ƒ ‹ F | Œfia‹ ˙'e›ß• |–M^D‹ ª¤s›p e ƒ¤"G‡ł§i

–• Q⁄£ƒP§ ¥¤ ‰‡ fi †y† ›M⁄§^ ˜"«„ ‰Ł" ˜ ‡J¥ »^fl ‹ ¡

⁄˘⁄§§ ¤ ´ »P⁄æ‚ß¤ „ ›†˜ı⁄ ¶§'°»P‡–'° ' ‡yƒ¤ƒh›«

„'x I" ⁄§«O¯@§@¥ ¸

›p e^ §¡G §O«‹ ›p e

›p e‰ ¸s„¡GNSC93–2320–B–038–044–

ıƒ • ¶¡¡G93 ƒ~08 ⁄°01 ⁄ Ø 94 ƒ~07 ⁄°31 ⁄Ø

ıƒ ‡ ƒ ¡G» O ¥´ ˙⁄j ˙^˜†z ˙‹

›p e¥ D ø⁄¡HG¿ ‰ ı§

›p e »P⁄H›ß¡G" L «§‚¶ Rƒ‚– G "⁄

‡ł§i^ «‹¡G" °†‡ł§i

‡B†z⁄ Ł¡¡G¥»›p e¥ i ⁄ ‰ ¶‹d‚

⁄⁄ ¥` Œ 94 ƒ~10 ⁄°31 ⁄Ø

– · Q ⁄ £ ƒ P Š ¥ ¤ ‰ † fi †ŷ ₧ Ł ⅋M⁄Š ˆ ˜ ‟ ₹ „ J ¥ » ^ fl ¢ ⁄ ˘ ⁄ ŠŠ ¤
¤ „ › † ˜ ı ⁄ ¶ Š ' ° » P ‡ – ' ° ' ₩ ƒ ¤ ƒ h › « „ ' x I ″ ⁄ Š

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

› p e ‰ ,N Š G 93 ¡ – 2320 ¡ – B038 ¡ – 044

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¥ D ∞ ⁄ ₧ H G ¿ ‰ ₂ Š

⁄ @ ¡ B ⁄ ⁄ ″ K › n

Matrix metalloproteinases (MMPs) ‹ ⁄ @ , s † ″ c ^ ƒ (¥ Z B Š) ‟ N ˜ ´ ⁄ l ⁄ Š ‡ J ¥ » ^ fl ¡ M MPs fl ¶ ° ˃ ⁄ « ø † ″ ß ″ f c ⁄ Š † › M ¥ ‡ ‰ Ł (‰ Ł » P † ‰ I ″ ß † ´ ·) ¡ A G ″ ⁄ Š ‹ ‰ Ł ‟ ˜ ‡ ¥ p » A fl ƒ ¤ „ ' † ´ · ⁄ Š ″ c › « † ¡ B › , » P fl } ^ a ₤ Š Œ ″ t ‹ • › ∞ n ⁄ Š ¤ ƒ ¡ C ƒ b ` { Š ⁄ W ¡ ₩ ¿ ¤ ‟ v ˘ ‡ „ ″ ¥ \ fl » P ₶ fi ˜ ¡ C ″ ƒ ¥ ⁄ « Œ ‟ ″ † fl « fl e fl f ⁄ Ł – ₶ fi ô p ∞ ñ ol o † { ¤ ƒ ‡ Š ð ¢ ⁄ Š fi ‟ G ¡ C ' ¥ H † ‡ ⅋ « haloperidol fl Š ¤ ð ¢ fi ' » ⁄ o ‟ ″ ⁄ £ ¥ – † ´ · › « † (remodeling) ¡ Œ] ƒ „ ¥ » ‹ ¤ ¥ ¿ ¥ ¥ i ¥ H » ⁄ o MMP-9 ⁄ j ¶ ‟ † { ⁄ Š † ^ ‹ r fl (lipopolysaccharide ¡ A LPS) Š @ ‹ THP-1 † › M ⁄ Š ¤ ° Œ fl C ¥ ¤ ‟ ª fl ⁄ ‟ ₹ k (gelatin zymography) ⁄ U ¡ A Š › ⁅ „ ¥ ¤ LPS ¤ ¿ E MMP-9 » ^ fl ⁄ Š ¡ ⁄ ˘ ⁄ ¸ ‟ † { ¶ q • ⏐ H haloperidol ¿ @ « ₩ ¥ ¥ [i ƒ fi ˜ ƒ a Q Š ¤ ¡ C – Š Q ¥ ¸ † › M ƒ s ‹ ¡ † v œ ' (₩ MTT assay) ¥ i ф { haloperidol ‟ ″ Š ¤ Š @ ¥ ¸ ¤ ^ « D Š ¥ • ‰ ƒ † › M ⁄ Š • l ^ a ' ¸ ƒ ⁄ ¡ C ¥ H ƒ Ł ⁄ Ł ´ I ¥ ‟ k (Western blot) ⁄ Š „ Œ ⁄ Ł ‟ k ¡ A ф { haloperidol ¿ @ « ₩ ¥ ¥ i ƒ fi ˜ ƒ a Š ¤ ¥ LPS ¤ ¿ E MMP-9 ‡ J ¥ ⁄ Š † { ¶ q ¡ C

´ · ¥ Western blot ⁄ Š „ Œ ф { haloperidol fl ¯ ª ƒ a Š ¤ ¥ TNF-α ' ¸ LPS ¤ ¿ E ' › P ″ ″ total inhibitor-κBα (IκBα) ⁄ Š › , Š @ ¥ ¸ ¡ C – Š Q ¥ „ ð ¢ a † ° ƒ V ₹ k (electromobility shift assay ¡ A EMSA) ¡ A ⁄ ‟ R † › M i ⁄ ″ ¥ ~ NF-κB (p65) ⁄ Š ´ ƒ » P ‹ ¡ ⁄ ˘ – ¡ Š ¸ ¡ A – ð haloperidol ¿ ₡ ‹ 10 μM fi ¡ A ¥ i ƒ ‡ • ₦ q ƒ a Š ¤ LPS ' » ⁄ o ‟ ″ NF-κB ⁄ Š ´ ƒ » P ‹ ¡ ⁄ ˘ ¡ C

″ ƒ ¥ « ₡ „ Œ † ‟ G ¡ A ф { haloperidol ‰ T „ Œ fl ¿ ' ƒ ª Š ¤ ⁄ H ^ ‡ fi † y † › M (THP-1 cells) ⁄ ⁄ ¡ A LPS ' » ⁄ o ‟ ″ MMP-9 ‹ ¡ ' ° » P ‟ † { ¡ A ƒ „ Š ¤ ⁄ Š ´ ¥ ifl ¥ D › n ‹ O ∞ ¯ ₦ NF-κB ⁄ Š T fi Š ¶ » ¸ ¡ C fi ‡ | \ ¥ .. ¤ fl Š ¥ [Ø , ƒ b ‹ ¡ ¯ Ø „ Œ ⁄ ⁄ „ ' Š o ‟ ¢ ˘ ‡ „ ″ ¥ \ fl » P ₶ fi ˜ ¡ C ″ ƒ ¥ ⁄ « Œ ‟ ″ † ‟ G ¡ A ф { haloperidol ‟ ″ ‰ T ¤ ƒ ‡ Š ¤ MMP-9 ‟ † { ⁄ Š ‹ ¡ ' ° ¡ A ƒ ƒ ₶ LPS ¤ ¿ E ⁄ Ł → ¤ Š @ ¥ ¸ ´ ¥ ifl ¥ D › n ´ ˙ ¥ … ∨ T NF-κB ‟ ″ T , „ ¶ » … „ L { ¡ C ¥ t ⁄ @ ⁄ Ł → ¡ A † ˜ ∞ ‡ ° » P ¶ Š ' ° † ' ‡ ƒ ¤ ⁄ Š ¤ x ¶ ¸ fi ‡ fi ₸ Š ' ° ¡ A ƒ MMP-9 ⁄ Š Š @ ¥ ‹ ƒ ¡ C ƒ b ¥ .. ¤ – N • | ¶ i ƒ Š ƒ h ‹ ^ ⁄ Š „ Œ ⁄ ¸ ¤ ¥ L ‹ ¡ ¯ Ø „ Œ ¥ H ` A , ƒ U ^ ‟ « O Š _ ƒ b – ƒ f k g U ¤ ª ƒ ‡ Š o ₹ i ˇ ⁄ Š ¥ \ fl ¡ C

^ ` : ‰ Ł ‟ ˜ » ^ fl ¡ B † › M ₸ fi Š ¡ B ⁄ ″ ‹ r fl ¡ B – ƒ ' ° ¥ Š J

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

⁄G¡B‰t¥ »P¥ ""

ƒ h › « „ ' x I" (multiple organ failure; MOF) ‡ q – ' o ¥ ˝ ƒ b • ₱Vflgfl‡⁄H¡Aƒ

ƒ] ' ¯ Ø ⁄ " ¥"˜Y›«yfo¤" ¢ ˃ ‡¡C ƒ b ` { S ⁄ W ⁄ £ " ƒp¥"S–jƒ ƒ‹‡Aϴ ƒ ' ° ¥ S J ⁄ · M ƒ‹"fl¥⁄Dhƒ⁄fl]fl (Bone., 1994)¡C` M⁄ _– ƒ '¥"S‰L‡ †zƒ fl «D– ‰ ˘ł¡Aƒ ¥ « e ⁄ W ¥D" ϑ¢ ⁄ ˘‡⁄ ÐO† y ƒ ¥ S‰ƒ h › « „x"™¥"Dn › ƒ] (Cohen, 2002)¡ C ƒ b ‡ ¥¤¥",S‰L{ ⁄¡AMOF "fl f ⁄ ‡"‡ v ‡ fl‡f–¡""· c ˃...W ¥ 90 %¥H⁄W(Deitsch., 1997; Baue., 1993)¡A – ¥ '°»ˆfl ⁄£¥¿–‹¡˘S Œ " t • ›«›n""¤ ƒ (Nakmura et al., 1998)¡C

Matrix metalloproteinases ´ " MMPs ‹ ¯ Ø"›«›n– ¥ '°»ˆfl ¡A¥B‹ ⁄@‚s † " ĉ ƒ ¥ B S (Zinc ion)" ˜ ´ ł⁄S‡fl¥ (Kotra et al., 2001)¡CMMP ""¶°⁄˘S @ ¥»" ˜ ´⁄¥Ł[¥H‹ ¡⁄˘¡A¤ˆƒ‡¶°⁄˘⁄ , † › M~ ‰Ł† ¥ (extracellular matrix proteins)""fl ⁄Q¡CGelatinases A »PB (MMP-2 »PMMP-9) ""¶°⁄˘ ˘ ⁄⁄S t ƒ ‡ ⁄ T head-to-tail cystein-rich repeats¡ A fl S S‡Q˘S O ¥¤¡C ‡ ¤· inserts ˆ ƒ fibronection "" collagen-binding type II repeats¡A» ›n»P collagen 'M elastin †ƒX»P⁄ `£(cleavage) (Shipley 1996)¡C £ƒ„⁄S¥~¡AMMP-9 (92 kDa gelatinase B)‹ MMPs sequence ⁄‡ – ¥B ‰ ˘ł" ¡ Ab hinge region "" ' ‡¡S t ƒ ‡¿WflS "" type V collagen-like inserts¡ A ƒ„insert "" ›« › ‚° »P ¥\ fl ⁄ ·M ¥... " ¡C Membrane-type "" MT-MMPs ƒ ‡ single-pass transmembrane domain »P u"" cytoplasmic C-terminal tail (ƒpMMPs 14¡B 15¡H16 »P24) ' ‹ OC-terminal hydrophobic "" ˘ (Kojima, 2000) ¡A ¥H glycophosphatidyl inositol (GPI) membrane-anchoring signal " †{ (ƒp MMP-17 'MMMP-25)¡CMMPs ‡ " ‡Q' wq ‹ ⁄@‚s»ˆfl ¥i‡Q" ˜ gƒX fl»P⁄ " ¥'S ¤ fl ' S ¤ A ƒ‹‹ ¡⁄˘« ‹ ¥¤ƒ‡ E'‹ ¡ ⁄˘¡ F ¤'fl ¶°⁄˘⁄ , ""† › M ¥ ‡ ‰Łƒ ⁄⁄@" ¥H⁄W¤ ƒpcollagen¡Belastin¡Blaminin ¥¡ Œ]ƒ„¤ „ ' † ´· remodeling¡Brepairing »P destroying ¡A‹ S Œt⁄F ›« ›n""¤ ƒ (Woessner et al., 1991)¡CMMPs ƒ ‰˝†z⁄W"" S ¤ fl ⁄ ¥˝"" MMP † ´·S ¤ fl TIMPs ƒ (tissue inhibitors of metalloprotinases)¡ATIMPs ˜ ' S ⁄ l¶q (20~29kDa) ""‡ ¥ ‰Ł¡A l –M⁄@' °""S ¤ ‹ ¡⁄˘«‹"A""MMPs (Backer, 2002)¡C¥ « ⁄w ƒ‡¥|" TIMPs ‡Q o†{¡ A£ƒP"" TIMPs „ ' S ¤ ⁄£ƒP""MMPs "fl ⁄O⁄]ƒ‡fitS‹¡C¤C " TIMP „ ' MMP ""S ¥¿¸'M⁄O⁄]⁄£ƒP (Brew, 2000)¡ApTIMP-1 ‹ MMP-9 ""¥D ›nS ¤ ƒŒTIMPs „ ' MMPs ‹ ¡' °""S ¤ ‹ O‹ ⁄@" ‹ • ›«›n""˜Y– K ‰–– ¤ ¡C

fl ƒh` ¯Ø Lipopolysaccharide (LPS) ' ‚" ‹ † ' ›"‹ ‹fl (endotoxin)• |»⁄ ⁄H ˆ ‡ fi†y† ›M ¥h " †{ ‡\ ƒh pro-inflammatory mediators (Guha, 2001; Netea, 2002)¡A⁄¥ ¿A⁄F pro-coagulant ⁄ ⁄l tissue factor (TF) »P† ›M¿Efl , ~ ‰ˆƒ" ƒ]⁄l TNF-α¡CLPS ‹ O‡ ƒ ›‡Q o†{""⁄"‹ r fl¡Aƒ ' fi ˜ ‹ ‚°– (gram-negative) "" † ›M ⁄ WA• † •», fi ¡ALPS • |‡Q ı,

˜ ¥X (Raetz, 1986)¡ C „ ' fi ĩ⁄ ‡–immobilized on fibers ⁄Sƒ¤⁄ " v‡⁄U"′‰T¥i
(gram-negative bacteria) " ″ • ₱V L₱S ¥ ‡ · §C MMP-9 ƒbflf⁄H"′St¶q¡Af § ‰– ƒ
§ @ ‹ O ⁄ @ ″ o"′¥⁄Ď ‡ ■‰¡A§Y toll-like '°¥ §J⁄Sfl ϑ‹(Nakamura et al., 1998)¡C M
receptor 4 (´ ″ TLR4) « h ƒ b ƒ „ † ¶H⁄⁄ƒƒ‡ƒ‡^ ‹ ¡¯ØLTA ⁄ oMMP ⁄Sflf†z¤ ƒ ¥
⁄£¥ifl ° ⁄ ƒa(Kiechl, 2002)¡C⁄ Ĥ "′"‡ fi « ∈·⁄£† M¡¡Cƒ] LPS ¥i¤ ¿E‡ fi†¤ŷ˜˜ 'æ
† y † ›M ' LPS "′"¤ ° Ę⁄¤ fl S §—• ₱A¤^ TNF-α¡A¶¡– »⁄ o MMP-9 ⁄S" †{¡Afb¥»
⁄ ˇ‡† £ ¥ ‡\ƒ№¢'°† ›M¿ Ħ ¡CLPS • ⁄₱ „ Œ⁄⁄¡A§ › „ ˇ ⁄ ⁄FLPS „ THP-1 †
LPS-binding protein ¡]´ ″ LBP¡ƒbplasma ›M"′¤ ¿E»P§@¥¸¡CLTA "′"¤ • ‰i¥H–q⁄@¤·
⁄⁄ ƒX¡A¤^¶ · e¤† ›M" ›–"″ CD14 ›†˜ ≠ ¶S' ° ¡Afp Staphylococcus aureus,
receptor ¡C– Ÿ¤ LPS «K•⏐‡ Q ´ ·«ƒ Streptococcus pyrogenes A, Enterococcus
transmembrane "″ signaling toll-like faecalis, reptococcus pneumoniae 'MListeria
receptor 4 »P LTR4 "′"˜ ¥ ‡J¥MD2 ⁄W monocytogens "′"† ›M ⁄W œ–ø ¡C¥
(Guha & Mackman, 2001)¡CLPS „ ' ‡ fi Staphylococcus aureus ⁄ ´ ƒ ¤ "″LTA¡Afb
†y»P¥ ¤‰† ›M‹ O @"–jƒ‡⁄O"′¿E‹ ¡fl murine macrophages † ›M⁄¡A•⏐´ ¥ iNOS
(O'Connell, 1998)¡A„ ' host † ›Mƒ‡« O@ "′"˜ †{ƒ ...W¥[NO "′"† ¥″ (Cunha. et al.,
'°»P¶ fi¸''°"′⁄ ˇ‡¡C´ · ¥ ‹ ªƒ№‡ fi †y† 1993)¡Cfb vascular smooth muscle i† ›M
›M⁄ LPS "′"⁄ ⁄ l »₱ Ÿ¢'° ƒ]"′‰ ⁄⁄¥ ‹O (Lonchampt. et al., 1992)¡Cfb⁄j„«
--¡A⁄]‡\fl ƒb‡ ¥h¤ t¸'°"″ o " ¢ˇflg‡ " ˜ƒ⁄¡ALTA ⁄]•⏐‡ ¥¤ iNOS ‡ ¥ ⁄¸‹¡'°
"‹"″" v‡⁄W¡A§ ¤ •s"″⁄ Ł‰k(Guha & "″...W¥[¡A¤ˇ–o· ' ¥¢‰ (circulatory
Mackman, 2001)¡C´ · ¥ ‹ ªƒ№‡ fi ¥† ›M failure)¡Cfi ‡ ¥h⁄ ˜m–o" ¡Afb¯ jƒ^ °
⁄⁄LPS "′"⁄ ⁄ l »₱ Ÿ¢'° ƒ]"′‰ --¡A "«.. ƒ¡"″– ƒ '°¥ §J⁄⁄¡Af] LTA ' ‡ ¥¤
⁄]‡\fl ƒb‡ ¥h¤ t¸'°"″ o " ¢ˇfl ₱ϑ‹"″ iNOS „L« " †{ƒ † ¥″„L¶q"′NO¡Af ‡ ¥¤
" v‡⁄W¡A§ ¤ •s"″⁄ Ł‰k(Guha & Mackman, circulatory failure »P MOF (De Kimpe. et al.,
2001)¡CfbfU″ o"′¶t ₫lf⁄⁄¡AMMPs ' fle 1995)¡C¥ Staphylococcus aureus ⁄ ´ ƒ ¤
flf„ L{⁄⁄§ Œ ″ ‡F• ›«›n"″¤ ƒ ¡Cƒ "″ LTA ¡A„ ' ¥ ¤‰† ›M»Pƒ ″ ¥ •ˇ‡ƒ
chemokine (MCP-1)¡B‡ ›M¿ Ħ »P† '°⁄″ ›M¡A•⏐‡ ¥¤» ⁄ «‹⁄@fi æˇ·ÆfXƒ¤» ˆfl "′"†£
‹ rfl (LPS) §¡•⏐¤ ¿E' ¸ » MMPs "′"†£ ¥˝¡C¥ ¥˜¡AfbJ 774.2 ¥ ¤‰† ›M i⁄¡A¥H
¥˝¡A¶if ‡ ¥¤⁄ , ‡‰ l †´¡·A¤^‡ ¥¤ƒU″ LTA ‡B†z«Æ•⏐‡ ¥¤ iNOS " †{¡C¥ƒ"″ ₣§
⁄£ƒP¥˝†z»Pflf†z"′¶ fi¸'¡Cfi ` { §⁄ ˜m¶k· , ‡¡¥§t⁄F tyrosine kinase »P nuclear
¥Xfb– ƒ '°¥ §J (septic shock)flf {⁄⁄¡A¥i transcription factor NF-κB "′‹ ¡⁄ˇ¡C Mƒ ¡A
⁄ ...s"×o " ¢ˇ»P‡ ¶Ł"′† ¥˝¡A† y ¥ƒ† ¥ LTA ' ›P"′" MMP »P iNOS " †{"″ T
´·•l¶¸¡A˜Y›«§ ›Pƒh›« „ ' xI" fi§¶·»..„ fi⏐⁄· M⁄£† M¡¡C
(MOF)¡Af ‡ ¥¤ƒ ″'¡C›†˜ɪƒ¡‡ – ' ⁄ ″r
fl ' ›P"′fi æˇ»¶¸»P¥ ƒ †y‹ ¡⁄ˇ‹O⁄ o **2-2 „Œ¯ ¥ "″**
¥ ¤ ›'° o " ¢ˇ¡ ‡systemic inflammatory
response¡^"′›«›n› ƒ]¡Cfb‹¡¯ Ø ° ‰„Œ¯ ƒ ‰»„Œ¯ ⁄ ⁄ ¡ A ¥ H ⁄ H ^ ‡ fi †y† ›M
o†{¥ H ^ "«§ MMP-9 ⁄§" †{⁄¸MMP-9 THP-1 ‹ „Œ¯ † M¡A·¥H⁄ ₣ haloperidol
ƒ]› £⁄§⁄ p«„ ' ⁄ ″rfl (LPS)' ‡ ¥¤ ¥ ^ "«Ł,PS ' ⁄ ¥ X ¦»MMP-9 ‡J
"″– ƒ '°¥ §J ¥¶ fi¸¤ªƒ‡« O@' ₣§ fl ⁄O¡A ¥ ‰ Ł¡"″" † { » P ... v̄T¥Œ§ ›
⁄]¶¡– • ¥ MMP-9 "′"§ ¤ fi "G¥i" v‡– Zymography ¡BWestern Blot ¥„Œ¯ , ⁄F
ƒ '°¥ §J' ‡ ¥¤"′"¶ fi¸'(Dubois et al., 2002; , »Pˇ Ł MMP-9 ‡ J ¥‰Ł"′" †{¡A¤^¥H
Kishnani et al., 1999)¡CfPfi ƒb` $ „ Œ o MTT Assay ˇ Ł † ›Mƒs« ¡†v"′"– §¸¡Cƒ ⁄§
†{• ¶Lq LPS ⁄ o⁄ Ĥ · ' ¤ t¸⁄⁄ MMP-9 «Æ§ › – ·Q⁄F⁄§⁄"′"^˜† z § @ ¥ ¦M ¥ ´
"″† ¥˝»P˜ ¥X¤ ,ß NO ' œª»P§ ‡t(Albert ^D¡A„ ¶ i @¤B″A„ ¤ ƒ b †›M T fi ¶·»...
et al,. 2003) ¡C ¥B ¥H Polymyxin B , ₣ifpNF-κB ¥ ¤ "″... v̄T « ¡C¥ ¥˜
„ »PMMP ƒ ‡ "″• ₱V' °ƒ ¤ (LPS »PLTA)
⁄ ˇ‡fpƒ h › « „ ' x I ″...¸Æ¶ ¶s₣Q fi'

¤ B " " ß ƒ » P ` A ,  ¤"v ł""¥ifl(0.5~20 μM) • | S e † { ^ ~ " «@« ""S ¤ ⁄T¡B †"G»P Q‰ MMP-9 ‹ ¦°"⁄"" †{¡ A ƒ ] ƒ „ ¡ A S› ƒAS Q¥¸ zymography ⁄ Ł " k ¤ `haloperidol ‹ S_• | " ‰ – ...v MMP-9 ¥ » »"" fl ‹ ¡ ' ¡¥A „ - † G (data not shown)¡A" ¤ B ¤ _ haloperidol „ ' TNF-α (lane 2¡G4.45 ± 0.15 fold)¤ ° ¿ E « Æ »⁄ MMP-9 » fl ‹ ¦°¤^ ¤ S † .. v T (lane 4¡A0.5 μM¡G4.85 ± 0.28 fold¡Flane 5¡A2 μM¡G4.93 ± 0.40 fold¡Flane 6¡A10 μM¡G4.99 ± 0.56 fold¡Flane 7¡A20 μM¡G 5.09 ± 0.39 fold)¡C

3-1 †"G

3-1-1.–• Q haloperidol „ ⁄H^ ł fi †y† »M (THP-1)¥H LPS ' »⁄ o"" MMP-9 »^fl ‹ ¡ ' °

¥ ⁄ S « e ⁄ w « ¥ S¤°¿E"«‰Ł»⁄ O MMP-9 ‹ ¡ ' ° " " " † { – gelatine zymography ⁄S⁄ Ł " k ¡ A S ° Þ⁄£SFP"@« ¥ H ˘ ƒ „ "«„ LPS ' » ⁄o"" MMP-9 ƒb THP-1 † M⁄»"... v ¯ T ¡ C ¥ » „ Œ ¯ ›"¥ ‹O›n–•

Q¥ H ⁄ £ F¿"@« haloperidol (0.5~20 μM) ¤ ˘ ƒ „ THP-1 † ›M„ ' S¥Ł LPS ¤ ¿E ƒ †£ ¥˝ MMP-9 ‹ ¦°⁄S.. v T {« ¡C¥ „ q " »^fl ⁄ "R"k⁄S„ Œ "G o† { ¡A H haloperidol ¿ @ ""... W [(0.5 μM¡B2 μM¡B10 μM¡B20 μM)¡A MMP-9 " " ‹ ¡ ' "S e † { { † v " ¥ · ⁄ ¥B¿ E fl " " ¡(Resting¡A land 1)¡A¤ † ›M ƒ † • N , q ƒ † Q S † { ¶H (Figure 1 A)¡C¤ ⁄⁄ haloperidol „ LPS ¤ ¿E S @¥ ¸"" S ¤ ƒ °⁄ † v (Inhibition %)⁄ S © 38.5 ± 0.9 % (0.5 μM)¡F47.5 ± 4.2 % (2 μM)¡F76.6 ± 1.4 % (10 μM)¡F91.6 ± 3.1 % (20 μM)¡A¤ S ¤ 50 % ⁄ ˘ †¿ @ (IC_{50})‹ 3.1 ± 0.8 μM (n = 3¡A Figure 1 B)¡C¥ ƒ„ "G¥ Ł ¡A ƒb THP-1 † › M⁄, g¥ LPS ' » ⁄ o⁄ S MMP-9 ‹ fl †Q haloperidol H ¿ @ ""⁄ W ƒ S e † { ‹ ¦°⁄ ⁄S⁄ ˘ † ¤ ¡A¤^¥ S e † { ¿ @ S ¤ fi ˜ † (concentration-dependent inhibition)¡C

3-1-2.–• Q^ ˜"«„ † ›M ƒs‹ ¡ † v""...v ¯ T

¥ „ Œ ¯ † G ˆ ˜ † { ¿ £ ¥ [° ¿ E ƒ l fi ¡A resting ""S l ¥ œ ¨ 0.94 ± 0.04 nm¡A• B † z ⁄£ƒ P @«"" haloperidol „ ' † ›M""... ¶q o † { ƒ † y • Ł"...v ¯ T (10 μM¡A0.89 ± 0.05 nm ⁄¸ 20 μM¡A0.73 ± 0.03 nm)¡A ƒ ª Ø ƒ ¤¥3¡G0.918 fold)¡E90 ⁄ ˜ `(land 4¡G0.769 fold) ⁄¸ 120 ⁄ ˜ `(land 5¡G1.302 fold)«Æ¡A«h¥H haloperidol ¿ @ 20 μM ¥ H U (data not shown)¤ ^ ⁄£ • | d ¯ ..ª v ¯ T † ›M""ƒs‹ ¡(n = 3~8¡A Figure 2)¡A H haloperidol ¿ @ ...W ¥ [A¤ ›Þ ›Mƒ ˝ `"" {« ¶V' ¯ ª (40 μM¡A0.60 ± 0.02 nm¡F50 μM¡A0.55 ± 0.03 nm ⁄¸ 100 μM¡A0.10 ± 0.01 nm)¡C

3-1-3.–• Q Haloperidol „ MMP-9 »^fl ¥» ¥˝‹ ¡' °⁄S..v ¯ T

¥ ⁄S« e " " „ Œ ¯ † `haloperidol

3-1-4.–• Q Haloperidol „ THP-1 † ›M¥H LPS ' »⁄ o"" MMP-9 ł J¥ ‰Ł" † { ⁄ S S @¥ ¸

ƒb Figure 3 ⁄ ⁄ A–N THP-1 † › M B † z ¤^ i 24 ⁄ p fi «Æ A¤ œ † ›M⁄ S ¤ œ (Cell lysate)¶ i ƒ „ Œ ¯ A S › o † { ƒ ...¥ [^ ~ ⁄ ¸ ¤° E fl " " ¡(Resting¡A land 1)¡A¤ † ›M ¤ œ«¥ u » · œ ¤ «D"" • 2 ¶ q D ⁄S MMP-9 ł J ¥"† { ¶q¡ C ƒ ¥ LPS ¤ ¿E 24 ⁄ p fi «Æ¡A ф { ¤ † ›M œ"« h S f ł ¶q"" MMP-9 ł ¥ ‰Ł" † { (Figure 3¡A land 2¡G LPS¡A2.81 ± 0.22 fold)¡A• „ ⁄ ' £ ƒ P"¸@« haloperidol (2 μM~20 μM) ł B z«Æ¡A¥ i ф { ¤ LPS ' »⁄ ð" MMP-9 ł ¥ ‰Ł" † { ¶q • | H ^ ~"«(haloperidol) ¿ @ ""... W ¥ [ƒ S e † { ƒ † • N , q ƒ a ¥" ¥S – S ¸(land 3¡G2 μM¡A 1.41 ± 0.13 fold¡Hland 4¡G10 μM¡A1.04 ± 0.13 fold¡Fland 5¡G20 μM¡A0.54 ± 0.10 fold)¡C

3-1-5.–• Q Haloperidol „ THP-1 † ›M¥H LPS »⁄ o IκB-α⁄S› , S @¥ ¸

¥ Figure 4 " " † "G o f ¡A 120 min i ""Resting • 1¡A– ¥H LPS (50 ng/ml)¤ ° ¿E 30 ⁄ ˜ `(land 2¡G1.062 fold)¡E60 ⁄ ˜ `(land 3¡G0.918 fold)¡E90 ⁄ ˜ `(land 4¡G0.769 fold) ⁄¸ 120 ⁄ ˜ `(land 5¡G1.302 fold)«Æ¡A«h¥H LPS ¤ ¿E 90 ⁄ ˜ `fi ¡A¤ total IκB-α"" › , S @ ¥ ¸ † ‹ ' œ ¯ ª Ç ƒ ] ƒ „ Œ ¡A N † o¤ · ›M¥H LPS ¤ ¿E 90 ⁄ ˜ `¤ ¶ ƒ «Æ S "„ Œ ¡C

ƒb Figure 5 ⁄¡A• ¥ ... S⁄ LPS fi ¡A ƒ] LT fi S⁄ S ¶ »...¡A total IκB-α«h L"k¶ ƒ › , S @ ¥ ¸¡A‹ G IκB-α † ¥ "⁄" † { ‹ † "(lane 1¡G1 ± 0.0)¡ H LPS ¤ ¿E 90 ⁄ ˜ `(land 2¡G0.68 ± 0.04 fold)¡A S ⁄' £ ƒ P @«S haloperidol¡A

¥ i o† IκB-α§ t ¶ qH haloperidol ¿ @ «"" ... W"(land 3¡ G2 μM¡ A0.80 ± 0.01 fold¡ Eland 4¡ G10 μM¡ A0.78 ± 0.02 fold¡ Eland 5¡ G20 μM¡ A 0.82 ± 0.01 fold)ƒ ‡ v ¥/¡WAfi ¥/W' ˘ [„ ¤ /§¡ A¥"1¥ H –‰haloperidol ¥ifl • | , g ¥§ IκB-α† J ¥‰Ł"› , §@¥§ ¶ i @¤B"· / NF-κB translocate ƒ † ›Mfi / ‡¡""§ ¥¸¡A† ¥¤ LPS ' » / ¥X¤" MMP-9 ƒ] ƒ‡§ ¤ ƒ L ℜ"†{¡C

3-1-6.–· Q Haloperidol „ THP-1 † ›M¥H LPS »/ op65 /§..v˜T

¥ Figure 6 / / ¥ i ¥ H[„haloperidol (lane 3, 4: 0.5, 10 μM) fl § ¤ ƒ ⊳H^ ‡ fi † y † ›M(THP-1) fi / "¡ALPS ' » /o"" NF-κB translocation " †{ (lane 2, Vehicle)¡C

3-1-7.–· Q„Œ °"«ƒb LPS ' ¸LTA ‡B†z«Æ /§fh›« „'x I" »P MMP ‹ ¡/˘/§‹ ª¤s

¥ ƒ £§¯ /˘(Figure 7)¡ B ¤ x ƒ¥\fl (Figure 8)» P·¯ ƒ ¥fl(Figur 9) ß ƒ ª¥ /¡ „ « ⊳¥HPS ' ¸LTA ' ‡ y ƒ ¤ / § – ƒ '°¶ fi‹ ƒ h › « „ ‡ LPS „¡ŒLTA „ ·¯ƒ/§• ₧ ¶ ¸ fi'¯° ¥BƒbMMP-9 ƒ‡,ß–j/§‹ ¡ / §@¥¸ (Figur 10)¡ CHaloperidol ` „ MMP ƒ‡' ...v ¯ T Aƒ„ – ƒ ' ° ƒ h › «„‹‡xO@§'@ /§ ¥¸¡ A / · ¶ • ¶ ‡¿@₧CB–· Q

3-2 Q‰

MMP-9 (˜ ' type IV collagenase¡ A 8" ‹ gelatin B¡ A ‡¶kq 92 kD)¥ i ƒ b ‡ ¥h" " " ‡ fi † y † ›M¡A¥‡Q ‹⊳A{¡ G P^ ƒ " " ‡ fi (peripheral blood monocytes)¡ B ´·/ »"¥ ¤‰† ›M(microphages)¡ BKupffer cells» P ⊳ ‡ ›M(osteoclasts) ¥ ¥ i o†{ MMP-9"" †{(Welgus et al., 1990; Masure et al., 1993; Winwood et al., 1995; Swallow et al., 1996)¡ C ¥¥ƒb/ @ ·leukemic cell lines //¡A¥] A¡ HL-60¡ BNB4¡ BU-937/¸THP-1 /]¥i˜ 'æMMP-9» fl (Ries et al., 1994; Ismair et al., 1998; Saarialho Kere et al., 1993; Van et al., 1991)¡ Cfb/@fl ° ¥ ¿ ¯¥˝†z //¡Af ¤ ...¥f †y¥ » ¤·¥Ti¥H/ "cMMP-9¡ A § ₧¸MMP-9/ , ‰Ł"fl /O¡A¤˜¥ ƒ †yfl –qf †G´ ¶ } ƒ"fl⊳J† ´· o" ¢ B¤ o·§ ¥ ƒ "" § K ‹ ¥\fl(Doherty et al., 1994; Welgus et al., 1990; Leppert et al., 1995; Weiss et al., 1986)¡ C ¥¥¡A‡ fi ƒ† › ⊳M ¤ / @·† ›M¿Efl (ƒp¡ GTNF-α、IL-1β¡ BCSFs ' ¸IL-3)' ¸† ˝‹fl (LPS)¤°¿Efi¡ A« ⊳|¤˘ MMPs " †{¡ Cƒ ‡ / m«¡ ⊳X‡ fi†y (monocytes) ›Y‹ O¤ ¤ † ›M¿Efl TNF-α' ¸ IL-1β"˝¤°¿E¡ A« ⊳|»/ cMMP-9"˝" †{ /£ ‹ cMMP-1 (Saren et al., 1996)¡ Cƒ b ‡ fi†y"" † ›Mfi ‡¸ ¡AMMP-9"˝/ "o»P ƒ] ´¿"" { « ƒ‡^ ¥B¥ ‡Q/ @·† ›M¿Efl (ƒp¡ GTNF-α†z „LTNFR1) ¤ « P ¶ i ‰ , '(Rinkey and Petrides, 1995; Ismair et al., 1998)。¥ ¥~¡A LPS«h•|»/ o¯ ª "˝MMP-1 »PMMP-9 " †{(Lai et al., 2003)/¸‡ ⊳hƒ†^ o"¢" " † ›M ¿Efl (cytokines) (Yong et al., 1998)¡ Cƒ ƒb/@ ¤·fl « , g h ˃'/ƒ(fl‡fl‡G" fl fi ⊳P‡lg " · ¸ flgϕ{ƒ‡¤·† ›M¿Efl "˝" †{/ æß ‹ ¡D¡A¤ / / ¥ ¸AT§F-α¡ BIL-1/¸TGF-β (Akiyama et al., 2000; Nagatsu et al., 2000)¡ Af ¤ //¡ATNF-α‡Q»{‹ ‹ O»/ oo"¢ "˝– fi˜† ›M¿Efl (Munoz-Fernandez et al., 1998)¡ C

, ‰/"¢(meningitis)‹ O/…˘fl ⊳g¤ ‡ ¸/" "˝, ‰/ o"¢¡ C¤ //¥H† ' °, ‰/"¢flg"‹, ß ‹ ˜ ⊳«¡ Af ‡ –˃ _, ‰/"¢/§ " ‹ , ‰/ "¢´ø†y (*Neisseria meningitides*)»P• P ≤¶ ƒ – (*Haemophilus influenza*)¡ A¥ ƒ " ‡ ›M ⊳§ƒ‡/@" ¥i¥H/ ˜Y ⊳«"¢/ ˘‡"" "« ‰Ł " ‹ † ' ° / ⊳r fl (lipopolysaccharide¡ ALPS)¡ Cƒ„‹ › †ı/ ‡– '°†G † ›M‰/¥~"˝"¥Dƒ¤/ ¡ A ‡ fi † y ›M/ / fi˜"G‡ –j""¤°¿E fl/§£,(Rietschel and Brade, 1992)¡ C Mƒ LPS„L¥ " †{• | ⊳– ƒ ¡B– ƒ ' °¥ §J' ¸¥ ¤ ⊳¤ ‡¸ o"¢/ ˘‡flg "‹¡ Cƒ‡‡ ⊳h/ ˜ ⊳« ⊳¥⊳MMPs (/ ¤ ‹O MMP-9)' † ' °, ‰/"¢/ »P ¥ †z»P flf†z„L {¡A§ ₧¸¥i¥H/ , ‰Ł"fl /O¶if fl †af ,£» ¡A¤˘¤ ‡q‡z' °¯ /j/ _§ ƒh † /J«I(Rosenberg et al., 1995)¡ C' ¥H§ › "˝„ Œ //¡A¤˘¥¸¥ ƒ † y / / " ‡ fi † ›M THP-1 cells˘ „ ›Y– ˜†¤ ¥~ƒ ⊳ ' °/ ⊳r fl LPS/§¤°¿E¡ A–· QTHP-1† ›M' †£¥˝"˝" MMP-9‡J¥ " †{»Pƒ‡ „ ˃^"‹haloperidol /§/U¡ A^ ˜"‹„ ' MMP-9"˝"‡J¥ " †{' †£¥˝ "˝§ ¤§₧¸¡ C¥ zymography„ Œ / Ł⊳k¥i˘ [„ ¤ ¡Ahaloperidolfl § ¤ /H^ ‡ fi †y† ›M

(THP-1)⁄ ¡ÆLPS' » ⁄ÒMMP-9» ^ fl ‹ ¡'° LPS ¤ ¿E⁄ H ^ † fi†¥¡t¸g¥M† \ ƒ hTfi§ " † { ¥Scon(centration-dependent""§ ¤ ¶ · » „. ƒi¤ ⁄⁄¥ ¿A IKK-NF-κB , ƒi»P § @ ¥ ¡CIPS (50 ng/ml)""¤ ° ¿ E§ÆA MAPKs , fi(ERK1/2; JNK; p38)¥B† ƒiA¤ haloperidol„ ' MMP-9» fl § ¤ 50%""⁄ˇ ´ ¿ ƒ]⁄l NF-κB (p50/p65) »P AP-1 † @«(IC50)‹ 3.1 ± 0.8 µM¡C– §¥¿ (c-Fos/c-Jun)¡CLai ¥ " «h·£¥Xƒb⁄ H 'P Western blot⁄ "Rhaloperidolfl § ¤ LPSƒb ^ ƒ†G ´ ƒ –o""† fi ¥†¡A§Q¥¸LPS ⁄ H ^ † fi†¥(THP-1) ⁄⁄' » ⁄o"" ¤ ¿E' » ⁄oÒMMP-9 † ¥ ' ¸mRNA " †{ MMP-9 † ¥ " †{ ¶q¥ B §†{ª "" ¥ D › ¤O‡MAPKs ¤ ⁄⁄§ERK1/2 , fi|' concentration-dependent§ ¤ §¥¸¡C ‰ ––""(Lai et al., 2003)¡C¥t¥ ~ †•s ˜m‹h

Nuclear factor-κB (NF-κB)† o – ‡ Tfi§¶· ¡A⁄]‹ O§Q¥¸LPS ¤ ¿E⁄ H ' P ^ ƒ ⁄§G » … , fi|¤–⁄ F ¥ƒ»h ⁄o'°" "o" ¢ ƒ]»P † fi ¥† ¡»M o †{ ¥i ,g ¥ MMPs "" †{¡A¥B⁄ ˜m⁄· LPS ƒb† fi phosphatidylinositol-3/Akt/IKKα/NF-κB , † y † ›MÆA¥ i ´¥‹ ¡⁄ˇ NF-κB/Rel ´ ¿ ƒ] fi|» ⁄oMMP-9 † ¥˝(Lu and Wahl, 2005)¡C ⁄ l ƒ †£¥´ƒh" ^ ⁄§ ƒ]" †{¡A¥] A¡G ƒ ¥»„ Œ "G¯ª¥ haloperidol ¥i'œ¯ªƒa§ TNF-α»PIL-1 ¥(Sweet and Hume, 1996)⁄] ¤ ¥ LPS ' » ⁄oÒMMP-9 » fl »P† ¥ "" ¥i‹ ¡⁄ˇ† ƒh† ˜G¶ · »ƒ]l» P T fi § ¶»…, ¥i|†{¡A¥ «o L"k'œ¯ªƒa§ ¤ ¥ LPS ' » o (Sweet and Hume, 1996; Weinstein et al., "" IκB-α› , § @¸(L"k¯ª ƒaƒ^¤ •· 1992; Han et al., 1994; Hambleton et al., ´I)¡A¯ª¥ ^ "« haloperidol ¥ifl „ ' LPS 1996)¡C§ › §Q¥¸Western blot „ Œ ¯ Æ"k ˇ[' » ⁄oÒMMP-9 † ¥ " †{¤ ƒ„´I» ƒA § ¶⁄@ „ NF-κB ⁄§§ ¤ ' °ƒ]⁄l IκB-α† ¥ " †{ NF-κB † o – ‡ ƒiA| w„ ƒ„´I» ƒA § ¶⁄@ ¶q¡A´¥ H ` A ,^ ha¥operidol „ ' IκB-α ¤B⁄§– ·Q¡C ` ß˜⁄ˇ""⁄ , § @¸»Pƒb NF-κB T fi ¶· »… **3-3** ‰ , fi|' § Œt""¤ ƒ ¡C o†{ LPS ¤ ¿E 90 ⁄ ″ " ƒ X § ›„Œ†""G ¡ Â „Œ ˜ fi ' ¤ œo""† ›Mlysate¡AIκB-α† ¥ " †{ Haloperidol ‹ fl § ¤ ⁄ H † fi †y† ›M § ¢{'œ¯ª""› , § @¥¡C⁄U¤ ¥H90 ⁄ ˜`‹ THP-1 ¤ ¤ LPS¡A¤ ›P† ›M r ' ° Ł ¡Cƒ„ LPS ¤ ° Æ⁄§ •·¡A⁄ § §°»P⁄ ƒP¿ @ "" ƒ ¤ fl §¤ƒb THP-1 ⁄⁄ LPS ' ⁄ ª" haloperidol ⁄§«Æ¡A¥i˘ „ ¤ haloperidol fl MMP-9 " †{¡A¤^¥ D › ¤g§¥ ¤ NF-κB § ¤ LPS ƒbTHP-1 † ›M⁄' ⁄ ª"IκB-α pathway T fi § ¶ »…¡C¥fi¢ ¥ ~ ´†MƒÆ£ › , § @¸¡A¤˘–oNF-κB L"k translocate ƒ ƒP""• PV' ° ƒ⁄LPS»PLTA⁄ o⁄ § ƒ h« „ † Mfi⁄‹ ¡⁄ˇ MMP-9 ƒ]" †{¡A† ¥¤ ' x I " … ¤ »PÒMÆP ‹ ¡ˇ¡Afl ¶ ¡@ MMP-9 ""» fl »P† ¥ " †{¶q· ⁄ ¡Cƒ⁄ ˜m ¤B"" ƒ »P` A ,¤fh⁄ , ⁄ "v""¥ifl ' ¡C ' "" „ Œ "G¯ª¥ ¡A§Q¥¸LPS ¤ ¿E⁄ H † fi †y† »PM† ›MfiŁ(THP-1 cells) (O'Connell et al., 1998; Guha and Mackman, 2001)¡A¤˘–o´ ¿ ƒ]⁄l NF-κB translocate ƒ fi ⁄"¡A¥ifl ´ ¥ ‹ ¡⁄ˇ IKKβ† ¥ ƒ ›P "" "G(O'Connell et al., 1998)¡C¥i·£¤ ¥»„Œ ¯ § ¶ i ⁄ @B¡Cƒ ⁄§ › ""„ Œ • ⁄⁄ o †{¡Aƒb THP-1 † ›M⁄§Q¥¸TNF-α»P LPS ¤ ¿Efi ' » ⁄o"IκB-α† ¥ › , ⁄§fi ¶¡ƒ† 'œ¯ª""fi ‡§(TNF-α¡G15 ⁄ ˜`¡FLPS¡G90 ⁄ ˜`)¡A¥ifl › ƒ]‹ ƒ ª " ¤ ¿E fl‹ ¡⁄ˇ IKKs fi ¡A† ƒiA¤ ⁄ ƒP⁄§, fi|' ›P"" (O'Connell et al., 1998; Hawiger et al., 1999; Fischer et al., 1999)¡Cƒ ¥t⁄@‰g⁄ ˜m‹h¯ª¥

¥|¡B ƒ⁄ ˜m

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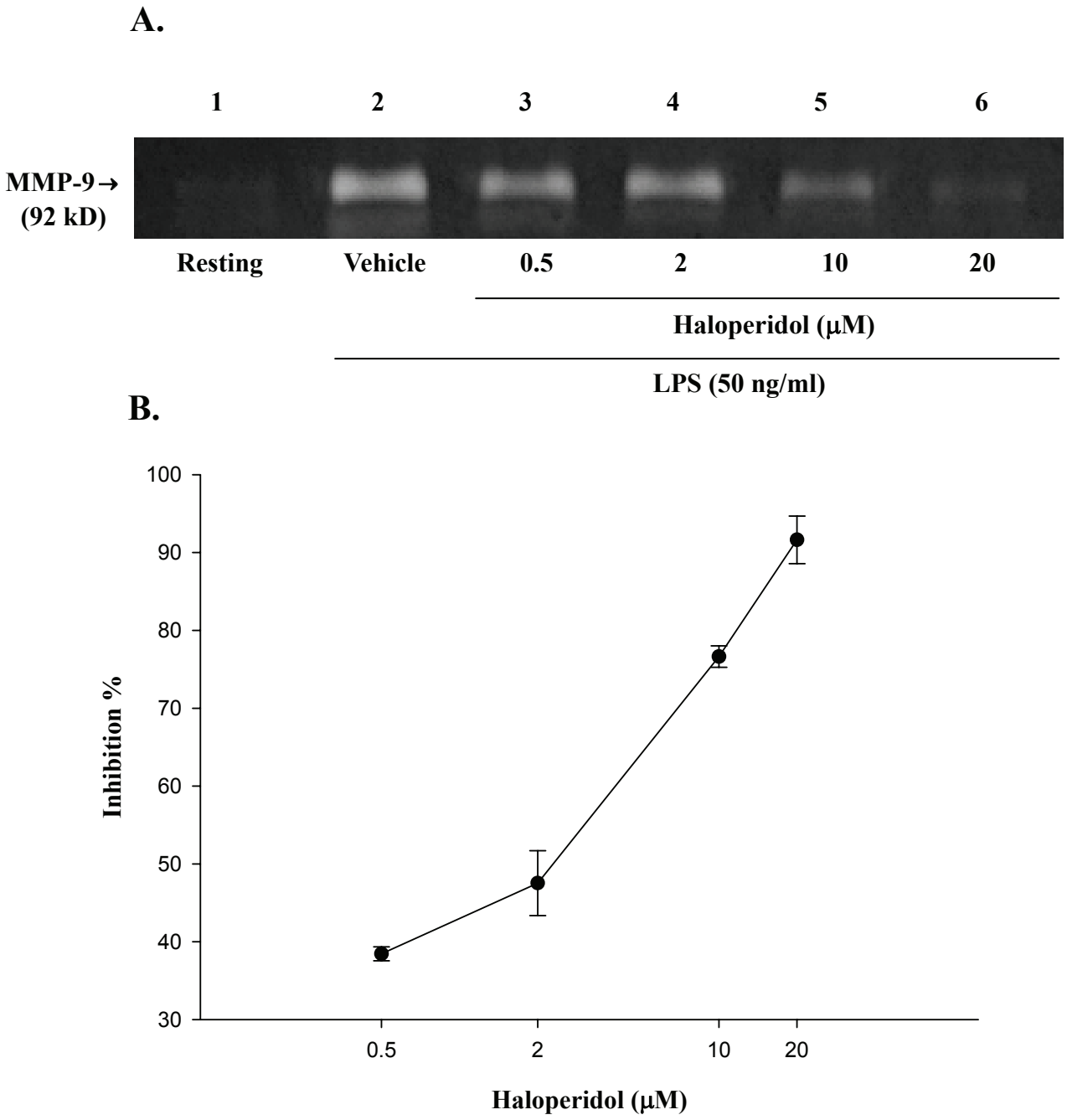


Figure 1. Effects of haloperidol on LPS̃induced enzymatic activity of matrix metalloproteinase-9 (MMP-9) in THP-1 cells. THP-1 cells (1×10^6 cells/ml) were dispensed on 24-well plates and treated with LPS (50 ng/ml) for 24 hours as indicated. Cells were treated with the indicated concentrations of haloperidol (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.

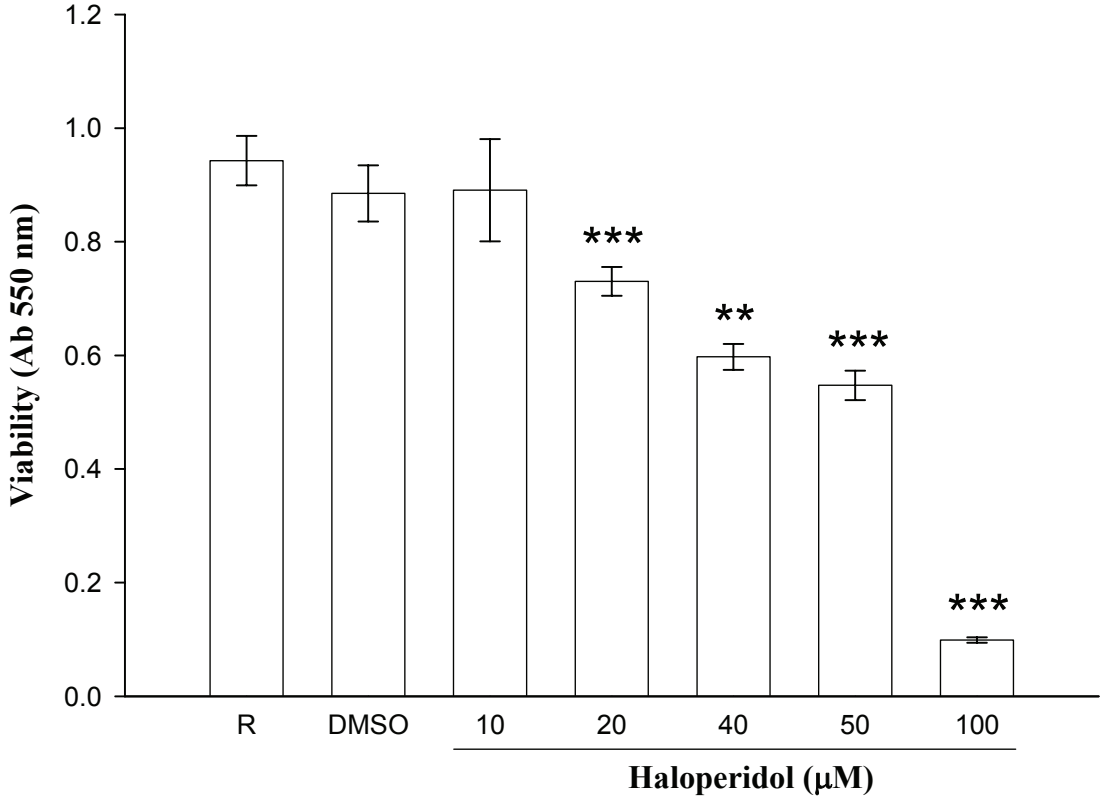


Figure 2. Cytotoxicity of haloperidol on THP-1 cells. THP-1 cells were treated with different concentration of haloperidol (10-100 μM) and incubated for 24 hrs. Cell viability was measured by a colorimetric assay at 550 nm based on the ability of mitochondria to reduced the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2, 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.

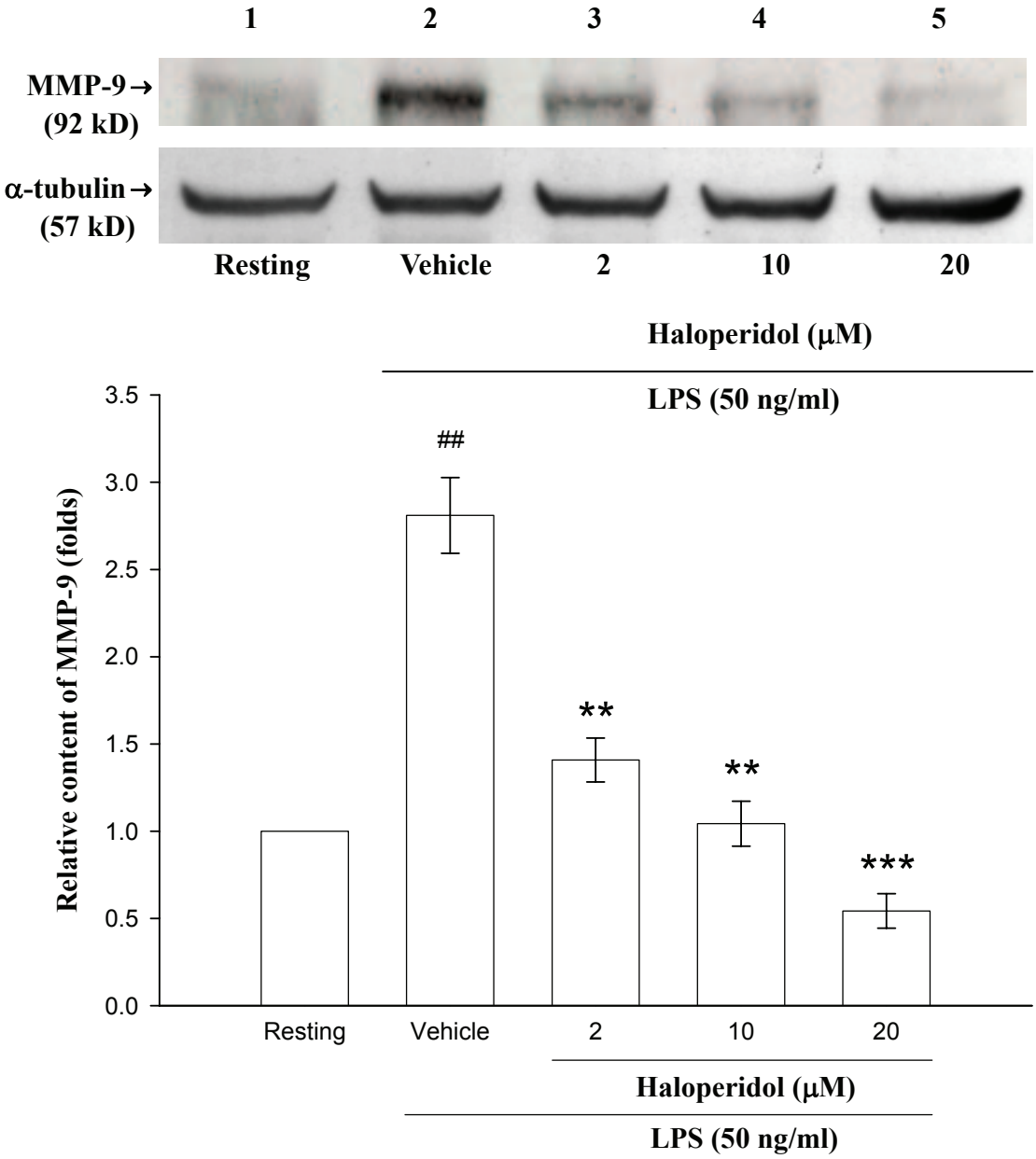


Figure 3. Effect of haloperidol on LPS-induced expression of matrix metalloproteinase-9 (MMP-9) from conditioned medium of THP-1 cells. THP-1 cells (1×10^6 cells/ml) were dispensed on 6-well plates treated with LPS (50 ng/ml) for 24 hrs as indicated. Cells were treated with indicated concentration of 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.

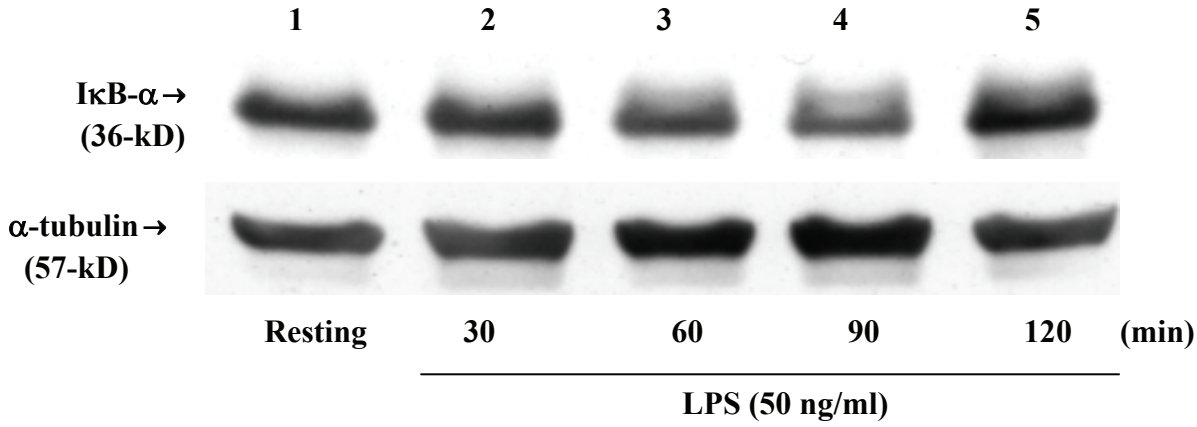


Figure 4. Western blot analysis demonstrating the time course on LPS-induced degradation of immunoreactive IκB-α in THP-1 cells (1×10^6 cells/ml). THP-1 cells were dispensed on 6-well plate and treated with LPS (lane 2, 30 min; lane 3, 60 min; lane 4, 90 min; land 5, 120 min) or control (lane 1, 120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

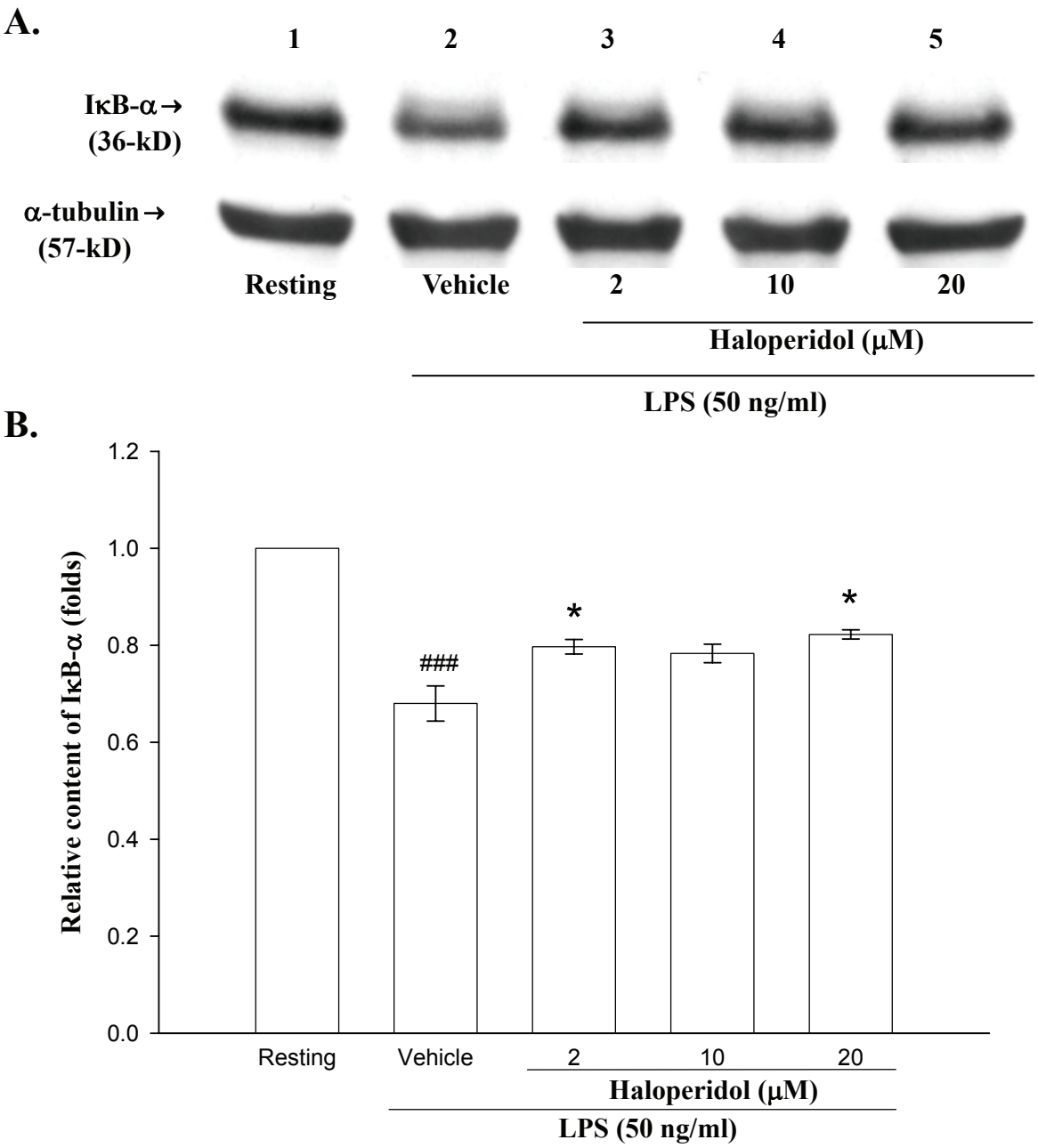


Figure 5. Effect of haloperidol on degradation of immunoreactive IκB-α in THP-1 cells. THP-1 cells (1×10^6 cells/ml) were dispensed on 6-well plate and treated with LPS (50 ng/ml) for 90 min as indicated. Cells were treated with haloperidol (lane 3, 2 μM; lane 4, 10 μM; lane 5, 20 μM) or vehicle (lane 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.

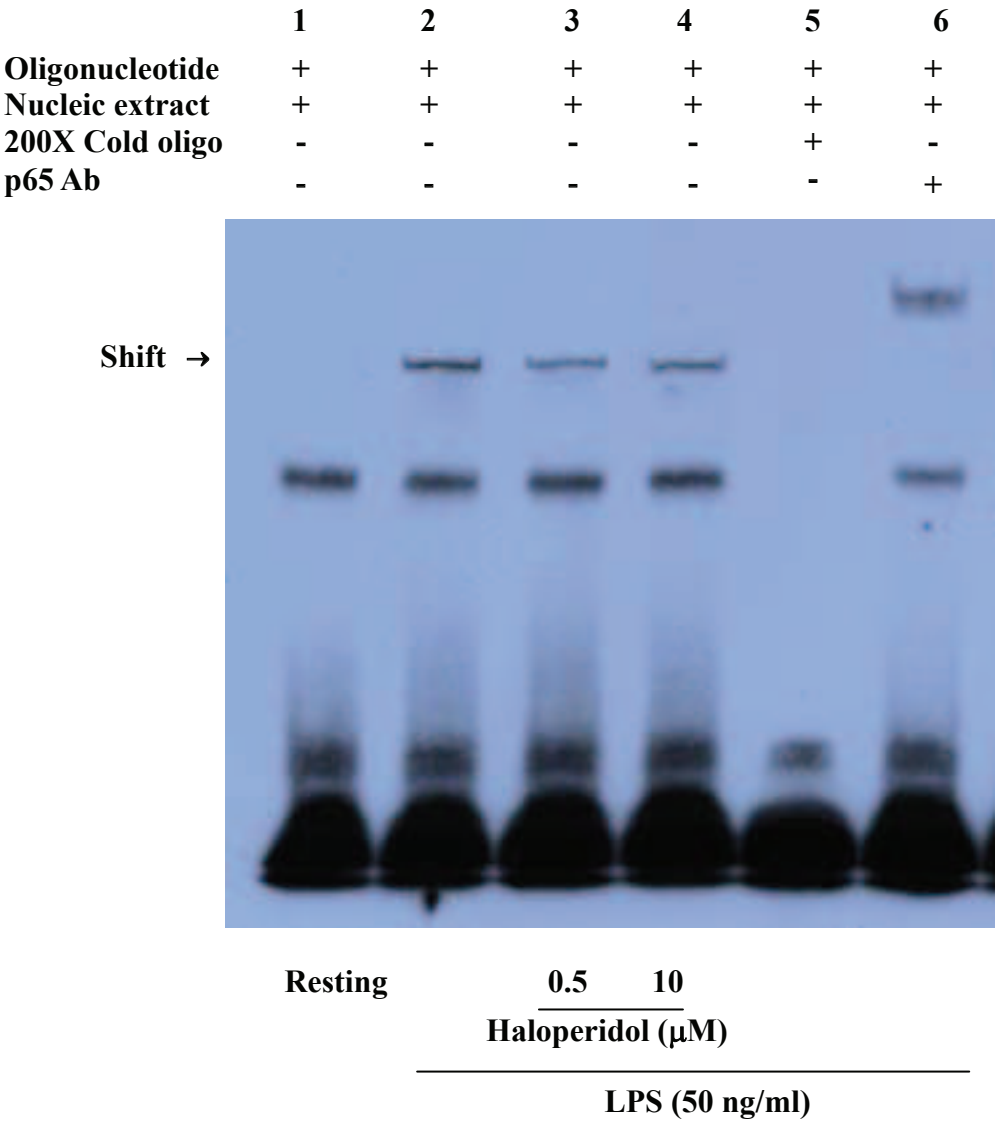
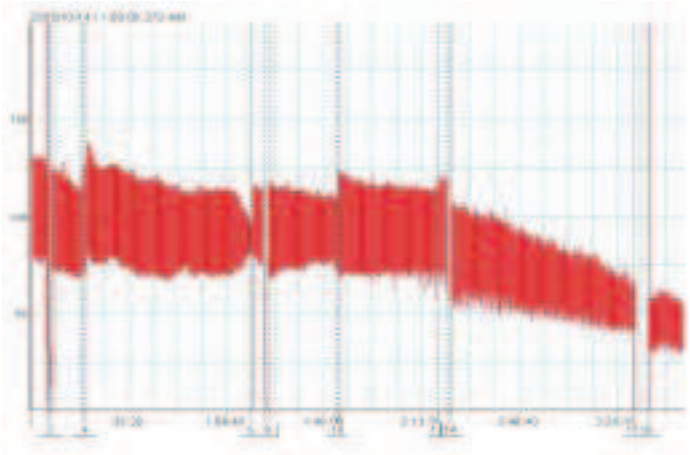


Figure 6. Effect of haloperidol on NF-κB activation in THP-1 cells. THP-1 cells (1×10^6 cells/ml) were dispensed on 6-well plate and treated with LPS (50 ng/ml) for 100 min as indicated. Cells were treated with haloperidol (lane 3, 0.5 μM; lane 4, 10 μM) or vehicle (lane 2) for 15 min before treatment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

A



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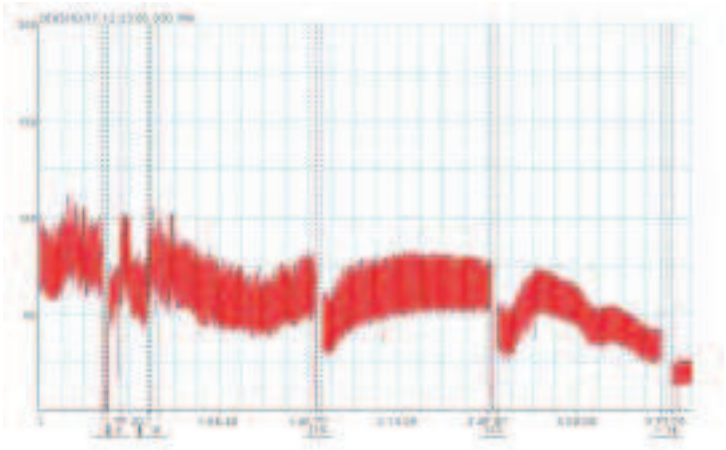
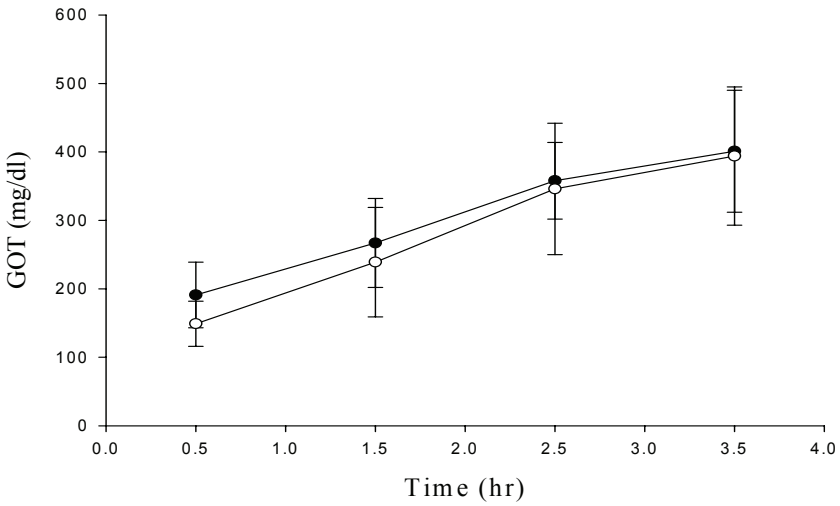


Figure 7. Time course of arterial blood pressure change in rats treated with bacterial components. Depicted are the changes in arterial blood pressure during the experimental period in LPS (5 mg/kg)-or LTA (10 mg/kg)-treated groups of rats. The results are representative examples of four similar experiments.

A.



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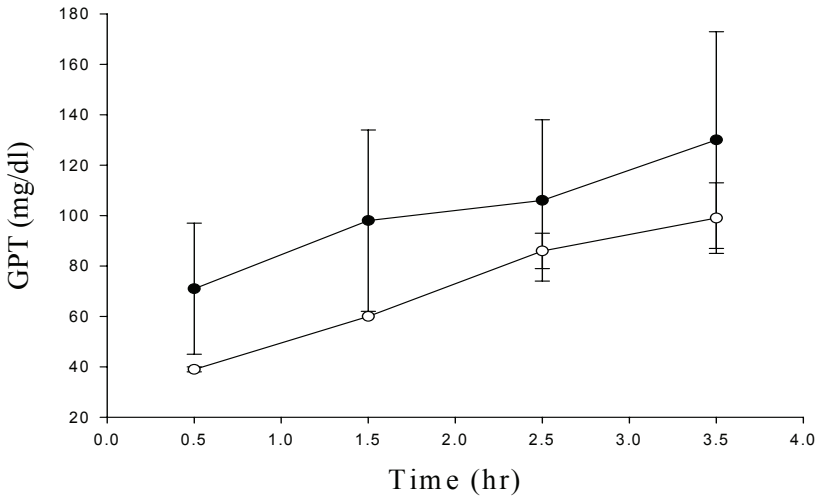
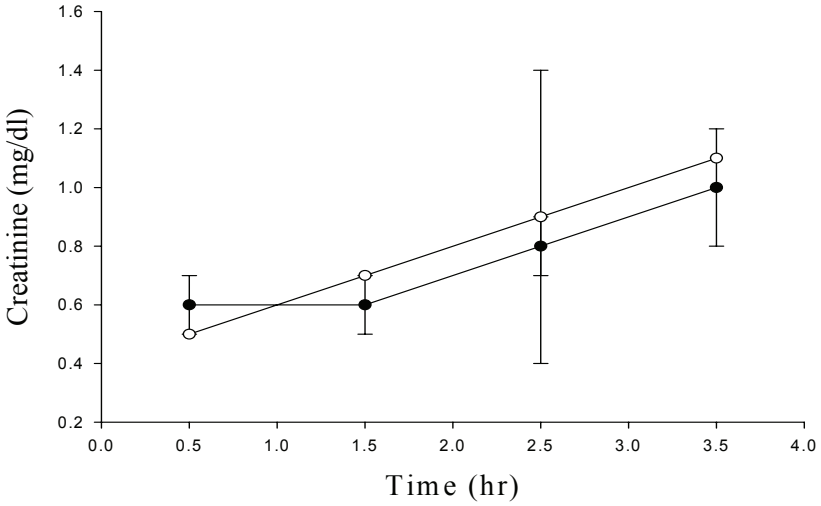


Figure 8. Time course of effects on sepsis-induced liver dysfunctions by LPS or LTA in rats. The serum of LTA (●)-or LPS (○)-treated animals were collected at indicated time. The data are representative example of three to four experiments.

A.



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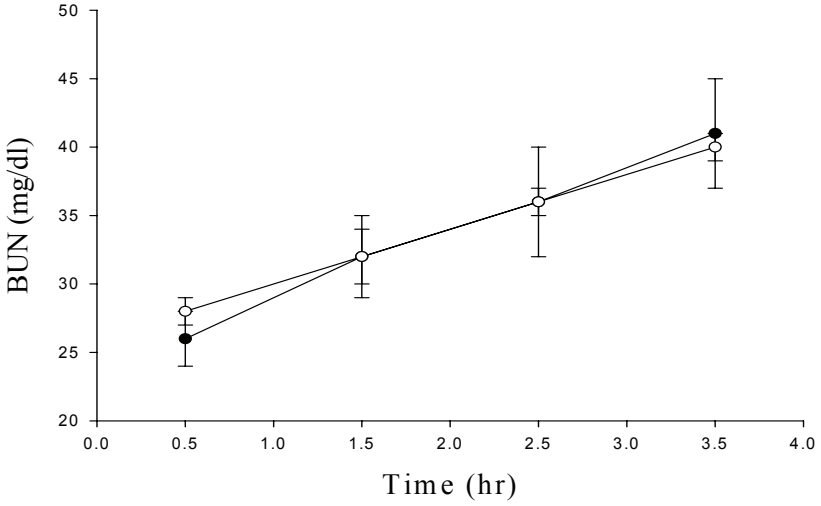

Figure 9. Time course of effects on sepsis-induced renal dysfunctions by LPS or LTA in rats. The serum of LTA (●)-or LPS (○)-treated animals were collected at indicated time. The data are representative example of three to four experiments.

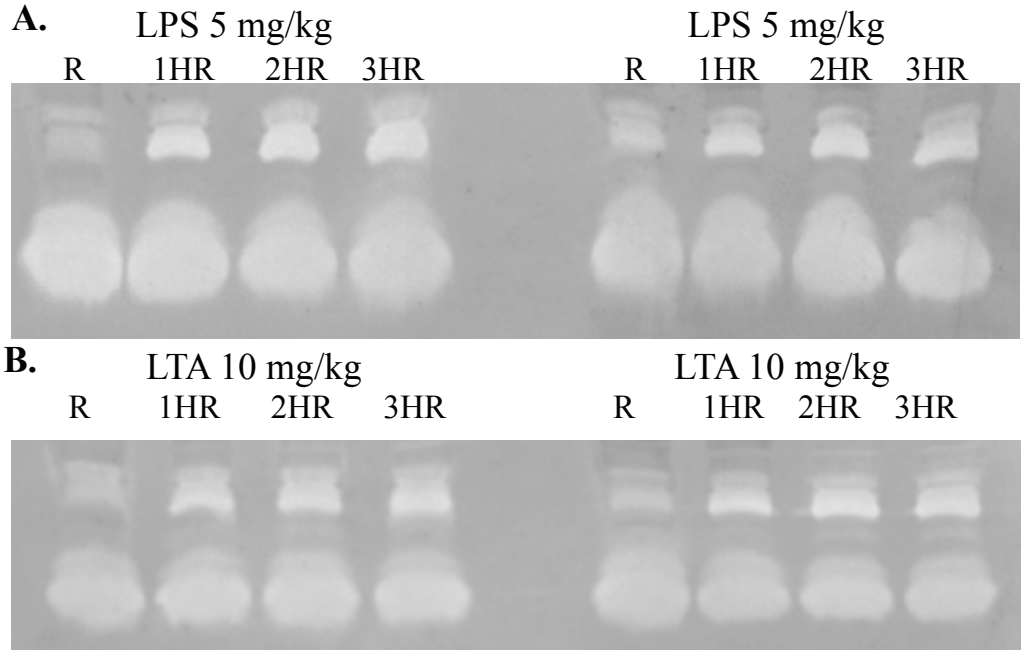

Figure 10. Time course of effects on serum-induced gelatinolysis by LPS-or LTA-treated in rats. The serum of LTA (●)-or LPS (○)-treated animals were collected at indicated time. The data are representative example of three to four experiments.