肝臟細胞色素 P450 2B1/2 對靜脈麻醉藥物 PROPOFOL 代謝所扮演之角色探討

ROLE OF HEPATIC CYTOCHROME P450 2B1/2 IN PROPOFOL METABOLISM

中文摘要

Propofol 是臨床常用之靜脈麻醉藥物，主要用於手術時之麻醉誘導與持續，亦可用於加護病房病人，做爲鎮靜藥物。先前研究證實，肝臟細胞色素 P450s (cytochrome P450s; CYPs)對此藥物之代謝扮演重要角色。但對於藥理及毒理研究常用之實驗動物模式，大鼠肝臟中，是何種 CYP 異構酶(isoforms)參與 propofol 之代謝，則尚無相關研究被提出。所以本研究主要將探討，於大鼠肝臟微粒體中，主要是何種 CYP 異構酶參與 propofol 之代謝。此外，肝細胞內 CYPs 對藥物代謝之效率，與藥物於細胞內之運輸情形有關，而細胞支架 (cytoskeleton)能影響細胞的藥物運輸，因此，本研究另一研究目標，將探討細胞支架是否會影響肝臟 CYPs 之酵素活性。

已知在不同物種中，CYPs 對 propofol 代謝均扮演著重要之角色，先前研究亦指出在人類及犬科動物，主要代謝 propofol 之 CYP 異構酶分別爲 CYP2B6 及 CYP2B11，皆屬於 CYP2B 次家族，因此本研究首要目標將探討大鼠肝臟中，CYP2B1/2 是否參與於 propofol 代謝。利用 CYP2B 誘導劑 phenobarbital (PB)，以腹腔注射方式，先對雄性 Wistar 大鼠(rat)連續施打 7 天，然後進行動物犧牲，取出肝臟並製備成微粒體。經 PB 處理後，大鼠肝臟微粒體之 pentoxyresorufin O-dealkylase (PROD)活性明顯增加，以免疫蛋白轉印分析顯示，經 PB 處理之大鼠肝臟微粒體，其 CYP2B1/2 蛋白量亦顯著增加。若以 PB 處理之大鼠肝臟微粒體，進行 propofol 之體外代謝，並以 HPLC 分析，可偵測到 propofol 代謝產物之形成，將代謝產物進一步以 LC/MS 分析後，證實此代謝產物爲 propofol 經氧化代謝所形成之 4-hydroxypropofol (4-OH-PPF)。相對於未經藥物處理之控制組，經 PB 處理之大鼠肝臟微粒體，有較強之 propofol 氧化代謝能力，4-OH-PPF 產量較高。更進一步以 CYP2B1/2 化學抑制劑 orphenadrine 處理大鼠肝臟微粒體，發現 4-OH-PPF 產量顯著下降，顯示其 propofol 氧化代謝能力降低。接著以 CYP2B1/2 專一性抗體處理大鼠肝臟微粒體，經抗體處理後，同樣造成 4-OH-PPF 產量顯著下降，顯示其 propofol 氧化代謝能力亦降低。

本研究之另一探討方向，以人類肝癌細胞株 HepG2 細胞爲研究模式，探討當細胞支架的 F-actin 受到調控時，CYP2B 活性是否會受影響。以抑制 F-actin 聚合藥物 cytochalasin D 處理細胞，以螢光標定物進行 F-actin 染色，利用共軛焦顯微鏡觀察並記錄細胞內的變化。經 cytochalasin D 處理後，HepG2 細胞

中 F-actin 結構受到干擾而呈點狀分佈。而利用共軛焦顯微鏡進行活細胞之代謝分析，記錄連續時間下，細胞內螢光產物形成之動態變化可知，經 cytochalasin D 處理後，同時會造成細胞的 PROD 活性降低，顯示 HepG2 細胞內由 CYP2B 參與之 pentoxyresorufin 代謝能力受到了抑制。
本研究結果顯示，經 PB 處理後，大鼠肝臟之 CYP2B1/2 活性及蛋白量都會增加。以 PB 處理之大鼠肝臟微粒體，能有效率的將 propofol 氧化代謝成 4-OH-PPF。而此氧化代謝反應會受到 CYP2B1/2 化學抑制劑及專一性抗體所抑制。因此得知，CYP2B1/2 對大鼠肝臟代謝 propofol 扮演著重要的角色，爲主要參與 propofol 氧化代謝之 CYP 異構酶。另外，經本研究結果亦得知，當 HepG2 細胞中細胞支架的 F-actin 聚合被干擾，細胞中 CYP2B 代謝活性亦會受到抑制，細胞支架 F-actin 可能與 CYP2B 代謝受質的能力有關。

英文摘要

Propofol, an intravenous anesthetic agent, is widely used for induction and maintenance of anesthesia during surgical procedures. Propofol can be used as a sedative agent for intensive care unit patients. Previous studies had reported that human cytochrome P450s (CYPs) play critical roles in the metabolism of propofol. Rat is a common animal model for pharmacological and toxicological studies of drugs. However, it is not determined which CYP isoforms contribute to the metabolism of propofol in rat liver. Thus, one of specific aims of this study is to determine which CYP isoforms in rat liver has a major role in propofol metabolism. The drug transport from extracellular environment to intracellular endoplasmic reticulums will determine if CYPs can effectively metabolize these drugs. Previous study has reported that cytoskeleton could modulate drug transport. Another specific aim of this study is to evaluate the role of cytoskeleton in CYP activities. Previous studies revealed that CYP2B6 and CYP2B11 are the major CYP isoforms participating in propofol metabolism in human and in canine, respectively. Male Wistar rats were intraperitoneally injected with 80 mg/kg body weight/day phenobarbital (PB) for 7 days. Liver microsomes from control- and PB-treated rats were prepared. Our data revealed that PB significantly increased pentoxyresorufin O-dealkylase (PROD) activity and CYP2B1/2 protein levels in rat liver microsomes. Data from HPLC analysis revealed that PB-treated liver microsomes had much efficacy on propofol hydroxylation than control ones. LC/MS analysis demonstrated that the major metabolite of propofol biotransformation by PB-treated liver microsomes was 4-hydroxypropofol (4-OH-PPF). Hydroxylation of propofol by PB-treated rat liver microsomes was significantly suppressed by orphenadrine, a CYP2B1/2-specific inhibitor. Pretreatment with a polyclonal antibody against rat CYP2B1/2 protein significantly reduced propofol hydroxylation by PB-treated liver

microsomes.

In this study, we further evaluated that that the modulation of F-actin cytoskeleton could affect CYP2B activity in HepG2 cells. Confocal microscopic analysis showed that cytochalasin D, an inhibitor of F-actin polymerization, could disrupt F-actin cytoskeleton of HepG2 cells. In parallel with the modulation of F-actin polymerization, cellular PROD activity was significantly decreased.

This study shows that PB can increase CYP2B1/2 activity and protein levels. Propofol is metabolized by PB-treated rat liver microsomes to 4-OH-PPF. And, a CYP2B1/2-specific inhibitor and antibody significantly inhibit the hydroxylation. Therefore, in rat liver microsomes, CYP2B1/2 plays a major role in propofol hydroxylation. This study has also shown that the modulation of F-actin cytoskeleton can regulate CYP2B activity in HepG2 cells. F-actin cytoskeleton may involve in the metabolism of its substrates by CYP2B.