口服聚合微膠體包覆 pMBP-Lac Z 之藥物動力學

Pharmacokinetics of oral administered pMBP-Lac Z with polymeric micelles

中文摘要

先前本實驗室曾使用非離子型聚合微膠體當作口服基因的載體，於 48 小時內投與六個劑量後，發現質體 DNA 不僅於腸胃道有表現，還能由血液分佈至較遠的組織及器官中，包括腦及脊髓。爲了進一步了解質體 DNA 於體內分佈的情形；合適的藥物劑量與多劑量給藥之間距等因此本實驗分別由靜脈及口服投與單一劑量(40 μg/150μL) pMBP-Lac Z 與同劑量經微膠體包覆的 pMBP-Lac Z 至裸鼠體內，評估 pMBP-Lac Z 之分布及其藥物動力學。於不同時間點下採集裸鼠血液及組織(包括十二指腸，肝臟，脊髓，與腦) ，經過萃取得到 total DNAs，使用同步定量 PCR (Q-PCR)分析檢品中 Lac Z 的含量。本實驗採用絕對定量方式分析檢品中 Lac Z DNA 的含量，因此以濃度爲 10^2 copies/μL 到 10^8 copies/μL 的純的 pMBP-Lac Z DNA 作爲標準曲線。在此濃度範圍內，標準曲線的對數值呈線性關係，並以同日內及異日間的數值來評估本實驗分析方法的精確性與準確性，其變異係數均小於 15%。實驗結果顯示，利用靜脈注射方式投與後，於血液檢品中發現，未包覆之 pMBP-Lac Z 的 area under the curve (AUC)爲 1.37x10^8 (min x pg/mL)，而微膠體包覆之 pMBP-Lac Z 於血 AUC 爲 3.88x10^7 (min x pg/mL)，兩者間無統計上的差異。但經過微膠體包覆後的 pMBP-Lac Z 的排除半衰期由 54.5 分鐘延長爲 210.5 分鐘。另一方面，在口服投與後於血液檢品中未包覆之 pMBP-Lac Z 其 AUC 値爲 9.29 (min x pg/mL)，使用聚合微膠體包覆者爲 6.09x10^3 (min x pg/mL)。此外可觀察到 pMBP-Lac Z 在體內的排除半衰期從 46.6 分鐘延長到 257 分鐘。由結果計算其不包覆與包覆之相對口服生體可用率時，約增加 600 倍左右。此外，聚合微膠體包覆 pMBP-Lac Z 經由靜脈注射後，其 AUC 於十二指腸、肝臟、脊髓及腦分別爲 170.6，108.3，1086.6，182.6 (min x pg/g gDNA)。未包覆者於上述四種器官中的 AUC 分別爲 127.6，199.0，463.8，187.4 (min x pg/g gDNA)。聚合微膠體包覆之 pMBP-Lac Z 經由口服投與後，於十二指腸、肝臟、脊髓及腦中的 AUC 分別爲 331.5，0.26，22.2，9.93 (min x pg/g gDNA)，除了小腸之 AUC 外，其於組織的 AUC 均小於靜脈注射之值。口服投與未包覆 pMBP-Lac Z 於十二指腸，脊髓，與腦中的 AUC，分別爲 57.5，1.39，1.8 (min x pg/g gDNA) ，其 AUC 値均小於相同投與途徑包覆之 pMBP-Lac Z。綜合上述結果，聚合微膠體包覆 pMBP-Lac Z 能增加 pMBP-Lac Z 之口服生體可用率。此外，利用口服投與聚合微膠體包覆質體 DNA，比未包覆者相比有較高的 AUC 值。然而，經靜脈投與後，於血液中 pMBP-Lac Z 有最高的 AUC 値。

英文摘要

We have previously used the nonionic polymeric micelles (PM) as a carrier for oral gene delivery given at six doses within 48 hours, and the results showed that the delivered gene expression was detected in GI, plasma, and other tissues and organs, including brain and spinal cord. In order to optimize the ideal dose and dose regimen, we evaluate the distribution and pharmacokinetic profile of pMBP-Lac Z DNA (40 μg/150 μL) delivered with or without polymeric micelles to nude mice orally and intravenously. The plasma and tissue samples (duodenum, liver, spinal cord, and brain) were collected at various time points, and total DNAs were extracted for Lac Z DNA quantitation using real-time quantitative polymerase chain reaction (QPCR). To absolutely quantitate Lac Z DNA in the samples, a standard curve was generated using purified pMBP-Lac Z DNA in a range of 10^2-10^8 copies/μL. Within the log linear range of standard DNA at 10^2 to 10^8 copies/μL, we obtained the CV% of within- and between-day assays were all less than 15%, indicating the precision and accuracy of the method used in this study. After IV administration, the area under the curve (AUC) of pMBP-Lac Z and formulated pMBP-Lac Z in plasma was 1.37x10^8 (min x pg/mL) and 3.88x10^7 (min x pg/mL), respectively, which were no significant different. But the formulated pMBP-Lac Z had prolonged elimination half-lives (from 54.5 to 210.5 min). On the other hand, the area under the curve (AUC) of pMBP-Lac Z and formulated pMBP-Lac Z in plasma was 9.29 (min x pg/mL) and 6.09x10^3 (min x pg/mL), respectively, after orally administration. In addition, the formulated pMBP-Lac Z had prolonged elimination half-lives (from 46.6min to 257min). The results indicated that the relative oral bioavailability of formulated pMBP-Lac Z was 600 folds of that of the naked pMBP-Lac Z. Furthermore, after IV administration, the AUC values of formulated pMBP-Lac Z in duodenum, liver, spinal cord and brain were 170.6, 108.3, 1086.6, 182.6 (min x pg/g gDNA), respectively. For naked pMBP-Lac Z, the AUC values in the above organs were 127.6, 199.0, 463.8, 187.4 (min x pg/g gDNA), respectively. The AUC values of formulated pMBP-Lac Z via oral administration in duodenum, liver, spinal cord, and brain were 331.5, 0.26, 22.2, 9.93 (min x pg/g gDNA). Most of the AUC values were smaller than that detected after IV administration, except that detected in duodenum. For the naked pMBP-Lac Z in duodenum, spinal cord and brain, the AUC values were 57.5, 1.39, 1.8 (min x pg/g gDNA) and were lower than that detected with formulated DNA. Overall, these results indicated that PM formulation enhanced the bioavailability of pMBP-Lac Z. In addition, PM-formulated pMBP-Lac Z shows higher AUC than naked pMBP-Lac Z in all tissues after oral administration. However, the highest AUC level of pMBP-Lac Z was observed in the plasma after IV administration.