靜脈麻醉藥物PROPOFOL對脂磷壁酸活化巨噬細胞生成一氧化氮之抑制作用研究

SUPPRESSIVE EFFECTS OF INTRAVENOUS ANESTHETIC PROPOFOL ON NITRIC OXIDE SYNTHESIS IN LIPOTEICHOIC ACID-ACTIVATED MACROPHAGES

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中文摘要

Propofol 是一種被廣泛使用的靜脈麻醉藥物，由於其具有短效且起始作用迅速的特性，因而常被用於全身麻醉之誘導與維持以及加護病房病患的鎮靜安眠。在加護病房中，嚴重的敗血症以及敗血性休克是最主要的致死原因。Nitric oxide (NO)是許多重要生理功能所必須的，但是過量的 NO 則被認爲是敗血症中導致組織傷害的重要因子。本實驗的主要目的是探討 propofol 對受到脂磷壁酸(lipoteichoic acid; LTA)活化之巨噬細胞產生 NO 的影響與其作用機轉。
將巨噬細胞以 25、50 及 75 ?M 的 propofol 或 1、5、10、20、50 及 75 ?g/ml 的 LTA 處理後發現，細胞的存活率與對照組相比並無明顯差異。將細胞以 100 μM 的 propofol、10 μg/ml 的 LTA 處理，或以 100 μM 的 propofol 與 10 μg/ml 的 LTA 同時處理巨噬細胞，會造成明顯的細胞毒性。接著以不同濃度的 LTA 刺激巨噬細胞，結果發現，NO 的產生與 LTA 的濃度成正比。再將 LTA 與不同濃度的 propofol 同時處理細胞，發現 propofol 可以抑制由 LTA 所誘導的 NO 產生，而且抑制的效果與 propofol 的濃度成正比。爲了探討 propofol 對 NO 生成的抑制機轉，所以接下來觀察 iNOS 蛋白與 mRNA 的表現。免疫轉印分析的結果顯示，propofol 可以抑制因 LTA 誘導所造成的 iNOS 蛋白增加，而反轉錄?連鎖反應分析的結果顯示，propofol 可以減低 LTA 刺激造成的 iNOS mRNA 上升。爲瞭解 propofol 抑制 LTA 誘導導致 iNOS 表現的機轉，接著觀察轉錄因子 nuclear factor-κB (NF-κB)的變化。免疫轉印分析的結果顯示，propofol 可以降低 LTA 誘導的 NF-κB 活化，而且 propofol 對於 dominant negative mutant inhibitor κB 所造成 NF-κB 轉位至細胞核的抑制作用有加成的效果。進一步探討轉錄因子 NF-κB 上游 inhibitor κB kinase 與 extracellular signal-regulated kinase1/2 的改變，結果發現，propofol 可以抑制 LTA 刺激產生的 inhibitor κB kinase 與 extracellular signal-regulated kinase1/2 活化。以上結果顯示，臨床濃度的 propofol 可以抑制巨噬細胞因 LTA

誘導所產生的 NO，此抑制作用與 propofol 降低 iNOS 蛋白及 mRNA 的表現有關。而對於 iNOS 表現的抑制作用，與 propofol 降低轉錄因子 NF-κB 的活化，以及 propofol 抑制 NF-κB 上游 inhibitor κB kinase 及 extracellular signal-regulated kinase1/2 的磷酸化有關。

英文摘要

Propofol, an intravenous anesthetic agent, is widely used for induction and maintenance of general anesthesia and sedation in the intensive care units because of its rapid onset and short acting. Severe sepsis and septic shock are the most important causes of death in intensive care units. Although nitric oxide (NO) is essential for several physiologic functions, excessive production of NO is believed to contribute to tissue damage in septic shock. The purposes of this study were to find out if propofol would affect NO synthesis in lipoteichoic acid (LTA)-activated macrophages and its possible mechanism.

Exposure of RAW264.7 macrophages to propofol at 25, 50, and 75 ?M or LTA at 1, 5, 10, 20, 50, and 75 ?g/ml did not affect cell viability. However, 100 ?M propofol, 100 ?g/ml LTA, and a combination of 100 ?M propofol and 10 ?g/ml LTA led to significant cell death. Exposure of macrophages to LTA at different concentrations increased the levels of nitric oxide in a concentration-dependent manner. While macrophages were treated with a combination of propofol and LTA, propofol could concentration-dependently decrease the NO production in LTA-activated macrophages. To explore the inhibition of NO production by propofol, the expression of inducible nitric oxide synthase (iNOS) protein and iNOS mRNA were examined. Immunoblotting analysis showed that propofol could decrease LTA-induced iNOS protein. Analysis by a reverse transcriptase-polymerase chain reaction revealed that the LTA-induced iNOS mRNA expression was inhibited by propofol. To clarify the mechanism by which propofol inhibited LTA-induced iNOS expression, transcription factor nuclear factor κB (NF-κB) was investigated. Immunoblotting analysis showed that propofol could inhibit LTA-induced NF-κB activation. Furthermore, the upstream inhibitor κB kinase (IKK) and extracellular signal-regulated kinase1/2 (ERK1/2) were examined. The results revealed that propofol could suppress LTA-induced NO production. These suppressive effects were associated with down-regulation of iNOS protein and mRNA by propofol. The inhibition of NF-κB activation as well as phosphorylation of IKK and ERK1/2 led to suppression of iNOS by propofol.