# 靜脈麻醉藥物氯胺酮調控人類臍帶內皮細胞一氧化氮及第一型內皮素表現之研究


## 中文摘要

氯胺酮 (ketamine) 是常用的靜脈麻醉藥物，但在臨床上使用常會造成患者有高血壓的現象。血管內皮細胞可分泌內皮細胞衍生釋放因子 (endothelium-derived releasing factor)，其中一氧化氮 (nitric oxide, NO) 及第一型內皮素 (endothelin-1, ET-1)對於人體血壓的調節，尤其扮演重要的角色。本研究探討氯胺酮是否能調控內皮細胞一氧化氮及第一型內皮素之生合成，以闡明氯胺酮對於血管內皮細胞可能產生的藥物作用。

本實驗以人類臍帶靜脈內皮細胞 (human umbilical vein endothelial cells) 做爲研究模式，首先利用 MTT 色度分析法來檢驗氯胺酮的細胞毒性，發現氯胺酮在臨床濃度 100 mM 下，不影響細胞存活率；接著以 Griess Reaction 來測量培養液中亞硝酸鹽 (nitrite) 的量，發現內皮細胞與氯胺酮共同培養 1 小時後，亞硝酸鹽產量約減少了 25%。而在處理 6 小時及 24 小時後，亞硝酸鹽產量分別減少了 28% 及 43%。再以免疫轉印分析法 (immunoblotting assay) 分析細胞內 eNOS 蛋白質的量，實驗顯示在以氯胺酮處理 1 小時、6 小時及 24 小時後，eNOS 蛋白質量各減少了 32%、44% 及 56%。以反轉錄聚合酶連鎖反應方法 (reverse transcriptase-polymerase chain reaction) 分析細胞內 eNOS mRNA，發現內皮細胞與氯胺酮共同培養 1 小時、6 小時和 24 小時後，內皮細胞中 eNOS mRNA 的表現分別減少了 43%、49% 及 72%。而以反轉錄聚合酶連鎖反應方法分析人類臍帶靜脈內皮細胞中 ppET-1 mRNA，結果顯示內皮細胞與 100 mM 氯胺酮共同培養 24 小時後，細胞中 ppET-1 mRNA 的表現量減少了 64%。再進一步以多重標定測讀儀 (multilabel light luminescence counter) 及共軛聚焦顯微鏡 (confocal laser scanning microscopy) 來監測細胞內鈣離子的濃度變化，發現氯胺酮會抑制細胞內鈣離子的移動，並降低細胞內鈣離子的濃度；最後將 Wistar Rat 腹腔注射 100 mg/kg body weight 的氯胺酮，在 3 小時後收集血液，控制組與注射氯胺酮的大鼠血清中第一型內皮素的濃度分別爲 3.93 pg/ml 及 2.97 pg/ml，證實了氯胺酮會減少血液中第一型內皮素的濃度。

經由本研究發現，氯胺酮在臨床濃度 100 mM 會抑制人類臍帶靜脈內皮細胞合成一氧化氮及第一型內皮素，且這個抑制機制應發生在前轉錄時期 (pretranslational mechanism)，而在後轉錄時期 (posttranslational)發現氯胺酮可抑制細胞內鈣離子的移動及降低細胞內鈣離子濃度，進而導致一氧化氮及第一型內皮素的表現量減少，也是可能的原因之一。

英文摘要

Ketamine (2-(2-Chlorophenyl)-2-(methylamino)-cyclohexanone) is a widely used anesthetic agent in clinics. However, induction of anesthesia with ketamine may raise blood pressure. Vascular endothelial cells can release endothelium-derived releasing factors, including nitric oxide (NO) and endothelin-1 (ET-1), which play important roles in regulation of blood pressure. The goal of this study was to evaluate the effects of ketamine on biosyntheses of NO and ET-1 using human umbilical cord endothelial cells (HUVECs) as our experimental model. Cell viability was assayed by a MTT colorimetric method. Exposure of HUVECs to 100 μM ketamine for 24 h had no effect on cell viability. Total amounts of nitrite, an oxidative product of NO, in the culture medium were quantified according to the Griess reaction method. The levels of nitrite were decreased in ketamine-treated HUVECs in a time-dependent manner. Immunoblotting analysis revealed that ketamine decreased the protein level of eNOS in a time-dependent manner. Analysis by RT-PCR showed ketamine decreased eNOS mRNA production. The effect of ketamine on ppET-1 mRNA expression in HUVECs was analyzed by RT-PCR. The results showed the expression of ppET-1 mRNA was decreased after exposure to ketamine for 24 h. Furthermore, we used a confocal laser scanning microscope and a fluorescence multilabel counter to analyzed intracellular calcium movement and concentrations. After stimulation by 200 nM bradykinin, a clinical relevant concentration of ketamine (100 μM) significantly decreased intracellular calcium mobilization and concentrations. Male Wistar rats were intraperitoneally injected 100mg/kg body weight ketamine and observed for 3 h. After scarification, blood was collected and ET-1 concentrations were quantified by a ET-1 chemiluminescent immunoassay. Treatment with ketamine significantly suppressed the levels of serum ET-1 concentration.

This study showed that ketamine at a therapeutic concentration can inhibit the biosyntheses of nitric oxide and endothelin-1. The suppressing mechanism may occur via a pretranslational mechanism and a reduction of intracellular calcium concentrations