細胞內穿透途徑的指標物質，發現在給予第一個吸入劑量後的 48 小時後，10%乙醇會同時分別增加胃(54%)及腸(41%)組織細胞間(Pmannitol)的穿透及胃(42%)與腸(141%)組織的細胞內(Pestradiol)穿透。我們也以同樣的吸入劑型併用 10%乙醇來傳遞含有人體囊性纖維膜蛋白序列的質體 DNA，結果用免疫染色的方式發現在肺組織內細支氣管周圍也會增加囊性纖維膜蛋白的表現。因此使用衰減全反射-傅立葉轉換紅外線光譜儀(ATR-FTIR)來進行胃腸組織內蛋白質及脂質的變化分析，可以發現組織內 amide I 波峰(1600 cm-1~1700 cm-1) 中，其蛋白質次級結構β-sheet/α-helix 比值會隨著乙醇的濃度與組織細胞間穿透係數呈線性增加。而隨著乙醇濃度的增加，其 C-H 波吸光值的降低(脂質流失) 也跟二種組織細胞間的穿透係數增加呈現相關性，但與細胞內穿透係數的改變不一致。同時以 P=O- 波峰(1237 cm-1)作爲組織脫水程度指標，可以發現在乙醇濃度 20%-40%時，會明顯的轉移到較低的波數(wave number)，顯示組織內的水分在乙醇濃度 20%-40%時會逐漸被乙醇取代。再進一步以體外組織重量分析，更可以確認在 20%和 40%乙醇影響下，胃腸組織的水分含量會明顯降低。因此綜合以上的結果，我們認爲利用 10%乙醇結合共聚合物微膠體系統的吸入劑型，可以有效的將基因傳遞到呼吸道及胃腸組織主要是由10%乙醇提高胃腸組織細胞內穿透作用所導致。但在較高濃度的乙醇(20%-40%)所造成的脫水作用會降低其組織細胞內穿透作用。

英文摘要

Modifications of both carriers and host barriers have been investigated for efficient inhalation gene delivery to lung. Here we used a biocompatible non-ionic

poly(ethyleneoxide) -poly(propyleneoxide)-poly(ethyleneoxide) (PEO-PPO-PEO) polymeric micelles (PM) as a carrier and combined it with ethanol (10-40%) to enhance tissue penetration of delivered DNA. The inhalation delivery with six 100 μg doses of pCMV-Lac Z with PM to nude mice in two days at 8 hr interval could enhance the gene expression in the lung around bronchioles (38%), trachea (32%), stomach (22%) and duodenum (26%) areas under only with 10% ethanol. Using intra-, intercellular penetration markers (estradiol and mannitol), 10% ethanol also increased the apparent intercellular permeability (Pmannitol) by 54% in stomach and by 41% in intestine, and increased the apparent intracellular permeability (Pestradiol) by 42% in stomach and by 141% in intestine at 48 hr after the first dosage of delivery. Also delivery of DNA encoding a functional human cystic fibrosis transmembrane protein (CFTR) using the same inhalation delivery method co-formulated with 10% ethanol, an increased expression of CFTR in lung was detected by immunostaining. Ethanol effect on biophysical alterations in protein and lipids of GI tissues was evaluated using attenuated total reflection/Fourier transform infrared (ATR/FTIR) spectroscopy. The IR spectra of both tissues showed that β–sheet/α-helix ratios of protein compositions in the secondary structure-dependent amide I band (1600-1700 cm-1) increased linearly with increasing concentrations of ethanol as well as tissue intercellular permeation. The decrease of absorbance in C-H stretching band, an indictor of lipid extraction was also correlated with intercellular permeation, but not consisted with intracellular permeation with increasing ethanol concentration. Using the P=O- (1237 cm-1) stretching band, an indicator of hydration state in tissues, both tissues only pretreated with 20%-40% ethanol was significantly shifted to lower wave number in IR spectra. Also the significant decrease of hydration level (HL) % in both tissues only with pretreated of 20%-40% ethanol was observed. Thus, we concluded that 10% ethanol co-formulated with the PM system could enhance inhaled gene delivery to airway and GI tract due to the intracellular transport of GI tissues increased. The dehydration ability of ethanol at higher concentration (20%-40%) may overshadow intracellular permeation of tissues with decreasing intracellular permeation.