嘉南藥理科技大學專題研究計畫成果報告

探討 Etanercept 對於脂多醣在兔子引起發燒之解熱作用

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摘　　要

Etanercept是將TNF-α接受器p75和TNF-α結合的部位和人類IgG1免疫球蛋白中的Fc部位融合在一起，而形成一個融合蛋白。目前etanercept已被應用於臨床上治療一些發炎性的疾病，如類風濕性關節炎。最近的研究顯示利用TNF-α阻斷劑etanercept先處理可減少心臟衰竭大白鼠下視丘視旁核中glutamate的釋放、活性氧（reactive oxygen species, ROS）之產生及COX-2蛋白之表現。就我們所知，目前對於etanercept減少LPS之發燒反應是否經由抑制兔子循環中TNF-α的產生及下視丘麩胺酸-氫氧自由基-前列腺素E_2之途徑，仍然所知有限。因此，本研究將探討etanercept是否經由抑制靜脈注射LPS誘導下視丘麩胺酸、氫氧自由基、前列腺素 E_2及血漿中TNF-α的產生，而達到解熱作用。主要是利用先前埋於兔子下視丘前視區(preoptic region of anterior hypothalamus, POAH)之微透析探針來收集細胞外之透析物。氫氧自由基之測量則是利用水楊酸鹽(salicylate)可和氫氧自由基形成穩定的2, 3-二基苯甲酸(2, 3-dihydroxybenzoic acid, 2, 3-DHBA)之特性，來測量下視丘中2, 3- DHBA，以代表氫氧自由基之量，而麩胺酸則利用高效液體層析法(high performance liquid chromatography, HPLC)結合螢光偵測器來測量；前列腺素E_2之濃度是以酵素連接免疫吸附法(enzyme-linked immunosorbent assay, ELISA)來測量。而且，我們將進一步利用西方墨點法探討IL-1ra減少下視丘前列腺素E_2之產生是否經由抑制下游環氧化酶-2 (cyclooxygenase-2, COX-2)蛋白表現所致。因此，本研究計畫結果將可揭露etanercept可能透過抑制LPS在兔子誘導產生循環中TNF-α及下視丘之麩胺酸、氫氧自由基及前列腺E_2，而達到解熱作用。

報告內容

（一） 研究目的：

生物體溫恆定之維持，主要是受到下視丘前視區(preoptic region of the anterior hypothalamus, POAH)的調控，因其內含有許多對溫度變化敏感的神經元，可以有效地整合來自中樞或週邊的體溫接受器(thermoreceptors)所傳入的訊息，感應外界環境溫度的變化，而使體溫維持在一定的溫度，即體溫設定點(set-point temperature)(Boulant, 1998)。發燒是臨床疾病上常被描述的症狀，尤其常見於因微生物感染所引起的發炎反應，其定義為下視丘體溫調控中樞之設定點(set point of thermoregulatory center)高於原來的設定點，使得體溫高於正常之恆定溫度(Kluger, 1991)。目前有關於熱原性發燒之研究，以細菌內毒素(endotoxin)或脂多醣(lipopolysaccharide, LPS)所引起發燒反應之研究最為詳盡。LPS 是革蘭氏陰性菌(Gram negative bacteria)細胞壁外膜的主要成份，細菌內毒素進入體內能引起各式各樣的臨床症狀，如畏寒(chills)、發燒、頭痛(headache)、敗血症(sepsis)、敗血性休克(septic shock)(Parrillo, 1993)。在美國，每年大約有21 萬人死於敗血症(Angus, et al., 2001)，而且敗血性休克的死亡率，更高達50 %(Salvo et al., 1995)。目前LPS引起發燒的機轉，可能是LPS刺激單核球(monocyte)或巨噬細胞(macrophage)產生一些致熱性細胞激素(pyrogenic cytokines)如TNF-α 、IL-1、IL-6，而來導致發燒(Luheshi,1998；Dinarello,1999)。至於這些血液中的pyrogenic cytokines 如何作用在腦部，而引起發燒反應。目前的可能機轉是這些pyrogenic cytokines 隨著血液循環進入位於第三腦室前面，視交叉(optic chiasma)上面的一個circumventricular organ，即所謂血管脈叢終端器官(organum vasculosum laminae terminalis, OVLT)，由於OVLT靠近下視丘體溫調控中樞，而且此區域含有豐富微血管及缺乏血腦障礙(blood brain barrier) (Hashimoto et al., 1994)，所以可允許一些血液中之致熱原進入此區域，特別是IL-1 ，而刺激OVLT附近的內皮細胞、微小膠細胞中cyclooxygenase (COX-2)基因之表現(Lacroix and Rivest, 1998; Quan et al., 1998)，進而產生前列腺素E_2 (prostaglandin E_2, PGE_2)(Komaki et al., 1992)，這些PGE_2 可直接擴散至下視丘前視區(Stitt,1986)或作用分佈在OVLT 內，而投射至下視丘的神經元(Ota et al., 1997)，進而提昇體溫控制中樞之設定點，使身體增加產熱、減少散熱，進而導致發燒的現象(Saper and Breder, 1994)。目前所知除了PGE_2是致熱源引起發燒的重要媒介物質外，最近研究亦發現glutamate 和熱原性發燒反應有關。利用SEA 靜脈注射至兔子內，並利用微透析(microdialysis)方法在OVLT區域收集腦脊髓液，結果發現SEA 在兔子內可引起發燒，並造成OVLT區域glutamate 之上升，而且SEA 所引起之發燒與glutamate 之釋放都可被NMDA receptor antagonist MK-801及ketamine所抑制(Huang et al., 2001)；除此之外，我們亦發現臨床常用的解熱劑acetaminophen 及aspirin 都可透過抑制glutamate 的釋放，而來達到解熱的作用(Huang et al., 2003a；Huang et al., 2003b)。

最近的研究亦發現麩胺酸(glutamate)及氫氧自由基(hydroxyl radical)和LPS的發燒有關（Huang et al., 2006; Kao et al., 2007a）。Abramov等人發現在cortical neurons因oxygen and glucose deprivation（OGD）造成glutamate的釋放，可透過增加細胞內Ca^{2+}的濃度，而活化NADPH oxidase，而造成ROS的增加（Abramov et al., 2007），而且在老鼠腦部紋狀體(striatum)

給予glutamate，利用微透析技術都可測得hydroxyl radical 有增加的現象(Lancelot et al., 1998a；Lancelot et al., 1998b)。同樣地，在體外(in vitro)實驗當中，亦發現NMDA 接受器之活化可造成細胞產生大量的oxygen-derived free radical (Lafon-Cazal et al., 1993；Lafon-Cazal et al., 1995; Culcasi et al., 1994；Gunasekar et al., 1995)。可見，LPS可能是透過glutamate之釋放，進而誘導hydroxyl radical之產生，而引起發燒反應。

ROS and Fever

所謂反應性氧自由基（reactive oxygen species；ROS）是泛指原子之電子軌道有不成對電子且具有高度反應性，可氧化或還原其他原子，其包含許多種類的自由基，例如：氫氧自由基（hydroxyl radical；$OH^{\cdot}$）、超氧自由基（superoxide radical；O_2^{-}）、過氧化氫（hydrogen peroxide；H_2O_2）、一氧化氮自由基（nitric oxide；$NO^{\cdot}$）等化合物（Bal-Price et al., 2002；Ciuseppe,2002；Sies,1993），這些自由基可由體內許多細胞產生，擔任重要生理功能。其中過氧化氫（H_2O_2）是一個很特別的分子，可自由通過細胞膜，且在金屬離子催化下Fenton reaction, Haber Weiss reaction）自行分解為氫氧自由基(hydroxyl radical；$OH^{\cdot}$），氫氧自由基是一種反應性超強的自由基，在正常生理狀態下，細胞本身所釋出的自由基具有防禦的功能，然而在異常大量產生情況下，由於其含有不成對電子，因此非常不穩定，經常會攻擊細胞內之大分子，如脂質、去氧核醣核酸、蛋白質之共價鍵，而引起脂質氧化、DNA受損及破壞電子傳遞鏈的酵素活性，因此造成細胞能量衰竭而死亡(Cuzzocrea et al., 2001)。除此之外，自由基亦和腦部缺血、中風所造成的神經傷害；內毒素所引起之休克、發炎反應、及動脈硬化(atherosclerosis)有關(Gunasekar et al., 1995; Guo and Ward, 2000; Podrez et al., 2000; Yang and Lin, 2002)。最近的研究亦發現reactive oxygen species 和熱原性之發燒有關。Riedel等人將LPS 週邊給予大白鼠或兔子可在血漿中測得oxygen radical 有增加現象，而且利用一些hydroxyl radical scavenger 如methylene blue、α-lipoic acid及aspirin都可抑制LPS 所引起之發燒及血漿oxygen radical 之產生(Riedel and Maulik, 1999; Riedal et al., 2003)。最近研究結果亦證實靜脈注射LPS可造成腦部hydroxyl radical的增加，而且和LPS的劑量呈現正相關，利用抗氧化劑α-lipoic acid及aspirin都可抑制LPS所誘導腦部hydroxyl radical 的產生，而達到解熱的效果（Huang et al., 2006; Kao et al., 2007b）。同樣地，我們發現中藥黃芩苷(baicalin）的解熱作用亦是透過抑制hydroxyl radical的產生所致（Tasi et al., 2006），由此可知在LPS發燒過程中確實會有hydroxyl radical之參與。目前許多證據顯示LPS可刺激腦部膠細胞產生ROS，而活化NF-κB (Dimayuga et al., 2007; Lee et al., 2006) 。最近，我們的研究發現NF-κB亦和熱原性發燒有關，利用NF-κB抑制劑如pyrrolidine dithio carbamate（PDTC）sodium pyrithione 及 N-acetyl-L-cysteine（NAC）靜脈注射至兔子內都可抑制LPS（Lee et al., 2003）及SEA（Shao et al., 2004）刺激週邊血單核細胞（peripheral blood mononuclear cells, PBMC）所產生之IL-1β、TNF-α及IL-6，進而抑制其所引起之發燒反應。Kozak等人將LPS靜脈注射至NF-κB基因缺乏的小鼠中，發現並不會引起發燒反應（Kozak et al., 2006），由此可知週邊NF-κB的活化在熱原性發燒扮演重要角色。先前研究顯示靜脈注射LPS, IL-1β, TNF-α都可在老鼠腦部內皮細胞及小神經膠細胞(microglia)偵測到COX-2基因之表現(Lacroix and Rivest, 1998；Quan et al., 1998)。事實上，先前的研究已證實LPS，金黃色葡萄球菌腸毒素A（staphylococcal enterotoxin A, SEA）及

IL-1β都可以透過活化腦部iNOS-COX-2途徑，而在兔子內引起發燒反應（Huang et al., 1997；Lin and Lin,1996），然而對於外生性致熱原LPS所引起之發燒是否透過腦中產生之ROS而活化NF-kB，並導致COX-2大量表現及PGE_2的產生，而引起發燒反應，目前仍然未知。

The clinical study of etanercept

TNF-α接受器主要有兩種型態，分別為p55及p75，而兩種型態接受器在許多細胞都有表現，如神經細胞、膠細胞（glia）（Benveniste and Benos, 1995）。Etanercept是經由基因工程方法將TNF-α接受器p75和TNF-α結合的部位和人類IgG1免疫球蛋白中的Fc部位融合在一起，而形成一個融合蛋白（fusion protein）（Moreland et al., 1997）。在融合蛋白中使用免疫蛋白Fc部位作為融合部位主要是讓本藥物具有較長的半衰期。Etanercept因能和TNF-α結合，而減少TNF-α和TNF-α接受器的作用，因此etanercept被認為是TNF結合至細胞膜接受器的強效競爭性抑制劑（Mease, 2002）。

Etanercept 因可和體液中的 TNF-α 結合，而減少對細胞膜上 TNF-α 接受器的刺激，進而阻斷其訊息傳遞，而降低發炎反應的發生。目前 etanercept 已被應用於臨床上治療一些發炎性的疾病，如乾癬性關節炎（Mease et al., 2000）、僵直性脊椎炎（Eou et al., 2006）、慢性氣喘（Howarth et al., 2005）及類風濕性關節炎（Kalden,2002;Bathon etal.,2000），而且 Korotkova 等人亦發現 etanercept 可抑制 LPS 刺激關節液中巨噬細胞 COX-2 之表現及 PGE_{-2} 的產生（Korotkova, 2000）。

Etanercept 作為 TNF-α 接受器的強效競爭性抑制劑，因此過去也將其應用到不同動物模式。在脊髓損傷（spinal cord injury）研究中顯示在脊髓受創後會造成水腫、嗜中性白血球匯集和細胞激素大量產生，而腹腔注射 etanercept 能夠有效降低發炎反應、組織損傷及嗜中性白血球匯集到損傷區。此外，治療後亦可減少脊髓損傷區一氧化氮合成酶（inducible nitric-oxide synthase, iNOS）、COX-2 及細胞激素 TNF-α 及 IL-1β 的表現，而且能夠恢復脊髓損傷小鼠後肢的行動功能（Genovese et al., 2006）。在腦部損傷的相關研究，Campbell 等人利用 IL-1β微量注射於大鼠腦室中，以模擬損傷後的發炎反應，藉此可引起急性反應（acute phane response），而使肝臟大量表現 TNF-α；而週邊給予 etanercept 能夠減少肝臟 TNF-α 的表現，因而降低腦部的損傷（Campbell et al.,2007）。

最近的研究顯示心臟衰竭（heart failure）大白鼠腦中下視丘視旁核（paraventricular nucleus, PVN）TNF-α 有大量增加，而且 TNF-α 可造成視旁核中 glutamate 及 superoxide 的增加，而引起交感神經過度興奮，並加速心臟衰竭的進行。利用 TNF-α 阻斷劑 etanercept 或 pentoxifylline 腦室注射可減少視旁核中 glutamate 的含量（Kamg et al., 2009）；在週邊給予 etanercept 或 pentoxifylline 可減少下視丘視旁核中活性氧（reactive oxygen species, ROS）之產生（Guggilam et al., 2007）或 COX-2 蛋白之表現（Kang et al., 2006），而改善心臟衰竭的狀況。

興奮劑胺基酸運輸器（excitatory amino acid transporter, EAAT2）是在中樞神經系統內負責將glutamate從突觸中清除（Tanaka etal.,1997），若glutamate之回收受損，將會導致過多細胞外glutamate的堆積，而造成神經細胞的死亡（Choi,1988）。最近的研究發現TNF-α可抑制EATT2的表現，而造成細胞外glutamate之增加（Pickeing etal., 2005; Sitcheran e tal., 2005）。最近本人研究亦發現腦室給予TNF-α會造成glutamate及hydroxyl

radical的增加，而引起發燒反應（Tasi et al.,2006; Huang e tal., 2008）。總之，本計畫之執行若能得到預期的結果，則對於etanercept之解熱機轉將有更深入之了解，除了透過抑制致熱性細胞激素之產生外，可能也透過抑制腦部glutamate及hydroxyl radical之產生，而影響下游COX-2表現及PGE_2之產生，而來達到解熱作用。

（二） 研究方法：

(1) Surgical Techniques

Adult male New Zealand White rabbits, weighing 2.0-3.2 kg at the start of the study, will be used. Animal chow and tap water will be available ad libitum. The ambient temperature (Ta) in the animal room was 22 ± 1oC; light and darkness will be alternated, with light on from 6:00 a.m. to 6:00 p.m.

The animals will be received a prophylactic injection of the antibiotic, gentamicin sulfate (18 mg/kg, im), and 1 h later will be anesthetized with sodium pentobarbital (30 mg/kg, iv). A sterile, microdialysis probe guide cannula with an indwelling stylet will be implanted stereotaxically into the left preoptic anterior hypothalamus (POAH) of each rabbit [coordinates, in mm, A2.5, L2.0, V15] or 6-mm-long, 17-gauge, thin-wall stainless steel into the right lateral cerebral ventricle [coordinates, in mm, P 4.0, R 3.0, V5] according to the atlas of Sawyer et al. (1954) and fixed to the skull with dental acrylic cement and four self-tapping, miniature, stainless steel screws. After surgery, the guide cannula will be plugged with a stylet, and animas will be returned to their cages for a minimal recovery period of 1 week.

(2) Animals and Pyrogen Assay

Experiments will be conducted between 0900 and 1900 h, with each animal being used atan interval of not less than 7 days. Throughout the experiment, colonic temperatures will be measured every minute with a copper constantan thermocouple connected to a thermometer（HR1300, Yokogawa, Tokyo, Japan）.The colonic temperature of each animal will be allowed to stabilize for at least 120 min before any injections. Only animals whose body temperatures are stable and in the range of 38.6 to 39.7 ℃ will be used to determine the effect of drug application. All experimental animals will be obtained from the animal center of Chi-Mei Medical Center. (Tainan, Taiwan, ROC). The animal protocol described here will be approved by the animal ethical committee of Ch-Mei Medical Center.

(3) Microdialysis for detection extracellular glutamate

At least 1 h before an experiment, the indwelling stylet of the guide cannula will be replaced by a CMA-12 microdialysis probe purchased from CMA/Microdialysis (RosLagsvägen, Stockholm, Sweden) so that its dialysis membrane tip protruded exactly 4.0 mm beyond the guide tube. Dialysate samples will be collected at 20-min intervals over a period of 10 hours, and stored at -70 ℃ until glutamate determination. For measurement of extracellular glutamate in anterior hypothalamus of rabbit brain, a CMA/12 microdialysis probe will be inserted into the guiding cannula. According to the methods described previously by Huang et al (Huang et al., 2004) with slight modification, an equilibrium period of 120 min without sampling will be allowed after probe

insertion. The microdialysis will be perfused at 1.2 l /min, and the dialysates will be sampled in microvials. The dialysates will be collected every 20 min in a CMA/140 fraction collector. Aliquots of dialysates (2 l) will be injected onto a CMA 600 Microdialysis analyzer for measurement of glutamate. The thermal experiments will be started after showing stabilization in four consecutive samples. In the present results, an equilibrium period of 4 h assures a stable level of the extracellular substance tested. Glutamate is enzymatically oxidized by glutamate oxidase. The hydrogen peroxide formed reacts with N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine and 4-amino-antipyrine. This reaction is catalyzed by peroxidase and yields the red-violet colored quinonediimine. The rate of formation is measured photometrically at 546 nm and is proportional to the glutamate.

(4) Determination of extracellular hydroxyl radical in the hypothalamus

For measurement of extracellular hydroxyl radical in the POAH, the implanted probe will be perfused with artificial cerebrospinal fluid (aCSF) containing 10 mM salicylic acid by a high pressure pump (CMA/Microdialysis; RosLagsvägen, Stockholm, Sweden) at a flow rate of 1.2 µl/min (Huang et al., 2006). It is known that salicylate can react with hydroxyl radical to generate stable dihydroxybenzoic acid (DHBA) derivatives, particular 2,3-DHBA, which can be used as in vivo indices of hydroxyl radical levels (Halliwell et al., 1991). The 2,3-DHBA in dialysates will be measured by high-performance liquid chromatography with a two-channel electrochemical detector (LC-4C, BAS, Bioanalytical System, West Lafacyette, USA).

(5) Measurement of prostaglandin E_2 in the hypothalamus

For measurement of hypothalamic PGE2, the dialysis system will be connected to microdialysis pump and perfused with artifical cerebrospinal fluid at a flow rate of 1.2 µl/min. The unanesthetized animals will be restrained in rabbit stocks for at least 120 min to achieve a stable dialysis level of PGE2. Dialysis samples from the POAH will be collected into microdialysis vial at 60 min intervals for 8 h and they will be stored at -80oC until analysed within 7 days. Immunoreactive PGE2 concentrations in dialysates will be determined using commercially available enzyme immunoassay kits (Cayman Chemicals Co, Ann Arbor, MI). Triplicate aliquots of 50 µl sample will be added to each well of plate and each sample will be assayed at a minimum of two dilutions. The limits of quantification for PGE2 is 20 pg/ml.

（三）結果：

一、腦室注射可抑制 LPS 發燒反應

如圖(一)及表（一）所示，靜脈注射LPS(2μg/kg)可引起雙峯之發燒反應，分別在90分鐘及180分鐘，而且這些發燒反應都可被腦室注射etanercept (10、50、100ng)所抑制，而且呈現劑量相關的抑制。

二、Etanercept 可抑制 LPS 在下視丘誘導麩胺酸及氫氧自由基之產生

圖(二)及圖(三)顯示，靜脈注射LPS(2μg/kg)，仍可誘導腦部麩胺酸及氫氧自由基之產生，而且呈現雙峰之現象，分別在90分鐘及在180分鐘，其上升與肛溫是同步，在LPS注射前1小時，在腦室注射etanercept (10、50、100ng)仍可有效抑制LPS在下視丘所產生之麩胺酸及氫氧自由基，並且呈現劑量相關的現象，如表（二）。

三、Etanercept 可抑制 LPS 在下視丘產生之 PGE_2

圖(四)顯示先用etanercept (10-100ng) 腦室注射，1小時候後再靜脈注射LPS(2μg/kg)可有效減少LPS刺激腦部產生之PGE_2。

（四）討論：

利用低劑量的致熱原如LPS可產生單峯之發燒反應，而如果使用高劑量之致熱原，則可產生雙峯之發燒 (Romanovsky, et al, 1998)，而且在很多動物都已經證實，包括兔子(Dinarello, 2004) 及大白鼠 (Romanovsky, et al, 1998)。本實驗利用低劑量(0.5μg/kg) 可產生單峯發燒，而高劑量(2-10 μg/kg) 可產生雙峯之發燒，其結果是和上述作者之實驗相符，其高峯是出現在LPS打後90分鐘及180分鐘。

Etanercept是經由基因工程方法將TNF-α接受器p75和TNF-α結合的部位和人類IgG1免疫球蛋白中的Fc部位融合在一起，而形成一個融合蛋白（fusion protein）（Moreland et al., 1997）。目前etanercept已被應用於臨床上治療一些發炎性的疾病，如乾癬性關節炎（Mease et al., 2000）、僵直性脊椎炎（Eou et al., 2006）、慢性氣喘（Howarth et al., 2005）及類風濕性關節炎（Kalden,2002;Bathon etal.,2000），而且Korotkova等人亦發現etanercept可抑制LPS刺激關節液中巨噬細胞COX-2之表現及PGE_2的產生（Korotkova, 2000）。

本實驗最重要的發現主要是etanercept可透過抑制LPS在腦部誘導所產生之麩胺酸、氫氧自由基及 PGE_2，而達到解熱的作用，而這在本人之其他實驗亦證實 baicalin (Huang et al, 2006)及curcumin (Huang et al, 2008) 都可透過相同途徑來達到解熱的作用。總之，以IL-10 1小時前注射，可抑制LPS之發燒反應，而其機轉主要是透過抑制LPS所誘導下視丘之麩胺酸-氫氧自由基途徑及 PGE_2所產生，而達到解熱之作用。

（五）實驗部份：

Fig.1

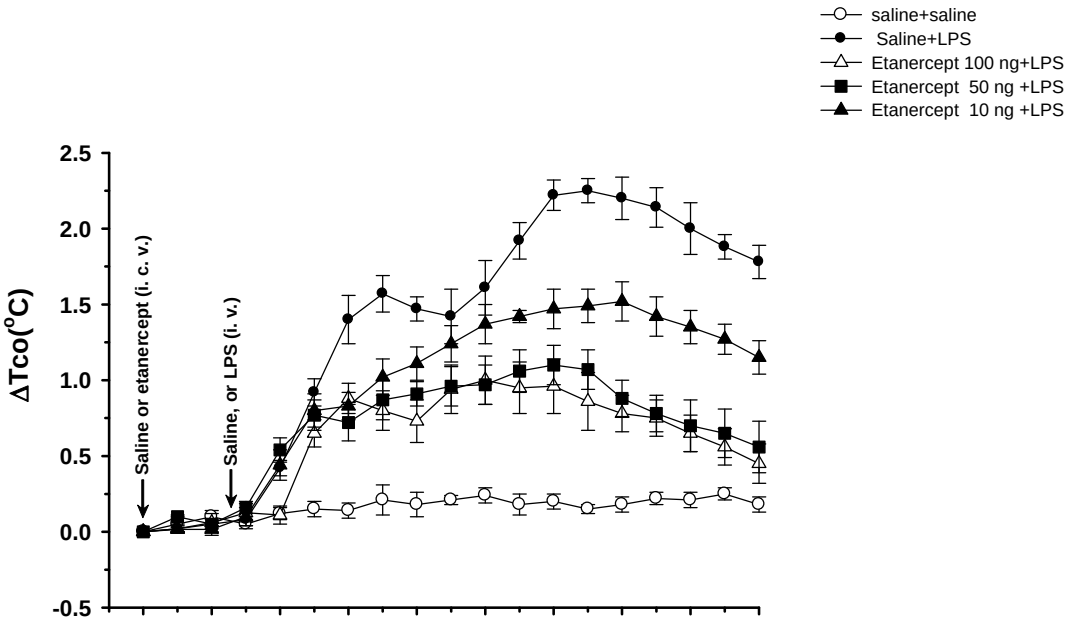

Fig. 1. Mean ± SEM changes in core temperature (Tco) in rabbits injected intracerebroventricular (i.c.v.) with either saline plus saline (i. v.)(○) (n=6), saline(i.c.v.) plus LPS (i. v.) (2 μg/ kg) (●) (n=6), Etanercept 100 ng(i.c.v.) plus LPS (2 μg/kg) (i. v.) (△) (n=6), Etanercept at 50 ng(i.c.v.) plus LPS (2 μg/kg) (i. v.) (■) (n=6), Etanercept at 10 ng(i.c.v.) plus LPS (2 μg/kg) (i. v.) (▲) (n=8). †P < 0.05, significantly different from corresponding control values (olive oil plus saline group) (ANOVA followed by Dunnett's test);* P < 0.05, significantly different from corresponding control values (olive oil plus LPS group) (ANOVA followed by Dunnett's test).

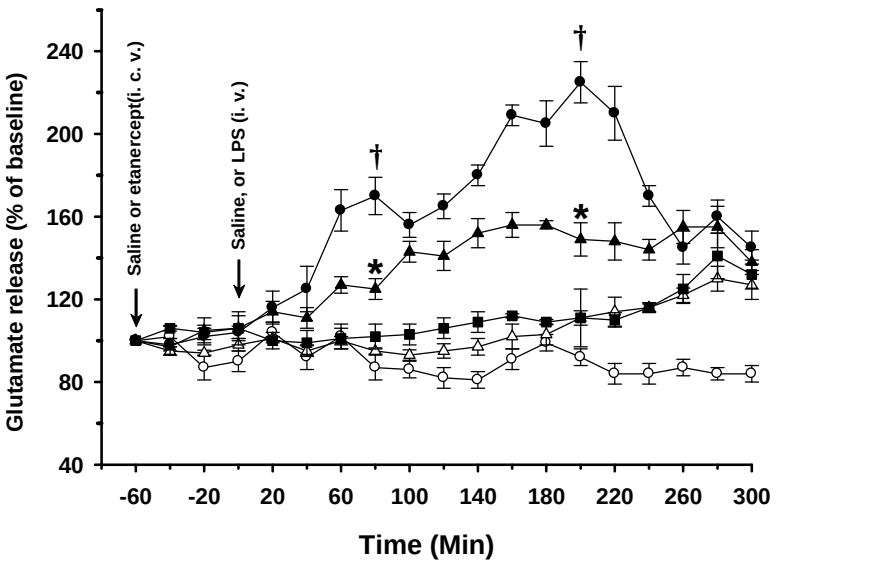

Fig. 2. Mean ± SEM changes in glutamate (Glu) release in POAH with in rabbits injected intracerebroventricular (i.c.v.) with either saline plus saline (i. v.)(○) (n=6), saline(i.c.v.) plus LPS (i. v.) (2 μg/ kg) (●) (n=6), Etanercept at 100 ng(i.c.v.) plus LPS (2 μg/kg) (i. v.) (△) (n=6), Etanercept at 50 ng(i.c.v.) plus LPS (2 μg/kg) (i. v.) (■) (n=6), Etanercept at 10 ng(i.c.v.) plus LPS (2 μg/kg) (i. v.) (▲) (n=8) † $P < 0.05$, significantly different from corresponding control values (olive oil plus saline group) (ANOVA followed by Dunnett's test); * $P < 0.05$, significantly different from corresponding control values (olive oil plus LPS group) (ANOVA followed by Dunnett's test).

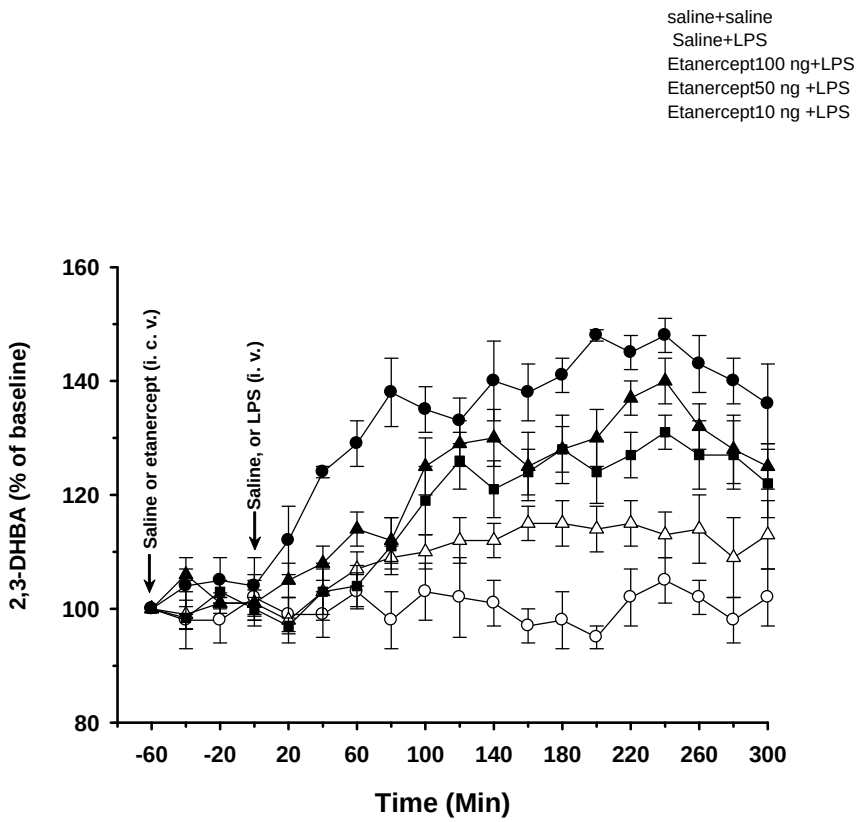

Fig. 3. Mean ± SEM changes in hydroxyl radical generation in POAH with in rabbits injected intracerebroventricular (i.c.v.) with either saline plus saline (i. v.)(○) (n=6), saline(i.c.v.) plus LPS (i. v.) (2 μg/ kg) (●) (n=6), Etanercept at 100 ng(i.c.v.) plus LPS (2 μg/kg) (i. v.) (△) (n=6), Etanercept at 50 ng(i.c.v.) plus LPS (2 μg/kg) (i. v.) (■) (n=6), Etanercept at 10 ng(i.c.v.) plus LPS (2 μg/kg) (i. v.) (▲) (n=8) † $P < 0.05$, significantly different from corresponding control values (olive oil plus saline group) (ANOVA followed by Dunnett's test); * $P < 0.05$, significantly different from corresponding control values (olive oil plus LPS group) (ANOVA followed by Dunnett's test).

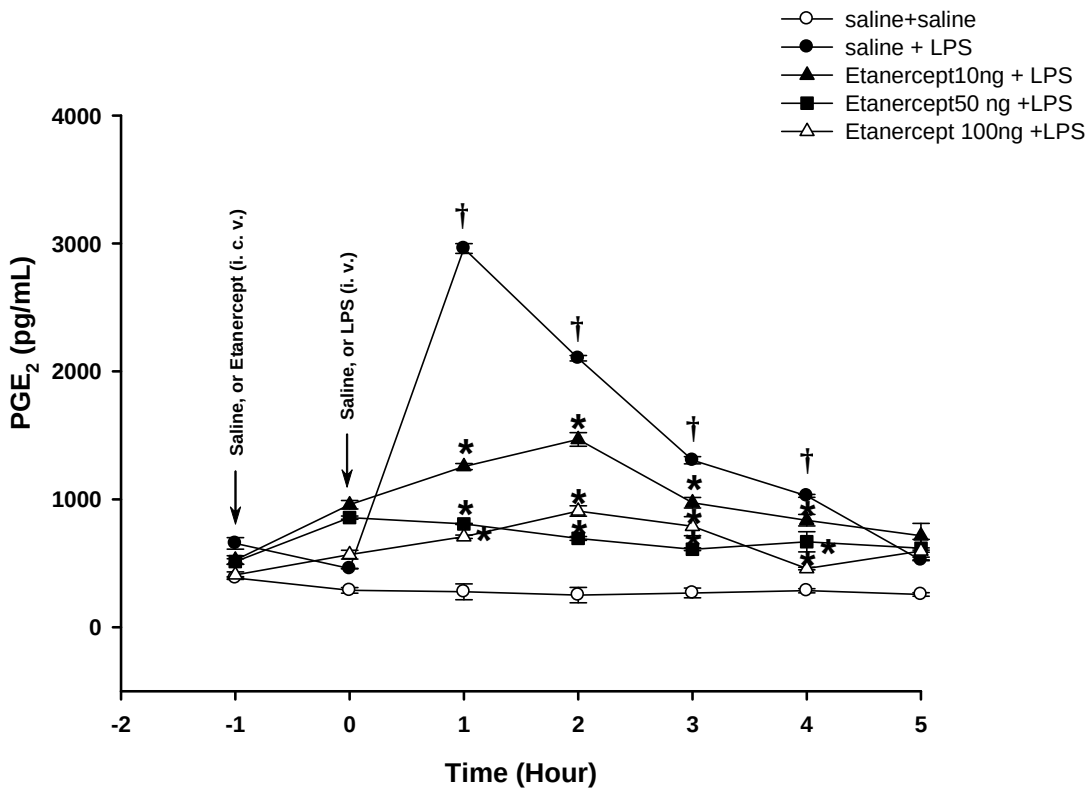

Fig. 4. Mean ± SEM changes in prostaglandin E_2 generation in POAH with in rabbits injected intracerebroventricular (i.c.v.) with either saline plus saline (i. v.)(○) (n=6), saline(i.c.v.) plus LPS (i. v.) (2 μg/ kg) (●) (n=6), Etanercept at 100 ng(i.c.v.) plus LPS (2 μg/kg) (i. v.) (△) (n=6), Etanercept at 50 ng(i.c.v.) plus LPS (2 μg/kg) (i. v.) (■) (n=6), Etanercept at 10 ng(i.c.v.) plus LPS (2 μg/kg) (i. v.) (▲) (n=8) †P < 0.05, significantly different from corresponding control values (olive oil plus saline group) (ANOVA followed by Dunnett's test); * P < 0.05, significantly different from corresponding control values (olive oil plus LPS group) (ANOVA followed by Dunnett's test).

Table 1. Effects of central administration of etanercept one hour before the LPS injection on the febrile response to intravenous injection of LPS in rabbits.

Treatments	Fever index (FI, °C · h)
Saline + Saline	FI = 0.98 ± 0.07
Saline + LPS (2 μg/kg)	FI = 8.15 ± 0.11[†]
Etanercept (10 ng, i.c.v.) + LPS (2 μg/kg)	FI = 7.38 ± 0.15[*]
Etanercept (50 ng, i.c.v.) + LPS (2 μg/kg)	FI = 4.49 ± 0.13[*]
Etanercept (100 ng, i.c.v.)+ LPS (2 μg/kg)	FI = 3.87 ± 0.11[*]

The values are means ± SEM of 8 rabbits per group. FI represent fever index for 6 h experimental observation.

[†]Significantly different from corresponding control value (vehicle plus vehicle group) ($P < 0.05$; two way analysis of variance followed by Dunnett's test);

[*]Significantly different from corresponding control value (vehicle plus LPS) ($P < 0.05$; two way analysis of variance followed by Dunnett's test)

Table 2. Effects of central administration of IL-10 one hour before the LPS injection on peak Tco, glutamate, and hydroxyl radicals elevation in POAH in response to LPS in rabbits.

Treatments	Response	
	early phase	late phase
	Peak Tco	(℃)
Saline + Saline	0.18± 0.0.05	0.23 ± 0.08
Saline + LPS(2 μg/kg)	1.57 ± 0.12†	2.25± 0.08†
Etanercept (10 ng, i.c.v.) + LPS (2 μg/kg)	1.52 ± 0.17	1.85 ± 0.12*
Etanercept (50 ng, i.c.v.) + LPS (2 μg/kg)	0.67± 0.07*	1.35 ± 0.11*
Etanercept (100 ng, i.c.v.)+ LPS (2 μg/kg)	0.62 ± 0.15*	1.15 ± 0.13*
	% of mean basal levels of glutamate	
Saline + Saline	104 ± 5	95 ± 9
Saline + LPS(2 μg/kg)	168± 9†	263± 7†
Etanercept (10 ng, i.c.v.) + LPS (2 μg/kg)	98 ± 8*	226± 7*
Etanercept (50 ng, i.c.v.) + LPS (2 μg/kg)	102± 12*	149± 6*
Etanercept (100 ng, i.c.v.)+ LPS (2 μg/kg)	155± 4*	135± 8*
	% of mean basal levels of hydroxyl radical	
Saline + Saline	102 ± 3	99 ± 3
Saline + LPS(2 μg/kg)	138± 6†	151 ± 2†
Etanercept (10 ng, i.c.v.) + LPS (2 μg/kg)	123 ± 4	136 ± 5*
Etanercept (50 ng, i.c.v.) + LPS (2 μg/kg)	115 ± 9*	115 ± 4*
Etanercept (100 ng, i.c.v.)+ LPS (2 μg/kg)	105 ± 4*	108 ± 3*

The values are means ± SEM of 8 rabbits per group. Rabbits injected LPS (2μg/kg) produced a biphasic fever which peaked at 80 mins (early phase) and 200 mins (late phase) after LPS injection.

† Significantly different from corresponding control value (vehicle plus vehicle group) ($P < 0.05$; two way analysis of variance followed by Dunnett's test);

* Significantly different from corresponding control value (vehicle plus LPS group) ($P < 0.05$; two way analysis of variance followed by Dunnett's test).

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