# Lycopene 與 Ketamine 抑制 LPS 引發大鼠微神經膠細胞產生發炎反應的作用機轉探討

# Mechanisms of Lycopene and Ketamine Involved in the Inhibition of Lipopolysaccharide Induced Inflammation in Rat Microglial Cells

## 中文摘要

Lycopene (茄紅素)是一種蕃茄類食物裡的天然色素，同時也是很強的抗氧化劑，在人體可以對抗多種退化性疾病；但我們的身體不會自行製造茄紅素，因此補充茄紅素可以幫助身體對抗因自由基所引起的疾病。Ketamine 是用於人或動物麻醉之一種速效、全身性麻醉劑，與 PCP (Phencyclidine)同屬芳基環己胺類結構，它是 N-methyl-D-aspartate (NMDA) receptor的一種非競爭性拮抗劑。革蘭氏陰性菌感染巨噬細胞所引發之發炎反應，主要是藉由細菌細胞壁表面的脂多醣體 lipopolysaccharide (LPS)所引發，這些反應主要是由巨噬細胞及單核球等釋出水溶性媒介物而造成；而發炎性媒介物包括 tumor necrosis factor-alpha (TNF-alpha、interleukin-1 beta (IL-1 beta、nitric oxide (NO)等。

本實驗利用大鼠腦部的巨噬細胞－微神經膠細胞爲材料，觀察 lycopene 及 ketamine 對於經由革蘭氏陰性菌 LPS 所引發的發炎反應後，細胞中 TNF-alpha、IL-1 beta 以及 NO 表現的影響，並進一步探討藥物在其中的作用機轉。我們發現在 LPS 以濃度及時間相關方式刺激 microglial cell釋放 NO，以100 ng/ml,24 小時可達到最佳刺激程度。當給予 lycopene (5-20 microM)及 ketamine (100-500 microM)，均會明顯並有統計意義的減少 LPS 所誘發產生的 NO。此外，在觀察蛋白質表現方面，lycopene 及 ketamine 也會抑制 iNOS protein 的表現量。而實驗更進一步發現，lycopene 與 ketamine 均能抑制 LPS 所引發的 Ikapa B alpha 降解作用。而在 MAPK 路徑中，lycopene 會抑制 ERK 1/2 及 JNK 蛋白質磷酸化反應。Ketamine 同樣會抑制 ERK 1/2 的磷酸化反應，但對 JNK 似乎沒有明顯的抑制效果。另外，在其他發炎物質方面，藉由 Western blot免疫轉漬分析法，lycopene 與 ketamine 都能有效抑制 LPS 所誘發產生的 IL-1 beta 以及 TNF-alpha 蛋白表現。

綜合以上實驗結果，我們得知 lycopene 與 ketamine 均具有抑制 LPS 誘發腦部巨噬細胞 microglia 釋放發炎物質 NO、IL-1 beta 以及 TNF-alpha 的作用。但除了調控 NO 的機轉方面較爲了解之外，其餘發炎物質的作用路徑尚待釐清。因此未來可能藉由 lycopene 與 ketamine 抑制 NO 產生的作用來治療敗血症所引發的發炎性傷害，並進一步了解其他發炎物質 IL-1 beta 以及 TNF-alpha 的作用機轉。

英文摘要
Lycopene is not only a natural pigment, but also a very potent antioxidant which exists in tomato. It could help us to fight against several degeneration disease. But lycopene can not be produced in our bodies by itself, so, to get more lycopene can help the bodies against the disease induced by free radical. Ketamine, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, is a widely used anesthetic in human and animals. It has the same chemical structure with PCP (Phencyclidine), and all belong the cyclohexane. Lipopolysaccharide (LPS), the major structural component of the outer wall of Gram-negative bacteria, is a potent activator of macrophages. Activated macrophages and monocytes produce soluble mediators and some inflammatory cytokines, like tumor necrosis factor-alpha (TNF-alpha), interleukin-1 beta(IL-1 beta), nitric oxide (NO).
The present study we used micioglial cells, the macrophages in rat brain, to investigate the effects of lycopene and ketamine, on the induction of TNF-alpha, IL-1 beta, and Nitric Oxide by LPS. According to our study, in microglial cells culture, LPS (100 ng/ml, 24 hours) could dose- and time-dependently induce NO production. Lycopene (5-20 microM) and ketamine (100-500 microM) caused a significant and concentration-dependent inhibition on the production of NO upon stimulation by LPS. In addition, pretreated with lycopene and ketamine by stimulation of LPS- caused a concentration-dependent reduction in iNOS protein expression. Furthermore, we found that lycopene and ketamine both can inhibit I B degradation. In addition, lycopene and ketamine significantly inhibited ERK 1/2 phosphorylation. But in another pathway—JNK/ SAPK, lycopene could be inhibited and ketamine didn’t. And in other cytokines, IL-1 beta and TNF-alpha, lycopene and ketamine both had the inhibition of LPS stimulated .
Therefore, based on the above observations, we suggested that lycopene and ketamine diminished LPS-induced inflammation in rat microglial cells. Except the mechanism of NO release, other inflammation cytokines pathway is unclear. These results suggest a possible role of lycopene and ketamine in managing septic inflammation through inhibition of NO induction, and to get more mechanisms of other cytokines like IL-1 beta and TNF-alpha.