科技部補助專題研究計畫成果報告
期末報告

神經恢復性藥物治療帕金森氏症之研發

計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 103-2320-B-004-002-
執 行 期 間 ： 103年08月01日至104年07月31日
執 行 單 位 ： 國立政治大學神經科學研究所

計 畫 主 持 人： 詹銘煥
共 同 主 持 人： 陳清漂
計畫參與人員： 碩士班研究生-兼任助理人員：林芊瑜
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報 告 附 件 ： 出席國際會議研究心得報告及發表論文

處 理 方 式 ：
1.公開資訊：本計畫涉及專利或其他智慧財產權，2年後可公開查詢
2.「本研究」是否已有嚴重損及公共利益之發現：否
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中 華 民 國 104 年 10 月 31 日

中 文 摘 要 ： 研究團隊的近期論文闡釋體外實驗中新合成新木脂素包含MH101及其衍生物可拮抗神經毒素引起神經細胞死亡，顯示他們具有神經保護功能。本研究使用新合成新木脂素(MH101-MH107)進一步探討這些新木脂素對於神經細胞的保護與滋養作用。透過腎上腺髓質嗜鉻細胞瘤 PC12 細胞預先處理新合成新木脂素，並以過氧化氫(H2O2)使細胞產生氧化壓力，之後利用活性氧檢測試驗(DCFH-DA assay)偵測細胞內活性氧(reactive oxygen species, ROS)的含量。實驗結果顯示，預先處理較高濃度(3-10 μM)的新合成新木脂素顯著降低過氧化氫所產生的氧化壓力。另以H2O2誘導PC12細胞死亡，並使用MTT試驗法，觀測新木脂素對於細胞存活的影響。結果顯示新木脂素顯著減少H2O2造成的細胞死亡，此結果與其抗氧化壓力有一致性結果。於神經滋養實驗，發現這些新木脂素本身無法直接誘導PC12細胞的神經突生長。因此，使用神經滋養因子(nerve growth factor, NGF)誘導PC12細胞神經突生長，發現新木脂素在低濃度(0.1-1 μM)顯著加強神經突生長。另一方面在體內動物實驗中，實驗結果表示新木脂素中MH101及MH102不僅可預防並且修補恢復因神經毒素6-OHDA引起的小鼠運動功能失調及多巴胺神經毒害。因此，據此推測新木脂素MH101及其新型類似物可能經由神經滋養特性以解救神經退化性疾病的神經傷害與肢體運動功能缺陷。然而，有關新木脂素(neolignan)的分子標的及藥理機轉仍然混沌不清，仍需要進一步的研究。

中 文 關 鍵 詞 ： 新木脂素，神經滋養因子，神經纖維增長、多巴胺、帕金森氏症

英 文 摘 要 ： Our previous report indicated that the new synthesized neolignans including MH101 and its novel derivatives exhibited the neuroprotective activity against neurotoxin-induced neuronal cell death in vitro. Thus, we predicted that these novel neolignans may conduct the trophic and protective effects on neurons. Using the PC12 cell model, it was observed that neolignans (MH101-107) at higher concentrations of 3-10 μM reduced the increases in reactive oxygen species (ROS) level and cell damage induced by hydrogen peroxide (H2O2) exposure. Neolignans at higher concentration (10-30 μM) also decreased the cell death by H2O2 exposure for 24 hours. As to the neurotrophic effects, neolignans alone did not induce neurite outgrowth. However, these neolignans significantly potentiated nerve growth factor (NGF) induced neurite outgrowth in a concentration-dependent manner (0.1-1 μM). In vivo studies, current data further demonstrated that the selective neolignans MH101 and MH102 could both prevent and restore the dopaminergic (DA) neuron damage and motor dysfunctions elicited by 6-hydroxydopamine (6-OHDA) in mice. Thus, it is likely that neolignan MH101 and its novel derivatives might exert their neuronal restorative actions and neurotrophic activity to rescue neuronal impairment and motor dysfunction in neurodegenerative diseases. However, the molecular targets

and pharmacological mechanism of neolignans are still unclear and required further studies.

英文關鍵詞：neolignan, neurotrophic factor, neurite outgrowth, dopamine, Parkinson’s disease

行政院國家科學委員會補助專題研究計畫 □期中進度報告 ■期末報告

（計畫名稱）

神經恢復性藥物治療帕金森氏症之研發

計畫類別：■個別型計畫　□整合型計畫
計畫編號：MOST 103－2320－B－004－002
執行期間：2014 年 8 月 1 日至 2015 年 7 月 31 日

執行機構及系所：國立政治大學 神經科學所

計畫主持人：詹銘煥
共同主持人：陳清漂
計畫參與人員：李美儀、林芊瑜

本計畫除繳交成果報告外，另含下列出國報告，共 _1__ 份：
□移地研究心得報告
■出席國際學術會議心得報告
□國際合作研究計畫國外研究報告

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中　華　民　國 104 年　10 月 30 日

一、中文摘要

關鍵詞：新木脂素，神經滋養因子，神經纖維增長、多巴胺、帕金森氏症

研究團隊的近期論文闡釋體外實驗中新合成新木脂素包含MH101及其衍生物可拮抗神經毒素引起神經細胞死亡，顯示他們具有神經保護功能。本研究使用新合成新木脂素(MH101-MH107)進一步探討這些新木脂素對於神經細胞的保護與滋養作用。透過腎上腺髓質嗜鉻細胞瘤 PC12 細胞預先處理新合成新木脂素，並以過氧化氫(H_2O_2)使細胞產生氧化壓力，之後利用活性氧檢測試驗(DCFH-DA assay)偵測細胞內活性氧(reactive oxygen species, ROS)的含量。實驗結果顯示，預先處理較高濃度(3-10 μM)的新合成新木脂素顯著降低過氧化氫所產生的氧化壓力。另以 H_2O_2 誘導 PC12 細胞死亡，並使用 MTT 試驗法，觀測新木脂素對於細胞存活的影響。結果顯示新木脂素顯著減少 H_2O_2 造成的細胞死亡，此結果與其抗氧化壓力有一致性結果。於神經滋養實驗，發現這些新木脂素本身無法直接誘導 PC12 細胞的神經突生長。因此，使用神經滋養因子(nerve growth factor, NGF)誘導 PC12 細胞神經突生長，發現新木脂素在低濃度(0.1-1 μM)顯著加強神經突生長。另一方面在體內動物實驗中，實驗結果表示新木脂素中 MH101 及 MH102 不僅可預防並且修補恢復因神經毒素 6-OHDA 引起的小鼠運動功能失調及多巴胺神經毒害。因此，據此推測新木脂素 MH101 及其新型類似物可能經由神經滋養特性以解救神經退化性疾病的神經傷害與肢體運動功能缺陷。然而，有關新木脂素(neolignan)的分子標的及藥理機轉仍然混沌不清，仍需要進一步的研究。

二、英文摘要

Abstract:

Keywords: neolignan, neurotrophic factor, neurite outgrowth, dopamine, Parkinson's disease

Our previous report indicated that the new synthesized neolignans including MH101 and its novel derivatives exhibited the neuroprotective activity against neurotoxin-induced neuronal cell death *in vitro*. Thus, we predicted that these novel neolignans may conduct the trophic and protective effects on neurons. Using the PC12 cell model, it was observed that neolignans (MH101-107) at higher concentrations of 3-10 μM reduced the increases in reactive oxygen species (ROS) level and cell damage induced by hydrogen peroxide (H_2O_2) exposure. Neolignans at higher concentration (10-30 μM) also decreased the cell death by H_2O_2 exposure for 24 hours. As to the neurotrophic effects, neolignans alone did not induce neurite outgrowth. However, these neolignans significantly potentiated nerve growth factor (NGF) induced neurite outgrowth in a concentration-dependent manner (0.1-1 μM). In vivo studies, current data further demonstrated that the selective neolignans MH101 and MH102 could both prevent and restore the dopaminergic (DA) neuron damage and motor dysfunctions elicited by 6-hydroxydopamine (6-OHDA) in mice. Thus, it is likely that neolignan MH101 and its novel derivatives might exert their neuronal restorative actions and neurotrophic activity to rescue neuronal impairment and motor dysfunction in neurodegenerative diseases. However, the molecular targets and pharmacological mechanism of neolignans are still unclear and required further studies.

三、研究緣由及目的

Parkinson's disease (PD), the most common adult-onset neurodegenerative movement disorder, is characterized by detriment of the pigmented dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) leading to a loss of striatal dopamine. The hallmark features of PD include akinesia, tremor, rigidity, and poor balance. Current treatments for PD rely heavily on dopamine replacement agents such as levodopa and some directly acting dopamine agonists[7]. Although levodopa is considered the gold standard for treatment of PD, multiple complications such as motor fluctuations, hallucinations, and psychosis arise from long-term therapy. For these reasons, the medicine capable for delaying onset of levodopa therapy or alleviating its

adverse reactions has been developed. Several levodopa sparing drugs such as dopamine agonists, monoamine oxidase (MAO), and catechol-o-methyltransferase (COMT) inhibitors, provide symptomatic benefit and allow a reduction in levodopa dosage. Anticholinergic agents are also commonly used to treat PD by restoring the cholinergic-DA balance. Amantidine with several proposed mechanisms show clinical improvement for PD[24]. However, since PD is a progressive neurologic disorder with motor and non-motor manifestations, development of disease modifying therapies that prevent development of disability, rather than provide symptomatic relief prompt the research for new therapeutic approaches to PD.

Because of the neuroprotective and neuronal function enhancing properties, neurotrophic factors are attractive candidates for treatment of neurodegenerative disorders. For PD, GDNF and its naturally occurring analogs have been shown to enhance survival and function of DA neurons both *in vitro* and in rodent and primate animal models of PD[8-9]. GDNF family ligands (GFLs), including GDNF, neurturin, artemin, and persephin, promote the survival, proliferation, differentiation, and function of various neuronal populations within the central and peripheral nervous systems[10]. Signaling transduction for GFLs is by activating the receptor complex consisting of a ligand binding GDNF family receptor α (GFRα) and the receptor tyrosine kinase Ret. In addition to Ret, the neural cell adhesion molecule (NCAM) has been identified as an alternative receptor for GDNF[11]. NCAM also plays an essential role in nervous system morphogenesis and affects neural regeneration and plasticity, including learning and memory. GDNF has been well documented for its potential to enhance neurites outgrowth in both normal and damaged DA neurons. Furthermore, the neuroprotective effect of GDNF is several times greater than those of other currently known neurotrophic factors. In the experimental PD animal models, subchronic administration of GFLs improved the motor dysfunctions and protected DA neuronal lesions caused by neurotoxins[12-14]. However, GFLs are far from the ideal pharmacological agents and limited in clinical use because the delivery and diffusion of GFL protein through BBB to the region of DA neurodegeneration is arduous. Thus, development of small molecules with function similar to GFL may solve the problems from the fact that GFL is a protein.

Recently, biphenyl neolignan compounds have been reported to enhance neurite outgrowth and increase release of neurotrophic factors[15-17]. Our current data showed subchronic administration of the novel synthesized neolignan MH101 could protect and restore the neuronal damage and motor impairment in PD mouse model. However, its pharmacological actions are still unclear. The neolignans with neurite outgrowth potentiating effects might be associated with their neuronal protective and restorative actions. Neolignans are proposed to produce the similar action of GFLs[17], particularly the biphenyl type with 5-allyl and 4'-hydroxyl groups. Therefore, activation of GFLs signaling raises great expectations as a potential therapeutic target for neolignans to treat PD. The present project is going to screen the novel synthetic biphenyl neolignans developed by Co-PI Prof. CP Chen (Department of Chemistry, National Dong Hwa University)[6] in hopes of characterizing functional similarities between neolignans and GFLs. Biphenyl neolignans might have the potent neurorescue effects *in vitro* and in PD animal model through activating the GFLs signaling. Further synthesis of the novel neolignan compounds with correct chemical properties to fit the biological interests and modification of the synthesis process with high production capacity will be carried out.

In this project, we will utilize the neurite outgrowth *in vitro* assay to screen the potent new synthetic neolignans (MH101-107), and then apply the potential compounds to PD mouse model. The specific aims for this proposal are to 1) evaluate the neurorescue and neurite outgrowth activity of potential neolignans, 2) characterize the neuronal restorative action of potent neolignans on DA

damage and motor dysfunction in PD animal model, 3) determine the effects of promising neolignans on the expression of GFLs, 4) identify the potent neolignans targeting at GFL downstream signaling, and 5) design and synthesize the novel potent neolignans promoting neuronal restoration and improve the synthesis procedure for potent neolignan with high production capacity. This study will develop the brand-new potent compounds of neolignans for treatment of PD through activating the GFLs signaling pathways. Clinical trials could be initiated after modification of the synthesis process with high production capacity and low production costs. The potent neolignans might be able to apply for the other neurodegenerative diseases.

四、結果與討論

(1) *Effects of neolignans on ROS production induced by H_2O_2*

The current results showed that the new synthetic neolignans (MH101-107) at concentrations of 3-10 μM protected PC12 cell damage and reduced the increases of reactive oxygen species (ROS) induced by hydrogen peroxide (H_2O_2) exposure. The ROS production was determined by 2',7' dichlorodihydrofluorescin diacetate (DCFH-DA) assay.

(2) *Effect of neolignans on enhancing NGF-induced neurite outgrowth in differentiated PC12 cells*

As to the neurotrophic effects, neolignans themselves did not induce neurite outgrowth. Importantly, these neolignans (MH101-107) significantly potentiated nerve growth factor (NGF)-induce neurite outgrowth at lower concentration of 0.1-1 μM.

(3) *Protective and therapeutic effects of MH101 and MH102 on rotational behaviors and nigrostriatal toxicity in 6-OHDA lesioned mice*

MH101 (0.1-10 mg/kg, i.p.) was administrated 30 min prior to or 7 days after 6-OHDA induced unilateral striatal lesion, subsequently given daily for 14 days. Results showed that contralateral rotations induced by apomorphine were reduced by subchronic pretreatment with MH101 (5-10 mg/kg) on the 15^{th} days after unilateral striatal 6-OHDA lesion. Consistently, subchronic pretreatment with MH101 recovered the reduction of tyrosine hydroxylase (TH)-positive fibers in lesioned striatum. Moreover, contralateral rotations induced by apomorphine were also reduced by MH101 and MH102 post-treatment for 7 and 14 days in unilateral striatal 6-OHDA lesion. Subchronic post-treatment with MH101 and MH102 for 2 weeks significantly restored the reduction of TH-positive fibers in lesioned striatum and substantia nigra.

Taken together, our results indicate that the novel synthetic neolignans have the potential to protect neuronal survival under oxidative stress and enhance NGF-induced neuritogenesis *in vitro.* Furthermore, our data further reveal that subchronic and daily pretreatment and post-treatment with MH101 and MH102 for 2 weeks significantly improved the motor impairments and rescued the nigrostriatal DA neurotoxicity by TH immunostaining in PD mouse model. Additionally, the signaling pathways and pharmacological mechanisms of subchronic neolignan treatment on reversing the neurotoxicity and behavioral deficits in PD mouse are under investigation. The present results demonstrate that the novel synthetic neolignans might have the higher therapeutic efficacy in PD mouse model. However, their molecular target and pharmacological mechanism are still unclear and required further studies.

五、參考文獻

Armstrong, R. J. & Barker, R. A. Neurodegeneration: a failure of neuroregeneration? *Lancet* **358**, 1174-1176, (2001).

Chen, H.-H., Lin, S.-C., and Chan, M.-H. The protective and restorative effects of magnolol on neurotoxicity in 6-hydroxydopamine-induced hemi-parkinsonian mouse model. Neurodegenerative Diseases **8**, 364-374, (2011).

Clarke, C. E. Neuroprotection and pharmacotherapy for motor symptoms in Parkinson's disease. *Lancet Neurol* **3**, 466-474, (2004).

Jenner, P. & Olanow, C.W. The pathogenesis of cell death in Parkinson's disease. *Neurology* **66**, S24-36, (2006).

Lin, Y. R., Chen, H. H., Ko, C. H. & Chan, M. H. Differential inhibitory effects of honokiol and magnolol on excitatory amino acid-evoked cation signals and NMDA-induced seizures. *Neuropharmacology* **49**, 542-550, (2005).

Lin, Y. R., Chen, H. H., Ko, C. H. & Chan, M. H. Neuroprotective activity of honokiol and magnolol in cerebellar granule cell damage. *Eur J Pharmacol* **537**, 64-69, (2006).

Lin, Y. R., Chen, H. H., Ko, C. H. & Chan, M. H. Effects of honokiol and magnolol on acute and inflammatory pain models in mice. *Life Sci* **81**, 1071-1078, (2007).

Lin, Y. R., Chen, H. H., Lin, Y. C., Ko, C. H. & Chan, M. H. Antinociceptive actions of honokiol and magnolol on glutamatergic and inflammatory pain. *J Biomed Sci* **16**, 94, (2009).

Ouchi, Y. *et al.* Microglial activation and dopamine terminal loss in early Parkinson's disease. *Ann Neurol* **57**, 168-175, (2005).

Teismann, P. *et al.* Pathogenic role of glial cells in Parkinson's disease. *Mov Disord* **18**, 121-129, (2003).

Whitton, P. S. Inflammation as a causative factor in the aetiology of Parkinson's disease. *Br J Pharmacol* **150**, 963-976, (2007).

國科會補助專題研究計畫出席國際學術會議心得報告

日期:2015年 7月28 日

計畫編號	MOST103－2320－B－004－002		
計畫名稱	神經恢復性藥物治療帕金森氏症之研發		
出國人員 姓名	詹銘煥	服務機構 及職稱	政 治 大 學 神經科學所 教授
會議時間	2015 年 6 月 30 日至 2015 年 7 月 3 日	會議地點	捷克布拉格
會議名稱	(中文) SEB(實驗生物學學會)2015 年年會 (英文) Society for Experimental Biology (SEB 2015)		
發表題目	(中文) 雙酚化合物對抗氧化壓力之神經保護作用及加強神經突生長 (英文) Neuroprotection against oxidative stress and potentiation of neurite outgrowth by biphenolic compounds		

一、會議背景簡介:

SEB(實驗生物學學會)在於促進和提高實驗生物學在科學界內的影響力,推動實驗生物學科學事業發展。本次年會在捷克布拉格市 Clarion Congress Hotel 舉辦，由 Michael Berenbrink (University of Liverpool) and Andrew Cossins (University of Liverpool)主辦，匯集歐美澳亞等洲各學術單位的研究成果，以演講、壁報、攤位展覽等方式交流，邀請的 keynote speakers 具有國際聲望，具高知名度足以匯集人氣參與交流。會議規模龐大，分成多重主題不同 session，不同廳場同時進行。

二、本次會議重要主題：

共有四個主題進行科學性發表與討論。(1) Animal Biology Sessions, (2) Cell Biology Sessions, (3) Plant Biology Sessions, (4) Education and Public Affairs Sessions。

Animal Biology Sessions

Conservation Physiology: How environmental influences on parents and early developmental stages determine "winners" and "losers"。

Integrative Physiology – Gen(om)es-to-Environments and vice versa: A Tribute to Andrew R. Cossins

Thermal Biology:　Oxygen- and capacity-limited thermal tolerance: a universal concept?
Toxicological Genomics – genes to ecology
Osmoregulation: From Magnesium to Mosquitoes: a tribute to Klaus W. Beyenbach
Neurobiology: Understanding intraspecific variation in animal phenotypes from genes to behaviour
Ecophysiology: Movement Ecology
Mechanics and biological functions of the Arthropod exoskeleton
Emerging models for studying the cardiovascular system
General Animal Biology
General Biomechanics

Cell Biology Sessions

The process view of life
Understanding and Engineering Biological Complexity
Cell Biology: Physical and Mechanical Signalling
Cross-Kingdom Immune Systems
Modelling Cells

Plant Biology Sessions

Retrograde signalling from chloroplasts in development and stress responses
Plants roots: new challenges in a changing world
Plant Biotechnology: Addressing the challenges for food security, health and sustainability
Linking N-terminal modifications to protein function in plants
Visualising Metabolism
Effector biology of beneficial and pathogenic microbes – a source to improve crop productivity

Education and Public Affairs Sessions

Science with Impact - “Rising CO2 – it’s not just about global warming”
Careers Day
Undergraduate Education
How to become an academic

三、參加會議人員概況

參加的場次多為 Animal biology 和 cell biology sessions。特別是學習到多樣性的動物行為模式及多種類物種間的差異，還有環境以及氣候的影響，對未來動物行為實驗有更多的選擇，且對於控制變因，如壓力，光週期、溫布、季節等因素會考慮更佳周詳。

四、發表論文摘要

Phenolic compounds, the well-known phytochemicals, are present in numerous vegetables and fruits and have been reported to possess the potent anti-oxidative and anti-inflammatory activity. Currently, the new series synthetic biphenolic compounds from our lab were found to prevent and reverse neuronal damage induced by neurotoxins in *in vitro* and *in vivo* studies. The purpose of this study was to investigate the neuroprotective and neurotrophic effects of novel synthetic biphenolic compounds and also to explore the underlying mechanisms. Results showed that biphenolic compounds (10-30 μM) protected PC12 cell damage induced by neurotoxins and reduced the increases in reactive oxygen species (ROS). As for the neurotrophic effects, biphenolic compounds alone did not induce neurite outgrowth. However, these biphenolic compounds significantly potentiated nerve growth factor (NGF)-induced neurite outgrowth of PC12 cells at low concentrations (0.1-1 μM). NGF sufficiently induced the signal of neurite outgrowth extracellular signal-regulated protein kinase 1/2 (ERK1/2) phosphorylation during 2-5 min after treatment. When PC12 cells were co-treated with NGF and biphenolic compounds, the phospho-ERK1/2 expression was prolonged to more than 10 min, although the maximal level of phospho-ERK1/2 expression was not influenced. Taken together, these results indicate that these novel synthetic biphenolic compounds have the potential to protect neuronal survival under oxidative stress and enhance NGF-induced neuritogenesis *in vitro*.

五、心得、感想及建議

參加國際性會議能促進國內研究學者與國際交流的機會，此次有多位台灣學者被選為口頭報告，另壁報論文也有多位台灣學者被徵選做口頭精簡報告，本人的壁報論文也為其中一篇，為台灣的生物學界研究做了很好的交流與宣傳。此次大會所安排的壁報論文口頭簡介，可讓與會學者先行知悉其論文概況，為本次會議一項創舉。

在此次會議中，原本對於實驗動物模式專注於嚙齒類，這個會議提供多元動物模式的認識，舉凡鱷魚、昆蟲、魚類等有別於傳統大小老鼠動物模式都是可應用的材料，其中特別關注斑馬魚的行為模式，將來可協助並改善先前本實驗團隊執行斑馬魚行為之實驗測試方法。

攜回會議議程，壁報縮小版數份、相關資訊簡介等等

科技部補助專題研究計畫出席國際學術會議心得報告

日期：104 年 7 月 28 日

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計畫名稱	神經恢復性藥物治療帕金森氏症之研發		
出國人員 姓名	詹銘煥	服務機構 及職稱	國立政治大學 神經科學所
會議時間	104 年 6 月 30 日至 104 年 7 月 3 日	會議地點	捷克布拉格
會議名稱	(中文) SEB(實驗生物學學會)2015 年年會 (英文) Society for Experimental Biology (SEB 2015)		
發表題目	(中文) 雙酚化合物對抗氧化壓力之神經保護作用及加強神經突生長 (英文) Neuroprotection against oxidative stress and potentiation of neurite outgrowth by biphenolic compounds		

一、會議背景簡介:

SEB(實驗生物學學會)在於促進和提高實驗生物學在科學界內的影響力,推動實驗生物學科學事業發展。本次年會在捷克布拉格市 Clarion Congress Hotel 舉辦，由 Michael Berenbrink (University of Liverpool) and Andrew Cossins (University of Liverpool)主辦，匯集歐美澳亞等洲各學術單位的研究成果，以演講、壁報、攤位展覽等方式交流，邀請的 keynote speakers 具有國際聲望，具高知名度足以匯集人氣參與交流。會議規模龐大，分成多重主題不同 session，不同廳場同時進行。

二、本次會議重要主題：

共有四個主題進行科學性發表與討論。(1) Animal Biology Sessions, (2) Cell Biology Sessions, (3) Plant Biology Sessions, (4) Education and Public Affairs Sessions。

Animal Biology Sessions

Conservation Physiology: How environmental influences on parents and early developmental stages determine "winners" and "losers" ◦

Integrative Physiology – Gen(om)es-to-Environments and vice versa: A Tribute to Andrew R. Cossins

Thermal Biology: Oxygen- and capacity-limited thermal tolerance: a universal concept?

Toxicological Genomics – genes to ecology

Osmoregulation: From Magnesium to Mosquitoes: a tribute to Klaus W. Beyenbach

Neurobiology: Understanding intraspecific variation in animal phenotypes from genes to behaviour

Ecophysiology: Movement Ecology

Mechanics and biological functions of the Arthropod exoskeleton

Emerging models for studying the cardiovascular system

General Animal Biology

General Biomechanics

Cell Biology Sessions

The process view of life

Understanding and Engineering Biological Complexity

Cell Biology: Physical and Mechanical Signalling

Cross-Kingdom Immune Systems

Modelling Cells

Plant Biology Sessions

Retrograde signalling from chloroplasts in development and stress responses

Plants roots: new challenges in a changing world

Plant Biotechnology: Addressing the challenges for food security, health and sustainability

Linking N-terminal modifications to protein function in plants

Visualising Metabolism

Effector biology of beneficial and pathogenic microbes – a source to improve crop productivity

Education and Public Affairs Sessions

Science with Impact - “Rising CO2 – it’s not just about global warming”

Careers Day

Undergraduate Education

How to become an academic

三、參加會議人員概況

參加的場次多為 Animal biology 和 cell biology sessions。特別是學習到多樣性的動物行為模式及多種類物種間的差異，還有環境以及氣候的影響，對未來動物行為實驗有更多的選擇，且對於控制變因，如壓力，光週期、溫布、季節等因素會考慮更佳周詳。

四、發表論文摘要

Phenolic compounds, the well-known phytochemicals, are present in numerous vegetables and fruits and have been reported to possess the potent anti-oxidative and anti-inflammatory activity. Currently, the new series synthetic biphenolic compounds from our lab were found to prevent and reverse neuronal damage induced by neurotoxins in *in vitro* and *in vivo* studies. The purpose of this study was to investigate the neuroprotective and neurotrophic effects of novel synthetic biphenolic compounds and also to explore the underlying mechanisms. Results showed that biphenolic compounds (10-30 μM) protected PC12 cell damage induced by neurotoxins and reduced the increases in reactive oxygen species (ROS). As for the neurotrophic effects, biphenolic compounds alone did not induce neurite outgrowth. However, these biphenolic compounds significantly potentiated nerve growth factor (NGF)-induced neurite outgrowth of PC12 cells at low concentrations (0.1-1 μM). NGF sufficiently induced the signal of neurite outgrowth extracellular signal-regulated protein kinase 1/2 (ERK1/2) phosphorylation during 2-5 min after treatment. When PC12 cells were co-treated with NGF and biphenolic compounds, the phospho-ERK1/2 expression was prolonged to more than 10 min, although the maximal level of phospho-ERK1/2 expression was not influenced. Taken together, these results indicate that these novel synthetic biphenolic compounds have the potential to protect neuronal survival under oxidative stress and enhance NGF-induced neuritogenesis *in vitro*.

五、心得、感想及建議

參加國際性會議能促進國內研究學者與國際交流的機會，此次有多位台灣學者被選為口頭報告，另壁報論文也有多位台灣學者被徵選做口頭精簡報告，本人的壁報論文也為其中一篇，為台灣的生物學界研究做了很好的交流與宣傳。此次大會所安排的壁報論文口頭簡介，可讓與會學者先行知悉其論文概況，為本次會議一項創舉。

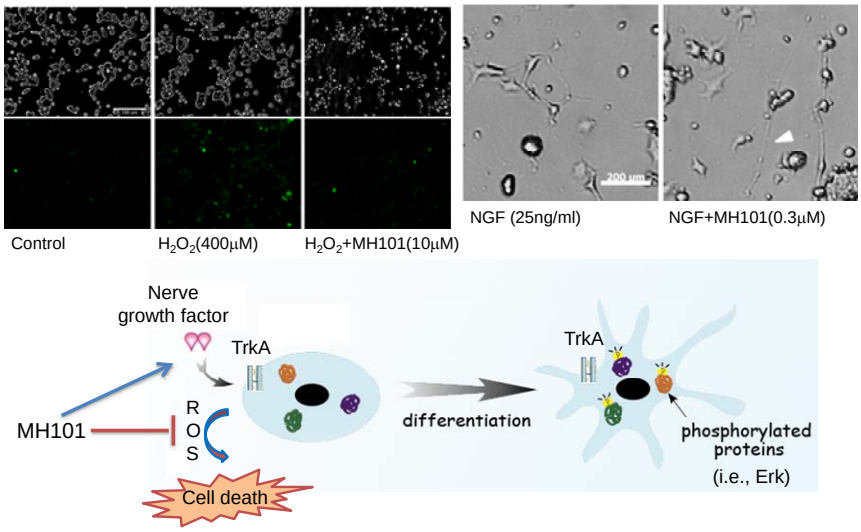
在此次會議中，原本對於實驗動物模式專注於嚙齒類，這個會議提供多元動物模式的認識，舉凡鱷魚、昆蟲、魚類等有別於傳統大小老鼠動物模式都是可應用的材料，其中特別關注斑馬魚的行為模式，將來可協助並改善先前本實驗團隊執行斑馬魚行為之實驗測試方法。

攜回會議議程，壁報縮小版數份、相關資訊簡介等等

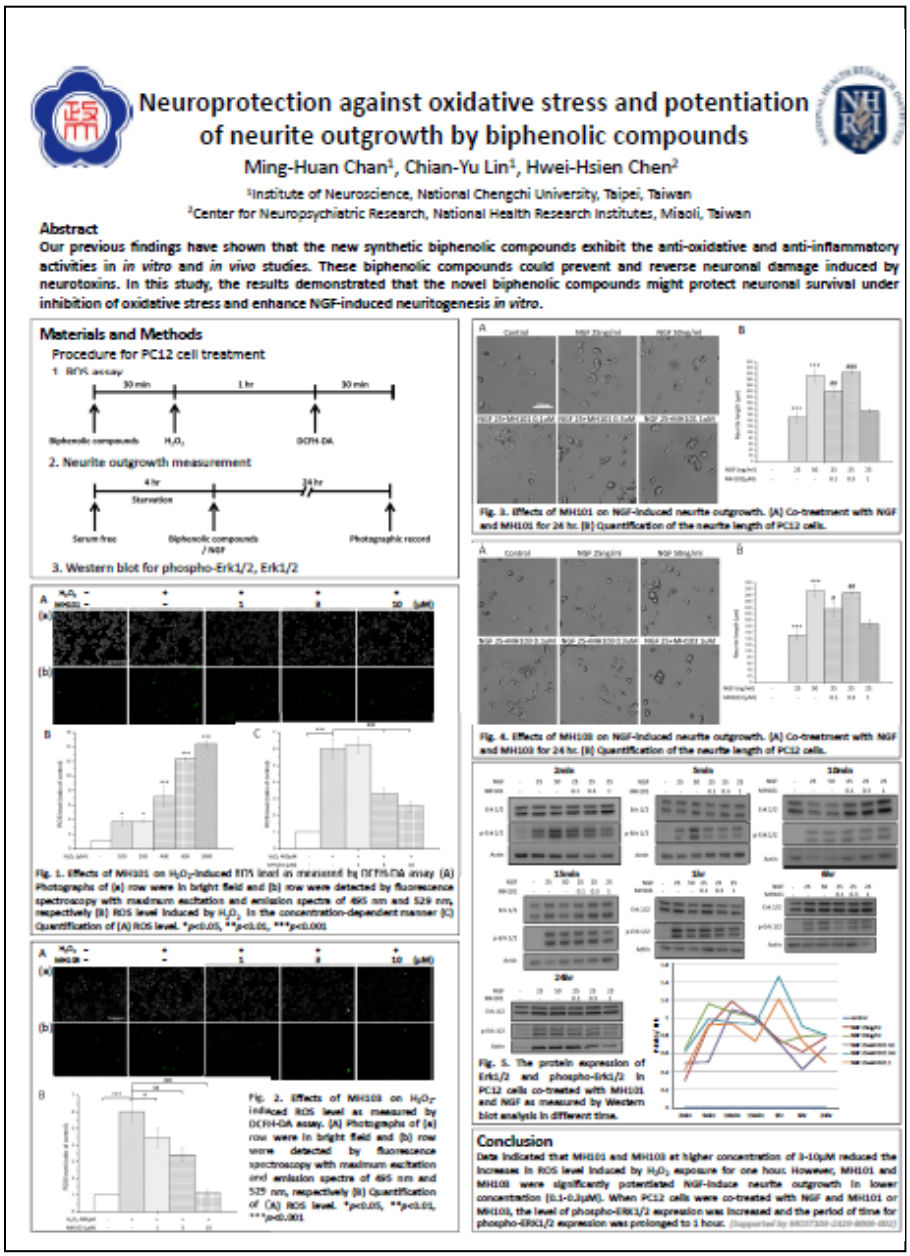
精簡報告

C3.27 Neuroprotection against oxidative stress and potentiation of neurite outgrowth by biphenolic compounds

Ming-Huan Chan, Ph.D. Institute of Neuroscience, National Chengchi University, Taiwan



壁報論文



Neuroprotection against oxidative stress and potentiation of neurite outgrowth by biphenolic compounds

Ming-Huan Chan[1], Chian-Yu Lin[1], Hwei-Hsien Chen[2]

[1]Institute of Neuroscience, National Chengchi University, Taipei, Taiwan
[2]Center for Neuropsychiatric Research, National Health Research Institutes, Miaoli, Taiwan

Abstract

Our previous findings have shown that the new synthetic biphenolic compounds exhibit the anti-oxidative and anti-inflammatory activities in *in vitro* and *in vivo* studies. These biphenolic compounds could prevent and reverse neuronal damage induced by neurotoxins. In this study, the results demonstrated that the novel biphenolic compounds might protect neuronal survival under inhibition of oxidative stress and enhance NGF-induced neuritogenesis *in vitro*.

Materials and Methods

Procedure for PC12 cell treatment

1. ROS assay
2. Neurite outgrowth measurement
3. Western blot for phospho-Erk1/2, Erk1/2

Fig. 1. Effects of MH101 on H_2O_2-induced ROS level as measured by DCFH-DA assay. (A) Photographs of (a) row were in bright field and (b) row were detected by fluorescence spectroscopy with maximum excitation and emission spectra of 495 nm and 529 nm, respectively (B) ROS level induced by H_2O_2 in the concentration-dependent manner (C) Quantification of (A) ROS level. *p<0.05, **p<0.01, ***p<0.001

Fig. 2. Effects of MH103 on H_2O_2-induced ROS level as measured by DCFH-DA assay. (A) Photographs of (a) row were in bright field and (b) row were detected by fluorescence spectroscopy with maximum excitation and emission spectra of 495 nm and 529 nm, respectively (B) Quantification of (A) ROS level. *p<0.05, **p<0.01, ***p<0.001

Fig. 3. Effects of MH101 on NGF-induced neurite outgrowth. (A) Co-treatment with NGF and MH101 for 24 hr. (B) Quantification of the neurite length of PC12 cells.

Fig. 4. Effects of MH103 on NGF-induced neurite outgrowth. (A) Co-treatment with NGF and MH103 for 24 hr. (B) Quantification of the neurite length of PC12 cells.

Fig. 5. The protein expression of Erk1/2 and phospho-Erk1/2 in PC12 cells co-treated with MH101 and NGF as measured by Western blot analysis in different time.

Conclusion

Data indicated that MH101 and MH103 at higher concentration of 3-10μM reduced the increases in ROS level induced by H_2O_2 exposure for one hour. However, MH101 and MH103 were significantly potentiated NGF-induce neurite outgrowth in lower concentration (0.1-0.3μM). When PC12 cells were co-treated with NGF and MH101 or MH103, the level of phospho-ERK1/2 expression was increased and the period of time for phospho-ERK1/2 expression was prolonged to 1 hour.

科技部補助計畫衍生研發成果推廣資料表

日期:2015/10/22

科技部補助計畫	計畫名稱: 神經恢復性藥物治療帕金森氏症之研發
	計畫主持人: 詹銘煥
	計畫編號: 103-2320-B-004-002- 學門領域: 藥理及毒理

無研發成果推廣資料

103年度專題研究計畫研究成果彙整表

計畫主持人：詹銘煥			**計畫編號：**103-2320-B-004-002-				
計畫名稱：神經恢復性藥物治療帕金森氏症之研發							
成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數（含實際已達成數）	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	2	0	100%		2015第30屆生物醫學聯合學術年會
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	1	0	100%	人次	
		博士生	1	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	0	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	0	100%		Society for Experimental Biology (SEB 2015)
		專書	0	0	100%	章/本	
	專利	申請中件數	0	1	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
其他成果（無法以量化表達之成果如辦理學術活動、獲得獎項、重要國		國際合作:客座教授Professor Sulie Chang 從美國回台，於2015年3至6月協助研究工作，促進雙方學術交流及合作。					

際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）	

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

科技部補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形：
   論文：□已發表 □未發表之文稿 ■撰寫中 □無
   專利：□已獲得 □申請中 ■無
   技轉：□已技轉 □洽談中 ■無
   其他：（以100字為限）
   第30屆生物醫學聯合學術會議(3月21-22日, 2015)發表研究成果。
   出席國際學術會議(Society for Experimental Biology, 2015),並發表研究成果。

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以500字為限）
   本研究成果發現新合成雙酚化合物具對抗氧化壓力之神經保護作用及加強神經滋養因子誘發神經突生長的能力，顯示這類合成物如實驗預期，具神經恢復之能力，未來將持續探討其藥理機制，進一步發展於臨床試驗。