靜脈麻醉藥物 midazolam 及 ketamine 對肝細胞細胞支架的影響

effects of midazolam and ketamine on cytoskeleton of hepatocytes

中文摘要

Midazolam 及 ketamine 是臨床上經常使用的靜脈麻醉藥物。Midazolam 與中樞神經的苯二氮平類（benzodiazepine）受器結合後，增進抑制性的神經傳導物（inhibitory neurotransmitter）的活性，例如 gamma-aminobutyric acid。Ketamine 是解離麻醉劑（dissociative anesthetic），其作用包括作爲 N-methyl-D-aspartate 受器的拮抗劑。這兩種藥物都是經由肝臟的單氧?酵素系統（monooxygease）代謝，主要參與代謝的是 cytochrome P450 3A4。細胞支架（cytoskeleton）與細胞功能息息相關，細胞支架與細胞形狀的改變、胞器（organelles）的分布以及代謝有關，細胞內的訊息傳導途徑也需要細胞支架才能專一又有效率。細胞支架的組成主要有三種蛋白質纖維，包括微絲（microfilaments）、微管（microtubules）以及中間絲（intermediate filaments），微絲由肌動蛋白（actin）聚合而成，微管由微管蛋白（tubulin）組成。由於麻醉藥物多爲高度脂溶性藥物，可穿過細胞膜，進入細胞內，前人的研究顯示麻醉藥物可改變細胞支架，而 midazolam 和 ketamine 對肝細胞細胞支架的作用，尚未被探討。所以，本研究以人類肝細胞株 HepG2 爲實驗模式，探討 midazolam 及 ketamine 對肝細胞細胞支架的影響。

實驗結果顯示，臨床濃度的 midazolam（0.5 μM）及 ketamine（100 μM）在 24 小時之內並不會影響肝細胞的存活率。在對微絲細胞支架的影響上，以免疫細胞染色法（immunocytochemistry）標定 HepG2 細胞的微絲後，在螢光及共軛焦顯微鏡下觀察，midazolam 及 ketamine 在 24 小時使得微絲的分布更集中在細胞膜周圍，而在螢光強度的分析上，在 24 小時，midazolam 及 ketamine 使得微絲螢光強度有意義的下降。爲探討藥物對微絲系統的影響是否與肌動蛋白的製造有關，以反轉錄聚合?連鎖反應（reverse transcriptase-polymerase chain reaction）分別定量 β-actin 及 α-actin mRNA 的合成發現，以 midazolam 處理過的細胞，α-actin mRNA 的合成增加，對 β-actin 則沒有影響。而 ketamine 對 β-actin 及 α-actin 都沒有影響。對微管細胞支架的影響，用 anti-α-tubulin-FITC 抗體作免疫細胞螢光染色後，再置於螢光及共軛焦顯微鏡下觀察，midazolam 及 ketamine 在 6 小時開始改變微管的分布，且降低螢光強度，在 24 小時的實驗中，此一效應更爲明顯，微管的排列更爲紊亂，且螢光強度更爲降低。

對肝細胞單氧?酵素系統的影響，本實驗檢測經過藥物處理後的 HepG2 細胞，其 cytochrome P450 3A4 及 2B6 的酵素活性及 mRNA 的表現量是否受影響。結果顯示，midazolam 不會影響 cytochrome P450 3A4 及 2B6 的酵素

活性及 mRNA的表現量，而ketamine在6及24小時造成cytochrome P450 2B6 mRNA的表現量下降，對cytochrome P450 3A4 的酵素活性及mRNA的合成則沒有影響。
綜合以上實驗結果可知，靜脈麻醉藥物midazolam及ketamine確實會影響肝細胞細胞支架，也可能進一步影響肝細胞的正常功能，至於二者的相關性及臨床上的影響則需進一步實驗釐清。

英文摘要

Midazolam, an imidazobenzodiazepine derivative, is utilized as an intravenous anesthetic agent. The hypnotic effect of midazolam probably is related to gamma-aminobutyric acid accumulation. Ketamine is an intravenous dissociative anesthetic agent, whose mechanism of action might be an N-methyl-D-aspartate receptor antagonist. Both drugs are metabolized by cytochrome P450-dependent monooxygenase system, mostly 3A4 isoform, in the liver.
Cytoskeleton is the major organelles in the cytoplasm, which is important for the architecture, motility, metabolism, and intracellular signal transduction of the cell. There are three types of protein filaments in the cytoskeleton, including microfilaments, microtubules, and intermediate filaments. Cytoskeleton is closely connected with plasma membrane; therefore, it is of interest when considering the effects of lipid-soluble anesthetics. Evidence showed that filament-disrupting agents, such as cytochalasin, alter cellular response, and certain general anesthetics modulated cytoskeletal organization. This study was aimed to elucidate the effects of midazolam and ketamine on cytoskeleton of hepatic cells, using human HepG2 cells as the experimental model.
The results demonstrated that, in the clinically relevant concentration, midazolam（0.5 μM）and ketamine（100 μM）did not affect viability of cells up to 24 hours. Cells were stained with TRITC-phalloidin that specifically binds filamentous actin, and observed using fluorescence microscopy and laser scanning confocal microscopy. Exposures to midazolam or ketamine for 24 hours changed microfilament distribution and reduced microfilament contents within cells. Reverse transcriptase-polymerase chain reaction assay was carried out to determine the effects of midazolam and ketamine on the synthesis of actin. Midazolam induced α-actin mRNA synthesis without affecting the transcription of β-actin. Neither β-actin nor α-actin mRNA production was affected by ketamine administration. Immunocytochemistry analysis was carried out using anti-α-tubulin-FITC antibodies to determine the effects of midazolam and ketamine on microtubule cytoskeleton. Microtubule structure was disorganized after exposure to either midazolam or ketamine for 6 and 24 hours.

Erythromycin N-demethylation and pentoxyresorufin O-dealkylase assays were carried out to determine the effects of midazolam and ketamine on enzyme activities of cytochrome P450 3A4 and 2Bs. Neither 3A4 nor 2Bs activity was affected by midazolam or ketamine. Reverse chain-polymerase chain reaction was performed to analyze the effects of midazolam and ketamine on the synthesis of cytochrome P450 mRNAs. Midazolam did not affect the production of cytochrome P450 3A4 and 2B6 mRNA. Ketamine inhibited cytochrome P450 2B6 mRNA synthesis after exposed for 6 and 24 hours.

Our results imply that hepatic cytoskeleton might be modulated by midazolam and ketamine. Changes in distribution of microfilaments and disorganization of microtubules in hepatocytes might affect normal hepatic function, e.g. cytochrome P450. Further studies are necessary to clarify the consequence and clinical importance.