氧化態低密度脂蛋白誘導腦血管內皮細胞 LOX-1 基因表現之訊息傳遞路徑探討

SIGNAL-TRANSDUCING MECHANISM OF OXLDL-INDUCED LOX-1 GENE EXPRESSION IN CEREBRAL ENDOTHELIAL CELLS

中文摘要

低密度脂蛋白(low density lipoprotein; LDL)容易被氧化形成氧化態低密度脂蛋白(oxidized low density lipoprotein; oxLDL)。oxLDL 會造成組織和細胞的損傷。Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1)是一種細胞膜蛋白，且爲內皮細胞最主要的 oxLDL 受體，負責將 oxLDL 胞飲(endocytosis)入細胞，並對細胞產生毒性。腦血管內皮細胞(cerebral endothelial cells; CECs)爲血腦障壁(blood brain barrier; BBB)主要組成之一。在 CECs 中，LOX-1 基因的表現量會被誘導，但是其中調控的機制卻還不清楚，因此本研究主要探討 oxLDL 誘導 CECs 中 LOX-1 基因表現之訊息傳遞路徑。

本實驗室先前的研究結果顯示，oxLDL 會導致 CECs 凋亡，因此我們首先探討 LOX-1 是否在此凋亡反應中扮演重要的角色。利用 LOX-1 siRNA 達到 knockdown 的效果，並以顯微鏡觀察細胞形態和利用流式細胞儀評估凋亡細胞所佔的比例，發現 LOX-1 siRNA 有效的抑制了 oxLDL 所導致的細胞凋亡反應，顯示了 LOX-1 可能參與調控此凋亡反應。接續以 RT-PCR 和 real-time PCR 分析腦血管內皮細胞中 LOX-1 mRNA，發現 oxLDL 的確會誘導 LOX-1 基因表現，並且有時間與濃度效應。

在訊息傳遞路徑探討上，於先前研究中發現 oxLDL 會造成 CECs 中活性氧分子(reactive oxygen species; ROS)上升，而 NF-κB 爲對氧化還原反應靈敏度高的訊息傳遞轉錄因子(redox sensitive transcription factor)，因此接續利用核質分離的方式分析氧化壓力相關的轉錄因子 NF-κB 之轉位(translocation)現象，發現 CECs 經 oxLDL 處理後，NF-κB 轉位入核的量有時間效應的增加，並在 3 小時達最大轉位量，進一步以 NF-κB 的抑制劑 Bay 11-7085 進行前處理後，oxLDL 誘導 LOX-1 mRNA 表現的情形會受到抑制，實驗結果顯示 oxLDL 的確會透過活化 NF-κB 誘導 LOX-1 基因表現。

接著依序往 NF-κB 訊息傳遞路徑上游探討，以免疫轉漬法測定 oxLDL 對 CECs 中 protein kinases 的磷酸化情形，結果顯示 CECs 經 oxLDL 處理後，IKK 磷酸化會在 30 分鐘時增加，ERK1/2 磷酸化的增加出現於 15 分鐘，而 MEK1/2 磷酸化增加出現在 5 分鐘，Raf 的磷酸化則在 1 分鐘後開始增加，以免疫沉澱的方式也可看到活化態 Ras 的增加。再進一步利用 LOX-1 siRNA 方式探討

LOX-1 受體在 oxLDL 誘導 LOX-1 基因表現之訊息傳遞路徑中所扮演的角色，實驗證實 LOX-1 siRNA 有效的抑制了 oxLDL 對 LOX-1 基因的誘導，同樣的 oxLDL 所引發的 NF-κB 轉位現象以及 Raf磷酸化也會受到抑制。
研究結果顯示，oxLDL 的確會透過 LOX-1 受體導致 CECs 產生細胞凋亡反應，並經由 LOX-1 受體活化細胞內 Ras/Raf/MEK/ERK 的訊息傳遞路徑，進而磷酸化蛋白激?IKK 導致轉錄因子 NF-κB 的轉位入核的量增加，使得 LOX-1 基因表現量的增加。

英文摘要

Low density lipoprotein (LDL) can be oxidized to oxidized LDL (oxLDL). OxLDL can damage a variety of tissues and cells. Lectin-like oxLDL receptor-1 (LOX-1) is a membrane protein specifically mediating endocytosis of oxLDL and its toxicity to cells. Cerebral endothelial cells (CECs) are important components in blood brain barrier (BBB). LOX-1 is inducible in CECs, but its regulatory mechanism is still unknown. This study is aimed to evaluate the signal-tranducing mechanism of oxLDL-induced LOX-1 gene expression using mouse CECs as the experimental model.
Previous studies in our lab revealed that oxLDL could cause apoptosis in CECs. Thus, one of the specific aims in this study was to determine if LOX-1 receptor had a critical role in oxLDL-induced apoptosis of CECs. Administration of CECs with oxLDL increased LOX-1 mRNA production in concentration- and time-dependent manners. In the LOX-1 siRNA experiment, we detect the distribution of apoptotic cells using microscope and flow cytometry. The data show that LOX-1 siRNA effectively inhibited oxLDL-induced apoptosis.
Thus, the other aim of this study was to evaluate the signal-tranducing mechanism of oxLDL on LOX-1 gene expression. Previous studies in our lab revealed that oxLDL could increase reactive oxygen species (ROS) in CECs. NF-κB is a redox sensitive transcription factor. In parallel with LOX-1 mRNA induction, oxLDL activated transcription factor NF-κB and promoted its translocation from cytoplasm into nuclei. Administration of Bay 11-7085, an NF-κB inhibitor, then consequently inhibited LOX-1 mRNA production.
NF-κB activated by upstream protein kinases. IKK, an upstream kinase for NF-κB activation, was activated after exposure to oxLDL for 30 min. ERK1/2, an upstream kinase for IKK activation, was activated after exposure to oxLDL for 15 min. When exposed to oxLDL for 5 min, MEK1/2, a kinase for ERK1/2 activation, was activated. Besides , Raf, a kinase for MEK 1/2 activation, was activated by oxLDL. Ras, a kinase for Raf, was also activated by oxLDL. We also observed that oxLDL-induced LOX-1 gene expression, NF-κB translocation and Raf phosphorylation were

inhibited in the LOX-1 siRNA experiment.

This study has shown that LOX-1 mediated oxLDL-induced apoptosis of CECs, and oxLDL induced LOX-1 gene expression via a Ras/Raf/MEK/ERK-dependent activation of NF-κB.