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论文

与CYPOR共表达CYP2D6*1和CYP2D6*10及其代谢活性比较

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摘要:

细胞色素P450 2D6 (cytochrome P450 2D6, CYP2D6) 是一种重要的氧化代谢酶, 呈基因多态性, 对药物的代谢呈现明显的个体差异和种族差异, CYP2D6*10在中国人群中的发生率高达51.3%。采用杆状病毒昆虫 表达系统, 采用与细胞色素氧化还原酶 (cytochrome oxidoreductase, CYPOR) 共表达的方式获得具有代谢活 性的CYP2D6*1/CYP2D6*10, 并初步研究其催化右美沙芬的代谢活性差异。构建重组质粒pFastBac-CYP2D6*1, pFastBac-CYP2D6*10和pFastBac-CYPOR, 并分别转化DH10Bac菌株构建重组Bacmid-CYPOR, Bacmid- CYP2D6*1和Bacmid-CYP2D6*10, 转染Sf9昆虫细胞, 获得重组杆状病毒用于病毒扩增和感染Sf9昆虫细胞。通过测定混悬蛋白催化右美沙芬的速率来优化重组CYPOR和CYP2D6病毒感染Sf9昆虫细胞的感染复数 (multiplicity of infection, MOI) 值和其比例, 获得活力较高的重组CYP2D6*1和CYP2D6*10。重组CYP2D6*1催化右美沙芬的K_m为 (26.67 ± 2.71) μmol·L^{-1} (n = 3), V_{max}为 (666.7 ± 56.78) pmol·nmol^{-1}(CYP2D6)·min^{-1} (n = 3), 清除率为25.0; CYP2D6*10催化右美沙芬的K_m为 (111.36 ± 10.89) μmol·L^{-1} (n = 3), V_{max}为 (222.2 ± 20.12) pmol·nmol^{-1} (CYP2D6)·min^{-1} (n = 3), 清除率为2.0。利用杆状病毒昆虫细胞系统表达的重组CYP2D6*1和CYP2D6*10代谢药物具有活性差异, 可用于体外研究其催化某些药物的代谢差异, 这有助于理解药物代谢的个体差异、预测药物之间的相互作用。

关键词: 昆虫表达系统 细胞色素P450 2D6*1 细胞色素P450 2D6*10 细胞色素氧化还原酶 代谢差异

CYP2D6*1, CYP2D6*10 co-expressed with CYPOR in Bac-to-Bac expression system and activity determination

Abstract:

CYP2D6 is an important drug-metabolizing enzyme. The polymorphism of CYP2D6 leads to metabolism difference and the different reactions of drugs in the individuals and different races are normal phenomenon in clinical medication. CYP2D6*10 is an important subtype in Asian people and 51.3% Chinese are classified with this subtype. To obtain recombinant active CYP2D6*1/CYP2D6*10 in baculovirus system by optimizing coexpression with CYPOR, and detect their activity to catalyze dextromethorphan, three recombinants pFastBac-CYP2D6*1, pFastBac-CYP2D6*10 and pFastBac-CYPOR were constructed and transformed into DH10Bac cell to obtain the recombinant Bacmid-CYPOR, Bacmid-CYP2D6*1 and Bacmid-CYP2D6*10. And then the recombinant CYP2D6*1 and CYP2D6*10 virus were obtained by transfecting Sf9. Then homogenate protein activity was determined with dextromethorphan as substrate. The multiple of infection (MOI) and its ratio of recombinant CYP2D6 virus to CYPOR virus were adjusted by detecting the activity of the homogenate protein. The K_m and V_{max} are 26.67 ± 2.71 μmol·L^{-1} (n = 3) and 666.7 ± 56.78 pmol·nmol^{-1} (CYP2D6)·min^{-1} (n = 3) for CYP2D6*1 to catalyze dextromethaphan. The K_m and V_{max} are 111.36 ± 10.89 μmol·L^{-1} (n = 3) and 222.2 ± 20.12 pmol·nmol^{-1}(CYP2D6)·min^{-1} (n = 3) for CYP2D6*10 to catalyze dextromethorphan. There is significant difference between CYP2D6*1 and CYP2D6*10 for V_{max} and K_m (P < 0.01). The clearance ratio of CYP2D6*1 is 25.0 and the clearance ratio of CYP2D6*10 is 2.0. The expressed CYP2D6*1 and CYP2D6*10 are useful tools to screen the metabolism profile of many xenobiotics and endobiotics *in vitro*, which are benefit to understand individual metabolism difference.

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