行政院國家科學委員會專題研究計畫　成果報告

探討不同抗巨噬單核球細胞之藥物對基質金屬蛋白酵素活化之抑制機轉與比較其對革蘭氏陽性與陰性菌所造成多重器官衰竭之保護作用

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探討不同抗巨噬單核球細胞之藥物對基質金屬蛋白酵素活化之抑制機轉與其對革蘭氏陽性與陰性菌所造成多重器官衰竭之保護作用

Investigate different anti-monocyte/macrophage agents on matrix metalloproteinase activation and comparison with protective effects on LTA- and LPS-induced multiple organ failure

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一、中文摘要

Matrix metalloproteinases (MMPs)為一群結構類似且含鋅(zinc)金屬離子之蛋白酵素。因 MMPs 能夠催化分解維持組織結構之細胞外基質蛋白(基質與結締纖維組織)，故稱之為基質金屬蛋白酵素，而其對於組織之結構重組、修補與破壞都扮演相當重要之角色。在臨床上，用來治療精神疾病方面的藥物 haloperidol 發現具有抗發炎之效果。所以假設藥物 haloperidol 能抑制發炎時所誘發的不正常組織重組(remodeling)。因此本研究使用可以誘發 MMP-9 大量表現之細菌類毒素(lipopolysaccharide，LPS)作為 THP-1 細胞之刺激劑。由電泳酵素分析法(gelatin zymography)下，我們觀察到由 LPS 刺激 MMP-9 酵素之活化及表現量會隨著 haloperidol 濃度增加皆可有效地被抑制。接著利用細胞存活率測定(MTT assay)可發現 haloperidol 的抑制作用並非完全源自細胞之損壞或死亡。以西方點墨法(Western blot)之實驗方法，發現 haloperidol 濃度增加皆可有效地抑制由 LPS 刺激 MMP-9 蛋白之表現量。

藉由 Western blot 之實驗發現 haloperidol 能顯著地抑制由 TNF-α或 LPS 刺激所導致的 total inhibitor-κBα (IκBα)之降解作用。接著利用電泳移動偏向分析法(electromobility shift assay，EMSA)，分析細胞核內外 NF-κB (p65)之轉位與活化情形，得知 haloperidol 濃度為 10 μM 時，可有意義地抑制 LPS 所誘發的 NF-κB 之轉位與活化。

綜合目前之實驗結果，發現 haloperidol 確實能選擇性地抑制人類單核球細胞(THP-1 cells)中，LPS 所誘發的 MMP-9 活性與表現，而此抑制之機轉可能主要是影響 NF-κB 之訊息傳遞路徑。期許未來能更加瞭解在活體實驗中對於抗發炎反應的功能與療效。綜合目前實驗的結果，發現 haloperidol 的確具有抑制 MMP-9 表現之活性，而在 LPS 刺激方面其作用機轉可能主要藉由影響 NF-κB 的訊號傳遞過程。另一方面，革蘭氏陰性與陽性細菌所造成之肝腎傷害有差異性，但 MMP-9 之作用相似。在未來將會進行更多相關之實驗及其他活體實驗以瞭解各藥物是否在敗血症下具有抗發炎療效之功能。

關鍵詞: 基質金屬酵素、細胞訊息、內毒素、敗血性休克

Abstract

Matrix Metalloproteinases (MMPs) are a family could catalyze and degrade tissue structure maintaining extracellular matrix protein (ECM), including ground substances and connecting fibers, they are named matrix metalloproteinase. Thus, it plays an important role in tissue structure remodeling, repairing and destroys. According to previous experiments, we found that haloperidol showed obviously inhibitory effect on MMPs activation. We observed that haloperidol significantly and concentration-dependently inhibit MMP-9 activation induced by LPS by zymographic method. Also, we found that the inhibitory

effect of haloperidol was not due to impairment of cellular viability by MTT tests. According to Western blot method, we found that various stimulator-induced expression of MMP-9 protein is concentration-dependent inhibition by haloperidol. At the same time, we investigated the mechanism of action of haloperidol in various signaling pathways. We found that haloperidol could significantly inhibit the degradation of inhibitor-κB-α (IκB-α) induced by LPS. Therefore, nuclear factor-κB (NF-κB) may not translocate for transcription. In summary, we found that haloperidol have inhibitory effect on MMP-9 expression, and its main mechanism of action might through NF-κB signal pathway on LPS stimulation. According to the septic injury *in vivo* studies, we found the functions of liver and kidney are graduately decay by LPS or LTA. It will be interesting to further investigate its anti-inflammatory therapeutic and septic profile *in vivo*.

Keywords: Matrix metalloproteinases, haloperidol, endotoxin, septic shock

二、緣由與目的

多重器官衰竭徵候群 (multiple organ failure; MOF) 通常發生在感染症病人，而導因於體內敗血性造成的嚴重發炎反應。在臨床上不管如何強而有力的改善，敗血性休克仍然成為病人死亡的主要因素(Bone., 1994)。雖然引起敗血性休克的病理因素非常複雜，但目前已知宿主的發炎反應乃是造成休克及多重器官衰竭的主要原因 (Cohen, 2002)。在造成休克的過程中, MOF 的病人死亡率會隨病情的惡化增高至 90 %以上(Deitsch., 1997; Baue., 1993), 而崩解性酵素不正常活化扮演相當重要的角色(Nakmura et al., 1998)。

Matrix metalloproteinases 簡稱 MMPs 為體內重要崩解性酵素，且為一群結構類似且含鋅(zinc ion)金屬離子之蛋白酵素(Kotra et al., 2001)。MMP 的催化作用需金屬離子加以活化，並有催化水解細胞外基質蛋白(extracellular matrix proteins)的能力。Gelatinases A 與 B (MMP-2 與 MMP-9)的催化區域中含有三個 head-to-tail cystein-rich repeats，而特別被區別出來。這些 inserts 類似 fibronection 的 collagen-binding type II repeats，需要與 collagen 和 elastin 結合與切除(cleavage) (Shipley 1996)。除此之外, MMP-9 (92 kDa gelatinase B)為 MMPs sequence 中最常且複雜者，在 hinge region 的底部含有獨特的 type V collagen-like inserts，而此 insert 的重要性與功能仍然未知。Membrane-type 的 MT-MMPs 有 single-pass transmembrane domain 與短的 cytoplasmic C-terminal tail (如 MMPs 14、15、16 與 24) 或是 C-terminal hydrophobic 的區域 (Kojima, 2000)，以 glycophosphatidyl inositol (GPI) membrane-anchoring signal 表現(如 MMP-17 和 MMP-25)。MMPs 最初被定義為一群酵素可被金屬螯合劑與內生性抑制劑所抑制，而為活化型可受有機汞所活化；其所能催化水解的細胞外基質蛋白至少一種以上, 例如 collagen、elastin、laminin 等。因此其對於組織 remodeling、repairing 與 destroying，皆扮演了重要的角色(Woessner et al., 1991)。MMPs 在生理上的抑制劑為內生性的 MMP 組織抑制劑 TIMPs (tissue inhibitors of metalloprotinases)，TIMPs 屬於低分子量(20~29kDa) 的蛋白質, 能夠專一性的抑制活化型態的 MMPs (Backer, 2002)。目前已有四種 TIMPs 被發現，不同的 TIMPs 對於抑制不同的 MMPs 的能力也有差別。每種 TIMP 對於 MMP 的作用親和力也不同(Brew, 2000)，如 TIMP-1 為 MMP-9 的主要抑制劑。TIMPs 對於 MMPs 活性的抑制是為一種相當重要的嚴密調控機制。

脂多醣體 Lipopolysaccharide (LPS)或稱為細菌性內毒素(endotoxin)會誘發人類單核球細胞去表現許多 pro-inflammatory mediators (Guha, 2001; Netea, 2002), 其中包括了 pro-coagulant 分子 tissue factor (TF) 與細胞激素腫瘤壞死因子 TNF-α。LPS 是最早被發現的內毒素, 位於格蘭氏陰性菌 (gram-negative) 的細胞壁上，當細菌溶解時，LPS 會被裂解

釋出 (Raetz, 1986)。對於格蘭氏陰性菌 (gram-negative bacteria) 的感染，LPS 可當作是一種發炎反應的主要源頭，而 toll-like receptor 4 (簡稱 TLR4) 則在此現象中有其不可缺少地位(Kiechl, 2002)。人類的單核球細胞對於 LPS 的刺激尤其特別敏感，並反應產生許多發炎性細胞激素。LPS 會與 LPS-binding protein（簡稱 LBP）在 plasma 中結合，並傳送到細胞表面的 CD14 receptor。接下來 LPS 便會被轉換至 transmembrane 的 signaling toll-like receptor 4 與 LTR4 的附加蛋白 MD2 上 (Guha & Mackman, 2001)。LPS 對於單核球與巨噬細胞是一種強有力的激活劑 (O'Connell, 1998)，對於 host 細胞有保護性與傷害性的反應。藉由研究在單核球細胞中 LPS 的分子機制與發炎性基因的調控，也許能夠在許多系統性的發炎反應症狀的治療上，找到新的方法(Guha & Mackman, 2001)。藉由研究在單核球細胞中 LPS 的分子機制與發炎性基因的調控，也許能夠在許多系統性的發炎反應症狀的治療上，找到新的方法(Guha & Mackman, 2001)。在各種發炎疾病中，MMPs 於疾病過程中扮演了相當重要的角色。而 chemokine (MCP-1)、細胞激素與細菌性內毒素 (LPS) 均會刺激或誘導 MMPs 的產生，進而造成分解結締組織，並造成各種不同生理與病理的傷害。根據臨床文獻指出在敗血性休克 (septic shock)病程中，可引起廣泛發炎反應與介質的產生，造成組織損傷，嚴重更導致多重器官衰竭 (MOF)，而造成死亡。革蘭式陰性菌內毒素所導致的氧化迫傷與白血球活化是引發全身性發炎反應（systemic inflammatory response）的重要原因。在活體動物實驗中發現以藥物抑制 MMP-9 之表現及 MMP-9 基因剔除之小鼠對於內毒素(LPS)所造成的敗血性休克等傷害具有保護抵抗能力，也間接暗示 MMP-9 的抑制效果可治療敗血性休克所造成的傷害(Dubois et al., 2002; Kishnani et al., 1999)。同時在臨床實驗發現微量 LPS 引發人類循環系統中 MMP-9 的產生與釋出其較 NO 明顯與快速(Albert et al,. 2003)。且以 Polymyxin B immobilized on fibers 之成分治療下的確可減低 MMP-9 在病人的含量，而改善敗血性休克之症狀(Nakamura et al., 1998)。然而有關活體 LTA 引發 MMP 之病理角色目前仍不清楚。因 LPS 可刺激單核球並釋放 TNF-α，間接誘發 MMP-9 之表現，在本實驗中，我們對觀察了 LPS 對 THP-1 細胞的刺激與作用。LTA 的來源可以從一些革蘭氏陽性菌，如 Staphylococcus aureus, Streptococcus pyrogenes A, Enterococcus faecalis, reptococcus pneumoniae 和 Listeria monocytogens 的細胞壁上萃取得到。由 Staphylococcus aureus 分離而來的 LTA，在 murine macrophages 細胞中，會藉由 iNOS 的表現而增加 NO 的產生 (Cunha. et al., 1993)。在 vascular smooth muscle 培養細胞中亦是 (Lonchampt. et al., 1992)。在大鼠肺臟中，LTA 也會造成 iNOS 蛋白及活性的增加，使得循環失調 (circulatory failure)。根據許多文獻得知，在齧齒類動物模式的敗血性休克中，因 LTA 所造成 iNOS 過度表現而產生過量的 NO，而造成 circulatory failure 與 MOF (De Kimpe. et al., 1995)。由 Staphylococcus aureus 分離而來的 LTA，對於巨噬細胞與血管平滑肌細胞，會造成誘導型一氧化氮合成酵素的產生。另外，在 J 774.2 巨噬細胞培養中，以 LTA 處理後會造成 iNOS 表現。它的訊息傳導路徑包含了 tyrosine kinase 與 nuclear transcription factor NF-κB 的活化。然而，由 LTA 所導致的 MMP 與 iNOS 表現的訊息傳遞路徑仍然不清楚。

2-2 實驗目的

在本實驗中，以人類單核球細胞 THP-1 為實驗細胞，藉以了解 haloperidol 等藥物對 LPS 所引出或誘發之 MMP-9 蛋白質活性的表現與影響。我們以 Zymography 、Western Blot 等實驗試著了解與觀察 MMP-9 蛋白質的表現，並以 MTT Assay 觀察細胞存活率的情形。而之後我們探討了之中的藥理作用和機轉等問題，試著進一步的瞭解其在細胞訊息傳遞路徑如 NF-κB 等機制的影響程度。另外對與 MMP 有關的感染性成分(LPS 與 LTA)反應如多重器官衰竭徵候群傷害，能進一

步的評估與瞭解其治療的可能性。

三、結果與討論

3-1 結果

3-1-1.探討 haloperidol 對人類單核球細胞(THP-1)以 LPS 所誘發的 MMP-9 酵素活性

由之前已建立之刺激物質誘發 MMP-9 活性的表現條件的 gelatine zymography 之方法，投與不同濃度之藥物以觀察藥物對 LPS 所誘發的 MMP-9 在 THP-1 細胞中的影響。本實驗首先是要探討以不同濃度的 haloperidol (0.5~20 μM) 來觀察 THP-1 細胞對於利用 LPS 刺激而產生 MMP-9 活性之影響程度。由電泳酵素分析法之實驗結果發現，隨著 haloperidol 濃度的增加(0.5 μM、2 μM、10 μM、20 μM)，MMP-9 的活性表現呈現逐漸減少且有意義地被抑制之現象(Figure 1 A)。其中 haloperidol 對 LPS 刺激作用的抑制百分率(Inhibition %)分別為 38.5 ± 0.9 % (0.5 μM)；47.5 ± 4.2 % (2 μM)；76.6 ± 1.4 % (10 μM)；91.6 ± 3.1 % (20 μM)，其抑制 50 % 反應濃度(IC_{50})為 3.1 ± 0.8 μM (n = 3，Figure 1 B)。由此結果可知，在 THP-1 細胞中經由 LPS 所誘發之 MMP-9 皆能被 haloperidol 隨著濃度的上升而呈現活性減少之反應機制，並且呈現濃度抑制效應(concentration-dependent inhibition)。

3-1-2.探討藥物對細胞存活率的影響

由實驗結果發現不加藥及刺激劑時，resting 的吸光值為 0.94 ± 0.04 nm，當處理不同濃度的 haloperidol 對於細胞的數量發現有稍微的影響(10 μM，0.89 ± 0.05 nm 及 20 μM，0.73 ± 0.03 nm)，但整體而言 haloperidol 濃度 20 μM 以下(data not shown)並不會明顯影響細胞的存活(n = 3~8，Figure 2)，隨著 haloperidol 濃度增加，其導致細胞死亡的程度越明顯(40 μM，0.60 ± 0.02 nm；50 μM，0.55 ± 0.03 nm 及 100 μM，0.10 ± 0.01 nm)。

3-1-3.探討 Haloperidol 對 MMP-9 酵素本生活性之影響

由之前的實驗結果得知 haloperidol (0.5~20 μM) 會呈現藥物濃度的抑制 MMP-9 活性的表現，因此，我們再利用 zymography 方法來瞭解 haloperidol 是否會直接影響 MMP-9 本身的酵素活性，由實驗結果得知(data not shown)，初步看起來 haloperidol 對於 TNF-α (lane 2：4.45 ± 0.15 fold)刺激後所誘發的 MMP-9 酵素活性並沒有影響(lane 4，0.5 μM：4.85 ± 0.28 fold；lane 5，2 μM：4.93 ± 0.40 fold；lane 6，10 μM：4.99 ± 0.56 fold；lane 7，20 μM：5.09 ± 0.39 fold)。

3-1-4.探討 Haloperidol 對 THP-1 細胞以 LPS 所誘發的 MMP-9 蛋白質表現之作用

在 Figure 3 中，將 THP-1 細胞處理並培養 24 小時後，取其細胞之萃取物(cell lysate)進行實驗，我們發現在未加藥及刺激劑的情形下(resting，land 1)，其細胞萃取物只偵測到非常微量的 92 kD 之 MMP-9 蛋白表現量。而以 LPS 刺激 24 小時後，發現其細胞萃取物則含有大量的 MMP-9 蛋白質表現(Figure 3，land 2：LPS，2.81 ± 0.22 fold)，當給予不同濃度的 haloperidol (2 μM~20 μM)處理後，可發現到 LPS 所誘發的 MMP-9 蛋白質表現量會隨著藥物(haloperidol)濃度的增加而呈現有意義地逐漸減少之情形(land 3：2 μM，1.41 ± 0.13 fold；land 4：10 μM，1.04 ± 0.13 fold；land 5：20 μM，0.54 ± 0.10 fold)。

3-1-5.探討 Haloperidol 對 THP-1 細胞以 LPS 誘發 IκB-α之降解作用

由 Figure 4 的結果發現，以培養 120 min 的 Resting 當 1，接著以 LPS (50 ng/ml)刺激 30 分鐘(land 2：1.062 fold)、60 分鐘(land 3：0.918 fold)、90 分鐘(land 4：0.769 fold)及 120 分鐘(land 5：1.302 fold)後，則以 LPS 刺激 90 分鐘時，其 total IkB-α的降解作用最為明顯。因此根據這些結果，將細胞以 LPS 刺激 90 分鐘來進行之後的實驗。

在 Figure 5 中，當未投予 LPS 時，因無訊息之傳遞，total IκB-α則無法進行降解作用，故 IκB-α蛋白的表現為最高(lane 1：1 ± 0.0)，以 LPS 刺激 90 分鐘(land 2：0.68 ± 0.04 fold)，投予不同濃度之 haloperidol，

可發現 IκB-α含量隨著 haloperidol 濃度的增高(land 3：2 μM，0.80 ± 0.01 fold；land 4：10 μM，0.78 ± 0.02 fold；land 5：20 μM，0.82 ± 0.01 fold)而逐漸上升，根據以上所觀察到之結果，可以推論 haloperidol 可能會經由抑制 IκB-α蛋白質的降解作用而更進一步的減少 NF-κB translocate 至細胞核內部的作用，造成 LPS 所誘導出來的 MMP-9 因此被抑制而無法表現。

3-1-6.探討 Haloperidol 對 THP-1 細胞以 LPS 誘發 p65 之影響

由 Figure 6 中可以觀察到 haloperidol (lane 3, 4: 0.5, 10 μM) 能抑制在人類單核球細胞 (THP-1) 核內，LPS 所誘發的 NF-κB translocation 表現 (lane 2, Vehicle)。

3-1-7.探討實驗動物在 LPS 或 LTA 處理後之多重器官衰竭與 MMP 活化之研究

由血壓之變化(Figure 7)、肝臟功能(Figure 8)與腎臟功能(Figur 9)評估顯示大鼠在以LPS或LTA所造成之敗血性傷害為多重器官衰竭。LPS 較 LTA 對腎臟之感受傷害性強且在 MMP-9 有較強之活化作用(Figur 10)。Haloperidol 雖對 MMP 有所影響，但對敗血性多重器官衰竭之保護作用，仍須進一步探討。

3-2 討論

MMP-9 (屬於type IV collagenase，又稱為gelatin B，分子量為92 kD)可在許多種類的單核球細胞中被發現，包括：周邊血液的單核球(peripheral blood monocytes)、組織中的巨噬細胞(microphages)、Kupffer cells與蝕骨細胞(osteoclasts)等皆可發現MMP-9的表現(Welgus et al., 1990; Masure et al., 1993; Winwood et al., 1995; Swallow et al., 1996)。另外在一些leukemic cell lines中，包括：HL-60、NB4、U-937及THP-1也可釋放MMP-9酵素(Ries et al., 1994; Ismair et al., 1998; Saarialho Kere et al., 1993; Van et al., 1991)。在一般正常生理中，成熟白血球本身就可以分泌MMP-9，利用MMP-9分解基質的能力，使白血球能從血液離開而滲入周邊組織發炎處來發揮它們的免疫功能(Doherty et al., 1994; Welgus et al., 1990; Leppert et al., 1995; Weiss et al., 1986)。另外，單核球細胞若受到一些細胞激素(如：TNF-α、IL-1β、CSFs或IL-3)或細菌內毒素(LPS)刺激時，便會使MMPs表現。有文獻指出，單核球(monocytes)若是受到細胞激素TNF-α或IL-1β的刺激，便會誘發MMP-9的表現而不是MMP-1 (Saren et al., 1996)。在單核球的細胞株裡，MMP-9的分泌與基因轉錄的程度有關且可被一些細胞激素(如：TNF-α透過 $TNFR_1$) 來促進調節製造(Ries and Petrides, 1995; Ismair et al., 1998)。另外，LPS則會誘發顯著的MMP-1與MMP-9表現(Lai et al., 2003)及許多有關發炎的細胞激素(cytokines) (Yong et al., 1998)。而在一些神經退化性疾病中(如：阿茲海默症與帕金森氏症)發現有些細胞激素的表現比較活躍，其中包括：TNF-α、IL-1及TGF-β (Akiyama et al., 2000; Nagatsu et al., 2000)，而其中，TNF-α被認為是誘發發炎的強效細胞激素(Munoz-Fernandez et al., 1998)。

腦膜炎(meningitis)是中樞神經系統內的腦膜發炎。其中以細菌性腦膜炎症狀較為嚴重，而最常引起腦膜炎之菌種為腦膜炎雙球菌(*Neisseria meningitides*)與感冒嗜血桿菌(*Haemophilus influenza*)，它們的細胞壁上含有一種可以引起嚴重發炎反應的物質稱為細菌性內毒素(lipopolysaccharide，LPS)。此為革蘭氏陰性菌細胞膜外的主成分，為單核球細胞中效果最強的刺激劑之一(Rietschel and Brade, 1992)。然而LPS過份表現會導致敗血、敗血性休克或全身系統發炎反應症狀。有許多文獻指出，MMPs (尤其是MMP-9)於細菌性腦膜炎中參與著生理與病理過程，利用可以分解基質的能力進而破壞血腦障壁，使其通透性變大引起更多細菌入侵(Rosenberg et al., 1995)。所以我們的實驗中，使用白血球中的單核細胞THP-1 cells觀察若接觸到外在細菌性內毒素LPS之刺激，探討THP-1細胞所產生的MMP-9蛋白表現與有給予藥物haloperidol之下，藥物對於MMP-9的蛋白表現所產生的抑制作用。由zymography實驗方法可觀察到，haloperidol能抑制人類單核球細胞

(THP-1)中，LPS所誘發的MMP-9酵素活性表現且呈現concentration-dependent的抑制作用。在LPS (50 ng/ml)的刺激之下，haloperidol對於MMP-9酵素抑制50%的反應濃度(IC_{50})為3.1 ± 0.8 μM。接著利用Western blot分析haloperidol能抑制LPS在人類單核球細胞(THP-1)中所誘發的MMP-9蛋白表現量且呈現顯著的concentration-dependent抑制作用。

Nuclear factor-κB (NF-κB)這條訊息傳遞路徑控制了許多誘發性的發炎基因與MMPs 的表現，且文獻中提到 LPS 在單核球細胞中，可藉由活化 NF-κB/Rel 轉錄因子而產生許多種類之基因表現，包括：TNF-α與 IL-1 等(Sweet and Hume, 1996)也可活化許多第二傳遞因子與訊息傳遞路徑(Sweet and Hume, 1996; Weinstein et al., 1992; Han et al., 1994; Hambleton et al., 1996)。我們利用 Western blot 實驗方法觀察 NF-κB 之抑制性因子 IκB-α蛋白表現量，藉以瞭解藥物 haloperidol 對於 IκB-α磷酸化的分解作用與在 NF-κB 訊息傳遞路徑所扮演的角色。發現 LPS 刺激 90 分鐘時所取得的細胞 lysate，IκB-α蛋白表現呈現明顯的降解作用。接下來以 90 分鐘為 LPS 刺激之基準，分別投與不同濃度的 haloperidol 之後，可觀察到 haloperidol 能抑制 LPS 在 THP-1 細胞中所引發的 IκB-α降解作用，使得 NF-κB 無法 translocate 至細胞核中活化 MMP-9 基因表現，造成 MMP-9 的酵素與蛋白表現量減少。有文獻所做的實驗結果顯示，利用 LPS 刺激人類單核球細胞與細胞株(THP-1 cells) (O'Connell et al., 1998; Guha and Mackman, 2001)，使得轉錄因子 NF-κB translocate 至核內，可能藉由活化 IKKβ蛋白而導致的結果(O'Connell et al., 1998)。可提供本實驗更進一步探討。而我們的實驗當中發現，在 THP-1 細胞中利用 TNF-α與 LPS 刺激時所誘發的 IκB-α蛋白降解之時間有明顯的差異(TNF-α：15 分鐘；LPS：90 分鐘)，可能原因為此兩種刺激劑活化 IKKs 時，牽涉到不同之路徑所導致的(O'Connell et al., 1998; Hawiger et al., 1999; Fischer et al., 1999)。而另一篇文獻則顯示 LPS 刺激人類單核球細胞可經由許多訊息傳遞路徑，其中包括 IKK-NF-κB 路徑與 MAPKs 路徑(ERK1/2; JNK; p38)且牽涉到轉錄因子 NF-κB (p50/p65)與 AP-1 (c-Fos/c-Jun)。Lai 等學者則提出在人類周邊血液分離而得的單核球細胞，利用 LPS 刺激所誘發的 MMP-9 蛋白或 mRNA 表現主要是受 MAPKs 其中之 ERK1/2 路徑所調控的(Lai et al., 2003)。另外最新文獻則提到，也是利用 LPS 刺激人類周邊血液之單核球細胞，發現可經由 phosphatidylinositol-3/Akt/IKKα/NF-κB 路徑誘發 MMP-9 產生(Lu and Wahl, 2005)。而本實驗結果顯示 haloperidol 可明顯地抑制由 LPS 所誘發的 MMP-9 酵素與蛋白的表現，但卻無法明顯地抑制由 LPS 所誘發的 IκB-α降解作用(無法顯著地回到基準點)，顯示藥物 haloperidol 可能對於 LPS 所誘發的 MMP-9 蛋白表現並非單純壓制 NF-κB 這條路徑，針對此點需再做更進一步之探討。

3-3 結論

綜合我們實驗的結果，證實 Haloperidol 皆能夠抑制人類單核球細胞 THP-1 受到 LPS，並與細胞毒性無關。此成分能抑制在 THP-1 中 LPS 所引發的 MMP-9 表現，並主要經由抑制 NF-κB pathway 訊息傳遞路徑。另外釐清有關不同的感染性成分 LPS 與 LTA 引發之多重器官衰竭徵候群傷害與 MMP 活化，能進一步的評估與瞭解其多元化治療的可能性。

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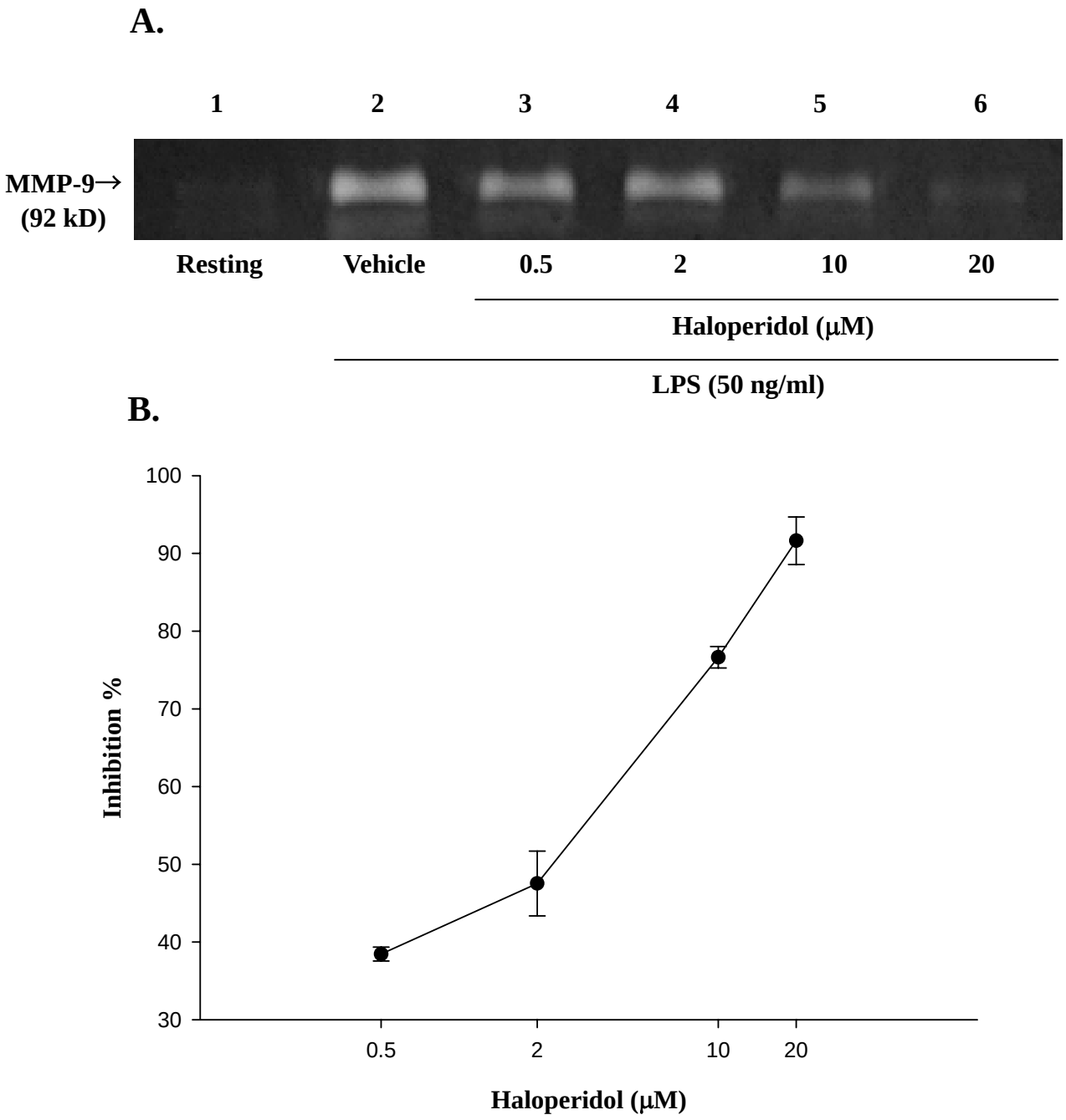


Figure 1. Effects of haloperidol on LPS̃induced enzymatic activity of matrix metalloproteinase-9 (MMP-9) in THP-1 cells. THP-1 cells (1×10^6 cells/ml) were dispensed on 24-well plates and treated with LPS (50 ng/ml) for 24 hours as indicated. Cells were treated with the indicated concentrations of haloperidol (lane 3, 0.5 μM; land 4, 2 μM; lane 5, 10 μM; land 6, 20 μM) or vehicle (land 2) for 15 minutes before treatment with LPS̃Cell-free supernatants were then assayed for MMP-9 activity by gelatin zymography, as detailed in "Methods" (land 1, control). Percent inhibition is presented as mean ± S.E.M. of three independent experiments.

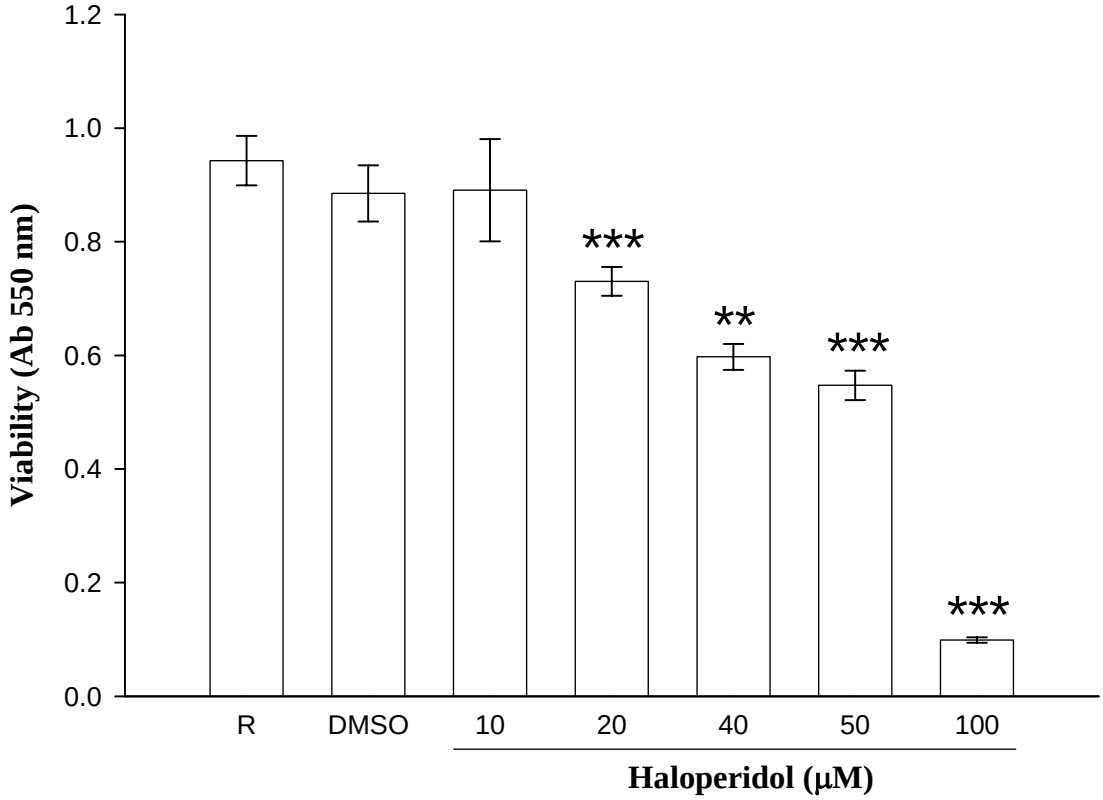


Figure 2. Cytotoxicity of haloperidol on THP-1 cells. THP-1 cells were treated with different concentration of haloperidol (10-100 μM) and incubated for 24 hrs. Cell viability was measured by a colorimetric assay at 550 nm based on the ability of mitochondria to reduced the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) in viable cells. Percentage of viability is presented as mean ± S.E.M. of three to eight independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ as compared with the resting.

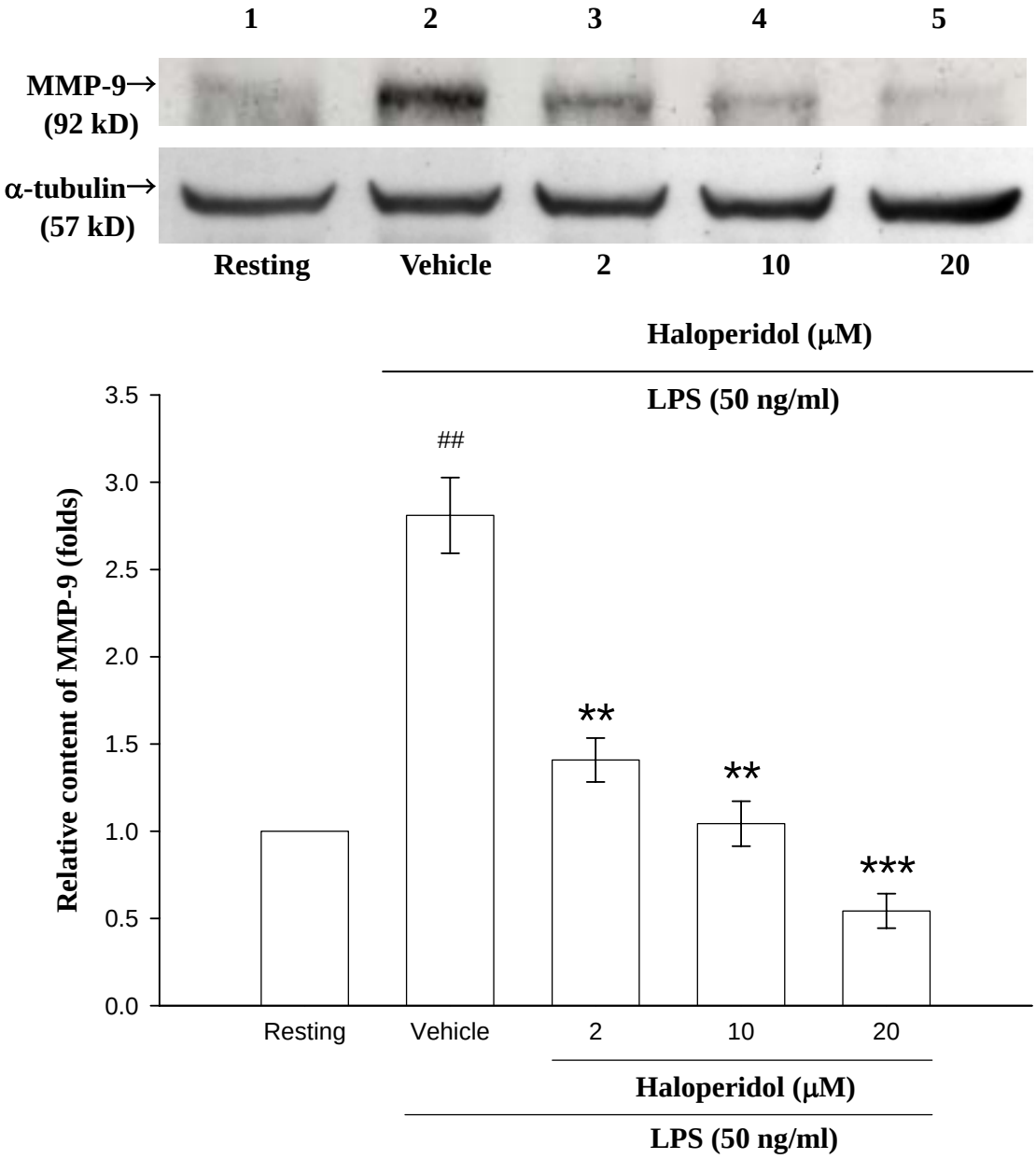


Figure 3. Effect of haloperidol on LPS-induced expression of matrix metalloproteinase-9 (MMP-9) from conditioned medium of THP-1 cells. THP-1 cells (1×10^6 cells/ml) were dispensed on 6-well plates treated with LPS (50 ng/ml) for 24 hrs as indicated. Cells were treated with indicated concentration of haloperidol (lane 3, 2 μM lane 4, 10 μM; lane 5, 20 μM) or vehicle (lane 2) for 15 min before treatment with LPS. Then the cell lysates were obtained and analyzed for MMP-9 protein expression by Western blot (lane 1, control). The data are representative example of five experiments. ### P<0.001 as compared with the resting; * P < 0.05; ** P < 0.01; *** P < 0.001 as compared with the vehicle.

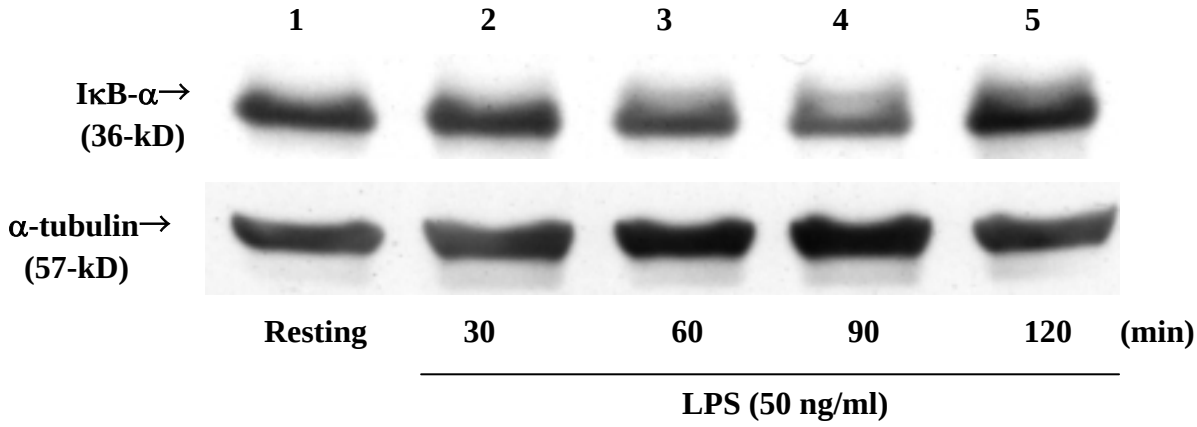


Figure 4. Western blot analysis demonstrating the time course on LPS-induced degradation of immunoreactive IκB-α in THP-1 cells (1×10^6 cells/ml). THP-1 cells were dispensed on 6-well plate and treated with LPS (lane 2, 30 min; lane 3, 60 min; lane 4, 90 min; land 5, 120 min) or control (lane 1, 120 min) as indicated.

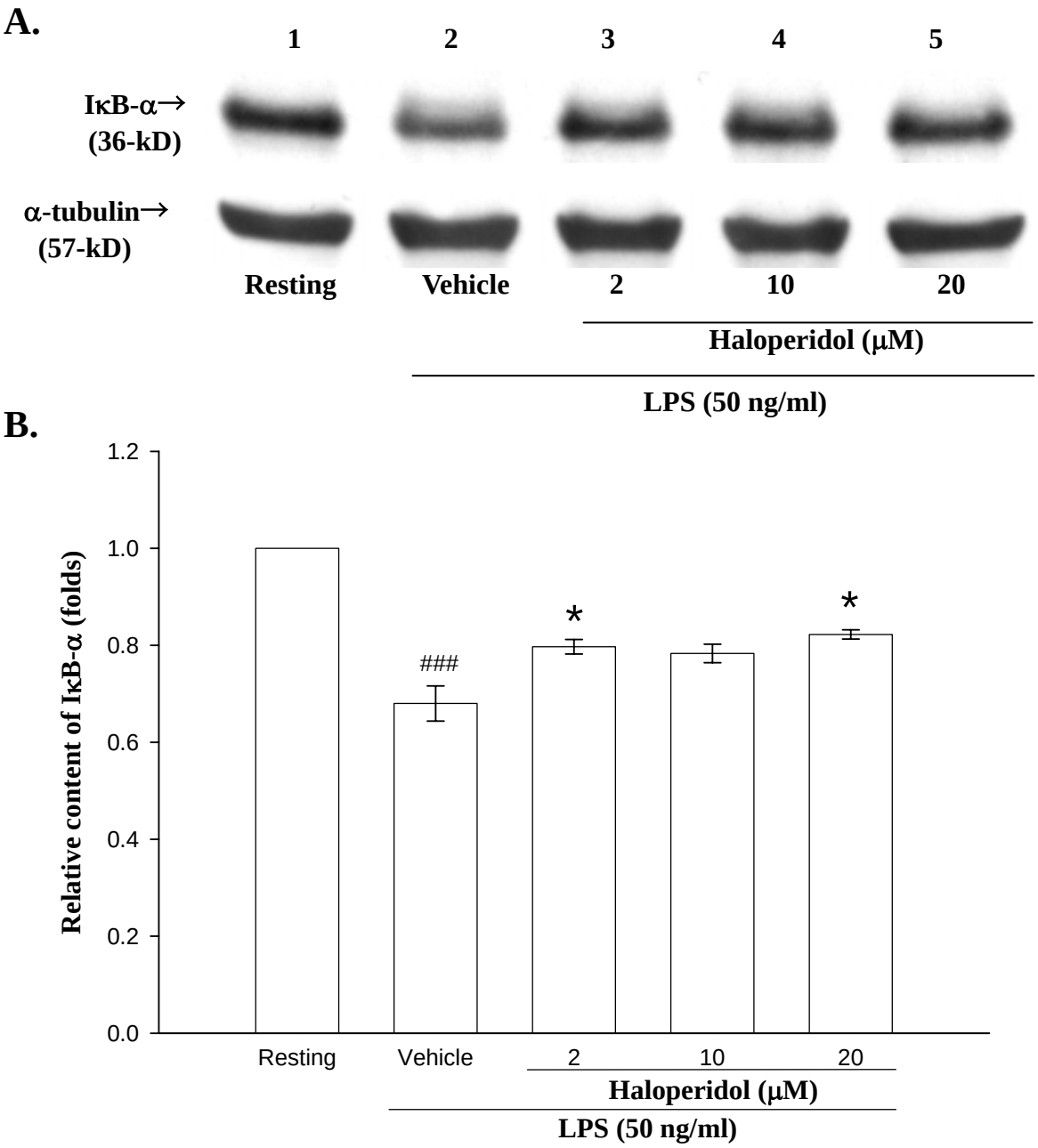


Figure 5. Effect of haloperidol on degradation of immunoreactive IκB-α in THP-1 cells. THP-1 cells (1×10^6 cells/ml) were dispensed on 6-well plate and treated with LPS (50 ng/ml) for 90 min as indicated. Cells were treated with haloperidol (lane 3, 2 μM; lane 4, 10 μM; lane 5, 20 μM) or vehicle (lane 2) for 15 min before treatment with LPS. Then cells were obtained and analyzed for IκB-α protein expression by Western blot (lane 1, control). The data are representative example of three to three experiments. $^{###}$ $P < 0.001$ as compared with the resting; * $P < 0.05$ as compared with the vehicle.

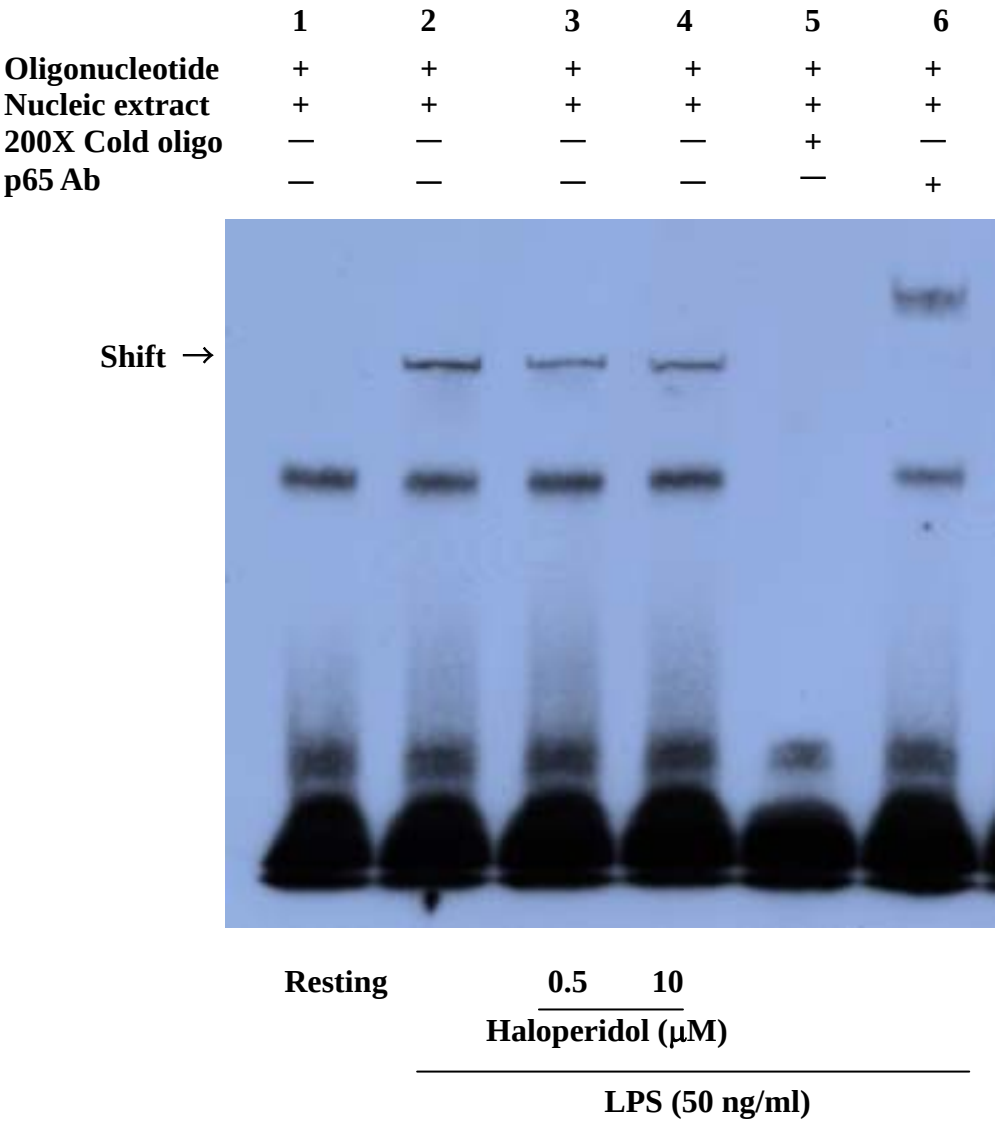
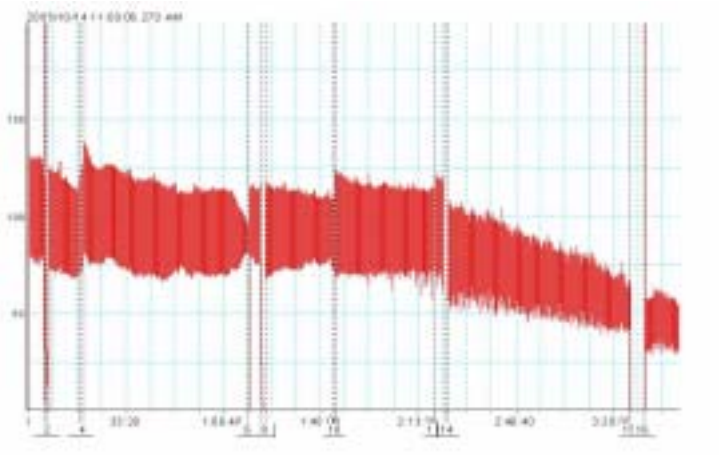


Figure 6. Effect of haloperidol on NF-κB activation in THP-1 cells. THP-1 cells (1×10^6 cells/ml) were dispensed on 6-well plate and treated with LPS (50 ng/ml) for 100 min as indicated. Cells were treated with haloperidol (lane 3, 0.5 μM; lane 4, 10 μM) or vehicle (lane 2) for 15 min before treatment with LPS. Then cellular nuclear extracts were prepared (8 μg) and analyzed for NF-κB activation by EMSA (lane 2, control).

A



B.

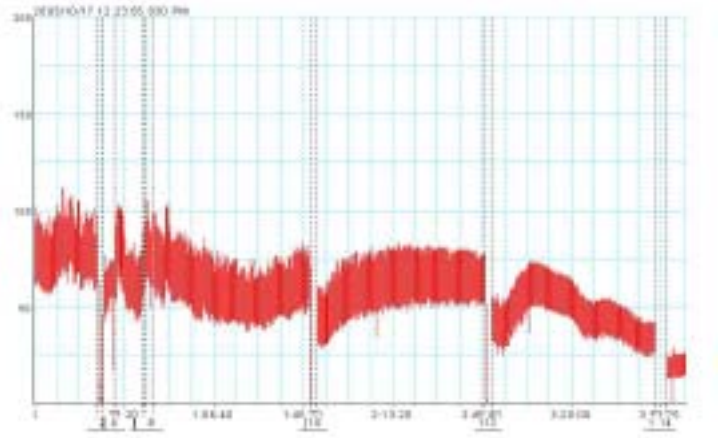
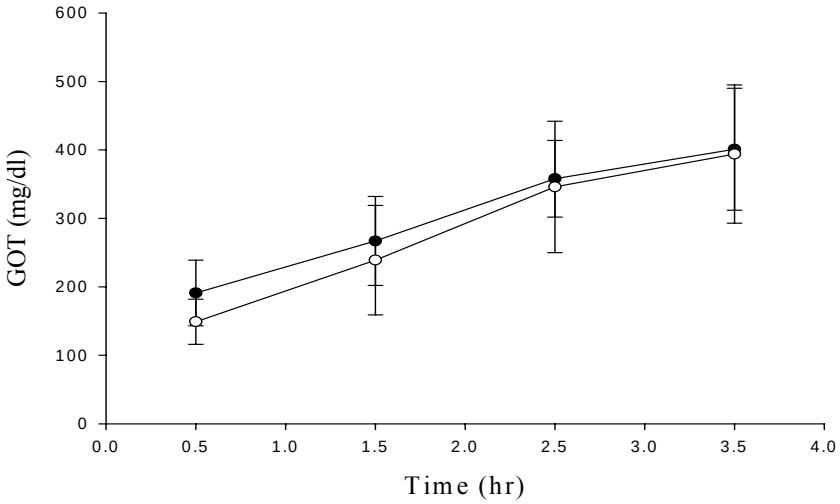


Figure 7. Time course of arterial blood pressure change in rats treated with bacterial components. Depicted are the changes in arterial blood pressure during the experimental period in LPS (5 mg/kg)-or LTA (10 mg/kg)-treated groups of rats. The results are representative examples of four similar experiments.

A.



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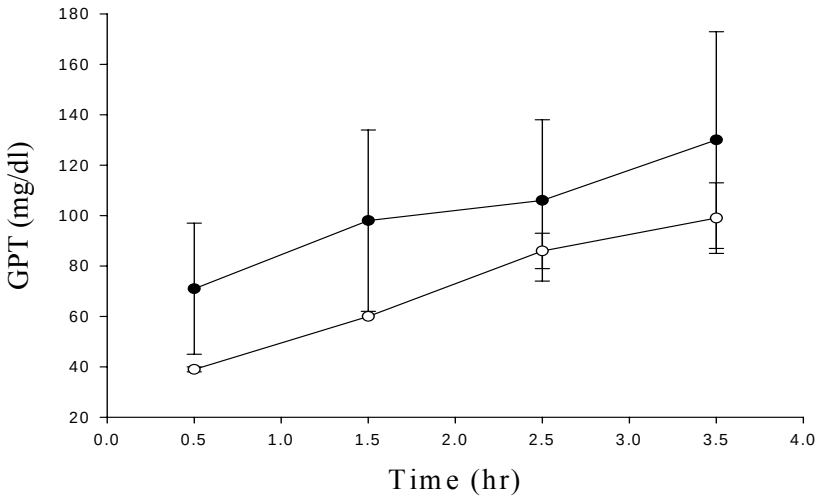
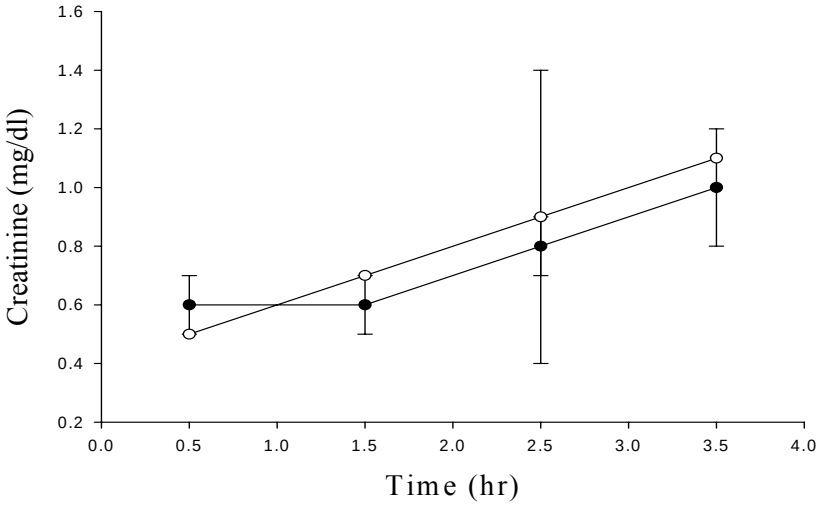


Figure 8. Time course of effects on sepsis-induced liver dysfunctions by LPS or LTA in rats. The serum of LTA (●)-or LPS (○)-treated animals were collected at indicated time. The data are representative example of three to four experiments.

A.



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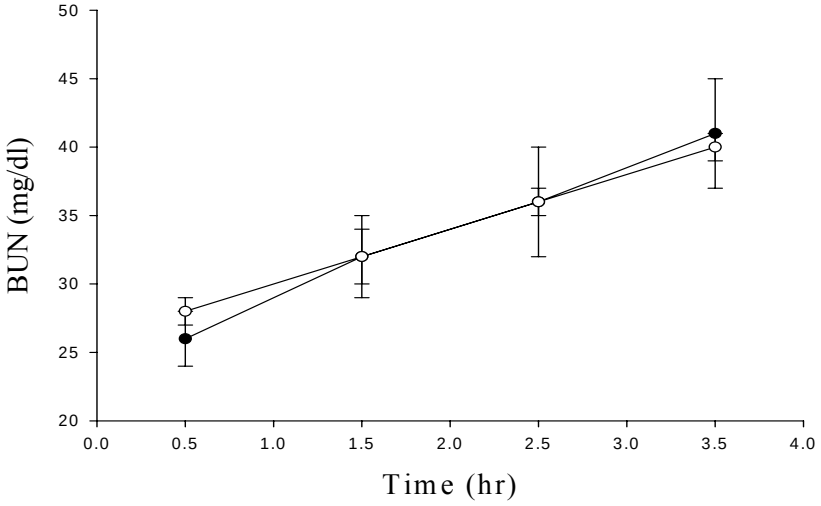

Figure 9. Time course of effects on sepsis-induced renal dysfunctions by LPS or LTA in rats. The serum of LTA (●)-or LPS (○)-treated animals were collected at indicated time. The data are representative example of three to four experiments.

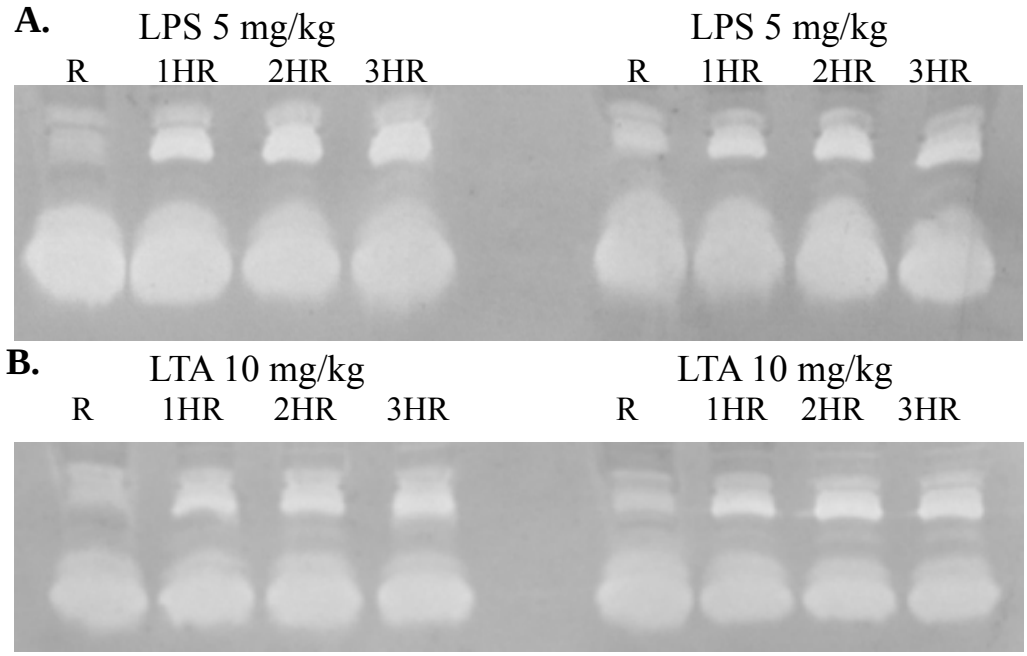

Figure 10. Time course of effects on serum-induced gelatinolysis by LPS-or LTA-treated in rats. The serum of LTA (●)-or LPS (○)-treated animals were collected at indicated time. The data are representative example of three to four experiments.