十字花科蔬菜衍生物對Lipopolysaccharide活化巨噬細胞所誘導之血管新生作用的影響

Effects of cruciferous vegetable derivatives in lipopolysaccharide activated macrophage induced angiogenesis

中文摘要

本研究主要探討十字花科蔬菜衍生物 benzyl isothiocyanate (BITC) 、phenylethyl isothiocyanate (PEITC) 與 indole-3-carbinol (I3C) 對於活化巨噬細胞所誘導之血管內皮細胞血管新生之影響。將細菌內毒素 lipopolysaccharide (LPS) 加入巨噬與血管內皮共同培養之細胞顯示，以 Greiss reagent 所測得培養液中一氧化氮之生成與以 matrigel 所分析之血管內皮細胞類血管生成皆有增加的現象，而 BITC、PEITC 與 I3C 的添加則可抑制 NO 與類血管的生成。爲探討此抑制之作用 是否因影響巨噬細胞之故，因而使用 LPS 與 BITC、PEITC 或 I3C 共同處理之巨噬細胞 condition medium (Co-BITC-CM, Co-PEITC-CM, Co-I3C-CM) 投予血管內皮細胞，結果指 Co-BITC-CM, Co-PEITC-CM 與 Co-I3C-CM 中含較低濃度之一氧化氮，且 Co-I3C-CM 可抑制血管內皮細胞之類血管生成的作用，且此抑制伴隨著較低血管內皮生長因子 vascular endothelial growth factor (VEGF)的釋放與較低基質金屬蛋白?MMP-9 (matrix metalloproteinase-9) 活性，此外投與 NO 抑制劑 L-NAME 亦有相類似之結果。然而 Co-BITC-CM 與 Co-PEITC-CM 雖可抑制血管內皮細胞類血管的生成與 MMP- 9 的活性，反而可以促進 VEGF 之釋放。此外 BITC、PEITC、I3C 與 L-NAME 對於活化之血管內皮細胞影響亦被分析，因而以 LPS 活化巨噬細胞之 condition medium (CM) 處理血管內皮細胞，並於 BITC、PEITC 與 I3C 之存在下偵測血管新生相關因子的影響。結果顯示，I3C 與 L-NAME 亦可抑制經 CM 誘導血管內皮細胞培養基內一氧化氮之生成、血管內皮細胞類血管生成、MMP- 9 之活性與 VEGF 之分泌，而 BITC 與 PEITC 亦可抑制經 CM 誘導血管內皮細胞培養基內一氧化氮之生成、血管內皮細胞之類血管生成與 MMP- 9 之活性，但亦增加血管內皮生長因子生成。總而言之，十字花科蔬菜衍生物 BITC、PEITC 與 I3C 可同時藉由抑制 LPS 所誘導巨噬細胞活化，以及影響血管內皮細胞的作用而具有抗血管新生之作用，且其中 I3C 之抑制作用主要藉由 NO 之抑制，而 BITC 與 PEITC 之抑制作用則與 NO 無關。

英文摘要

The aim of this study was to investigate the roles of cruciferous vegetable derivatives, BITC (benzyl isothiocyanate), PEITC (phenylethyl isothiocyanate), and indole-3-carbinol (I3C), in activated macrophage-induced angiogenesis. After co-culturing macrophages and vascular endothelial cells, we observed that the nitric

oxide (NO) production and tube formation were significantly enhanced by LPS (lipopolysaccharide), and cotreatment with BITC, PEITC, and I3C significantly inhibited such enhancement. To clarify the inhibitory roles of BITC, PEITC, and I3C on macrophages, LPS and BITC-, PEITC-, I3C-, or NO inhibitor- (Nitro-L-arginine methyl ester, L-NAME) treated macrophage condition medium was used to cultivate vascular endothelial cells. The tube formation, vascular endothelial growth factor (VEGF) secretion, and matrix metalloproteinase-9 (MMP-9) activity were suppressed by I3C and L-NAME, and tube formation and MMP-9 activity were suppressed but VEGF secretion was enhanced by BITC and PEITC in vascular endothelial cells. To understand whether BITC or PEITC or I3C or L-NAME also affected vascular endothelial cells, we used LPS-activated macrophages condition medium (CM) to treat to vascular endothelial cells. The results showed that addition of I3C and L-NAME inhibited CM-induced NO production, tube formation, VEGF secretion and, MMP-9 activity in vascular endothelial cells. BITC and PEITC inhibited CM-induced NO production, tube formation, and MMP-9 activity in vascular endothelial cells, but stimulated CM-induced VEGF secretion. In summary, we demonstrate that the cruciferous vegetable derivatives, BITC, PEITC, and I3C, not only inhibits LPS stimulated macrophage activation but also affects vascular endothelial cells to inhibit macrophage-induced angiogenesis. Additionally, the inhibitory effect of I3C is dependent upon NO production, whereas the effects of BITC and PEITC are irrelevant to NO.