科技部補助專題研究計畫成果報告
期末報告

Salidroside配合運動治療對睡眠呼吸終止症誘發大腦細胞凋亡的療效探討

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處 理 方 式 ：
1.公開資訊：本計畫可公開查詢
2.「本研究」是否已有嚴重損及公共利益之發現：否
3.「本報告」是否建議提供政府單位施政參考：否

中 華 民 國 104 年 11 月 30 日

中 文 摘 要 ： 背景。本研究的目的是確定是否紅景天對小鼠重度睡眠呼吸模型上缺氧誘發皮質廣泛分散的細胞凋亡的保護作用。方法。六十四C57BL / 6J小鼠5-6個月的年齡被分成四組，即對照組（21％O 2，24小時，每天，8個星期中，n = 16）;低氧組（缺氧：7％O 2 60秒，20％的O 2交替60秒，每天8小時，8個星期中，n = 16）;低氧+ S10和低氧+ S30組（缺氧對於第一4週，缺氧預處理為10mg / kg和30毫克/公斤紅景天通過每天口服管飼法對第二個星期4中，n = 16和16）。從四組的切除大腦，用Western blot檢測。結果。Fas ligand, Fas death receptors, Fas-Associated Death Domain (FADD), activated caspase 8, and activated caspase 3 (Fas pathways)（FAS途徑）明顯降低缺氧+ S10和缺氧+ S30。在線粒體途徑，BcLx, Bcl2, Bid (anti-apoptotic Bcl2 family)（抗凋亡Bcl2的家庭），缺氧+ S10和缺氧+ S30的蛋白水平明顯多於缺氧。Bax, t-Bid, activated caspase 9, and activated caspase 3較少在缺氧+ S10組和缺氧+ S30組比缺氧。結論。我們的研究結果發現紅景天苷對慢性間歇性低氧誘導的Fas依賴性和線粒體依賴性凋亡途徑在小鼠大腦的保護作用。

中 文 關 鍵 詞 ： apoptotic凋亡, caspase, brain 腦, hypoxia低氧, low oxygen, salidroside

英 文 摘 要 ： Background. The goal of this study is to determine if salidroside has protective effects on hypoxia-induced cortex widely dispersed apoptosis in mice with severe sleep apnea model. Methods. Sixty-four C57BL/6J mice 5-6 months of age were divided into four groups i.e. Control group (21% O2, 24 hrs per day, 8 weeks, n=16); Hypoxia group (Hypoxia: 7% O2 60 seconds, 20% O2 alternating 60 seconds, 8 hrs per day, 8 weeks, n=16); Hypoxia+S10 and Hypoxia+S30 group (Hypoxia for 1st 4 weeks, hypoxia pretreated 10mg/kg and 30mg/kg salidroside by oral gavage per day for 2nd 4 weeks, n=16 and 16). The excised brains from four groups were measured by Western Blotting. Results. Compared with Hypoxia, the protein levels of Fas ligand, Fas death receptors, Fas-Associated Death Domain (FADD), activated caspase 8, and activated caspase 3 (Fas pathways) were decreased in Hypoxia+S10 and Hypoxia+S30. In mitochondria pathway, the protein levels of BcLx, Bcl2, Bid (anti-apoptotic Bcl2 family) in Hypoxia+S10 and Hypoxia+S30 were more than those in Hypoxia. The protein levels of Bax, t-Bid, activated caspase 9, and activated caspase 3 were less in Hypoxia+S10 and Hypoxia+S30 than those in hypoxia. Conclusions. Our findings suggest that salidroside have protective effects on chronic intermittent hypoxia-induced Fas-dependent and mitochondria-dependent apoptotic pathways in mice brains.

英 文 關 鍵 詞 ： apoptotic, caspase, brain, hypoxia, low oxygen, salidroside

Protective effect of salidroside on cortex apoptosis in mice with chronic intermittent hypoxia

Ting Hua, Shin-Da Lee[2,10*]

Abstract

Background. The goal of this study is to determine if salidroside has protective effects on hypoxia-induced cortex widely dispersed apoptosis in mice with severe sleep apnea model. **Methods.** Sixty-four C57BL/6J mice 5-6 months of age were divided into four groups i.e. Control group (21% O_2, 24 hrs per day, 8 weeks, n=16); Hypoxia group (Hypoxia: 7% O_2 60 seconds, 20% O_2 alternating 60 seconds, 8 hrs per day, 8 weeks, n=16); Hypoxia+S10 and Hypoxia+S30 group (Hypoxia for 1st 4 weeks, hypoxia pretreated 10mg/kg and 30mg/kg salidroside by oral gavage per day for 2nd 4 weeks, n=16 and 16). The excised brains from four groups were measured by Western Blotting. **Results.** Compared with Hypoxia, the protein levels of Fas ligand, Fas death receptors, Fas-Associated Death Domain (FADD), activated caspase 8, and activated caspase 3 (Fas pathways) were decreased in Hypoxia+S10 and Hypoxia+S30. In mitochondria pathway, the protein levels of BcLx, Bcl2, Bid (anti-apoptotic Bcl2 family) in Hypoxia+S10 and Hypoxia+S30 were more than those in Hypoxia. The protein levels of Bax, t-Bid, activated caspase 9, and activated caspase 3 were less in Hypoxia+S10 and Hypoxia+S30 than those in hypoxia. **Conclusions.** Our findings suggest that salidroside have protective effects on chronic intermittent hypoxia-induced Fas-dependent and mitochondria-dependent apoptotic pathways in mice brains.

Key words: apoptotic, caspase, brain, hypoxia, low oxygen, salidroside

Introduction:

Obstructive Sleep Apnea (OSA), a sleep breathing disorder, is associated with nocturnal airflow disruption in humans [1]. OSA is a high risk factor of cardiovascular diseases [2] and could increase coronary heart diseases by 30% [3, 4]. Chronic intermittent hypoxia (CIH) led to multiple long-term cardiovascular pathophysiologic consequences similar to what we observed in OSA [2, 5]. One study showed that CIH leads to left ventrical myocardial dysfunction [6] and our previous study showed that 8 week CIH induced cardiac abnormalities and apoptosis [7].

Salidroside [2-(4-hydroxyphenyl)ethyl beta-D-glucopyranoside], active ingredients of Rhodiola rosea, was used for high mountain sickness to protect erythrocytes against oxidative stress, and improve resistance to stress and fatigue [8]. Salidroside were found to reduce cell apoptosis, improved cardiomyocytes glucose uptake, reduced ischemia/reperfusion-induced cardiomyocyte damage [9]. However, the effect of salidroside on cardiovascular health is still not totally understood.

Apoptosis, a cell death program, has long been recognized to be involved in cardiovascular diseases [10, 11]. The cardiomyocyte apoptosis is as one of predictors of cardiac diseases or neurodegeneration [12]. Cardiac widely dispersed apoptosis was found by our labatory in chronic cardiometablic or stressful conditions such as obesity [13-15], hypertension [11, 16, 17], diabetes [18, 19], ovariectomy [20], long-term hypoxia[7, 21, 22], and smoke [23]. The Fas receptor-dependent apoptotic (Type I) pathway is a major pathway triggering cardiac apoptosis [10, 24], and initiates binding the Fas ligand to the Fas receptor [24] [24-27]. This binding, followed by Fas-receptor oligomerisation leading to the death-inducing signal complex, starts with recruitment of the Fas-Associated Death Domain (FADD) adaptor protein [24]. The activated caspase 8 cleaves pro-caspase 3 then undergoes autocatalysis to form active caspase 3,

an effector caspase of apoptosis [24, 25]. The mitochondria-dependent (Type II) apoptotic pathway starts with apoptosis-regulating protein family exemplified by Bcl-2 family, such as anti-apoptotic Bcl-2 and pro-apoptotic Bad [24, 26, 27]. Pro-apoptotic Bcl2 family will enhance cytochrome *c* release from mitochondria [24, 26-28]. Cytochrome *c* release into cytosol activates caspase-9, then caspase-3 executes the apoptotic program [24, 27]. Besides, t-Bid was regarded as a main intracellular molecule signaling mediator from Fas to mitochondrial pathway because activated caspase 8 can cleave Bid to t-Bid then release cytochrome *c* to activate mitochondria-dependent apoptosis [24, 25]. In our previous study, the 8- week CIH were found to activate the Fas-dependent and mitochondria-dependent apoptotic pathways in rat brains [7]. The effect of salidroside on CIH-induced cardiac apoptosis in mice brains is not understood. We hypothesized that salidroside may prevent CIH-activated Fas-mediated and mitochondria-mediated cortex apoptosis in mice brains.

Materials and Methods

Animal model and Salidroside

The studies were performed on sixty-four C57BL/6J 5-6 month old male mice. Sixty-four C57BL/6J mice 5-6 months of age were divided into four groups, the Control group (21% O_2, 24 hrs per day, 8 weeks, n=16); Hypoxia group (Hypoxia: 7% O_2 60 seconds, 20% O_2 alternating 60 seconds, 8 hrs per day, 8 weeks, n=16); Hypoxia+S10 group and Hypoxia+S30 group (Hypoxia for the first 4 weeks, Hypoxia pretreated 10mg/kg and 30mg/kg salidroside by oral gavage per day for the second 4 weeks, n=16 and n=16). Salidroside in the current study was purchased from Venter International Co., Ltd. The salidroside was extracted from Rhodiola and was tested over 98% purity in high performance liquid chromatography fingerprinting analyses. Ambient temperature was maintained at 25°C. All mice were kept on an artificial 12-h light-dark cycle and the light period began at 7:00 A.M. The mice were provided with standard laboratory chow (Lab Diet 5001; PMI Nutrition International Inc., Brentwood, MO, USA) and unlimited water. All procedures were reviewed by the Institutional Animal Care and Use Committee, China Medical University, Taichung, Taiwan, and the principles of laboratory animal care (NIH publication) were followed.

Tissue Extraction

The brain tissue extracts were homogenized into a lysis buffer in a ratio of 100 mg tissue/1ml buffer for 1 minute to obtain cortex tissue extracts. The homogenates were place on ice for 10 minutes then centrifuged on 12,000 *g* used for 40 minutes twice. The supernatant was collected and stored at -70°C.

Electrophoresis and Western Blot

The Bradford Method was used to determine the protein concentration of cortex tissue extracts (Bio-Rad Protein Assay, Hercules, CA, USA). Protein samples (50

μg/lane) were separated on a SDS polyacrylamide gel (10%) electrophoresis (SDS-PAGE) with a controlled voltage of 75V. Electrophoresed proteins were applied to polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, 0.45μm pore size) with a Bio-Rad transfer apparatus. PVDF membranes were incubated in 5% milk with a TBS buffer. Primary antibodies using in the current study including Bcl-2 (BD Biosciences, San Jose, California, USA), Fas ligand, Fas receptor, FADD, Bcl-xL, Bax, Bid, t-Bid, caspase 8, caspase-9, caspase-3 (Santa Cruz Biotechnology, Inc. Santa Cruz, CA, USA), and α-tubulin (Neo Markers, Fremont, CA, USA) were diluted to a 1:500 ratio in an antibody binding buffer overnight at 4°C. The immunoblots were washed three times in TBS buffer for 10 minutes and then immersed in the second antibody solution containing goat anti-mouse IgG-HRP, goat anti-rabbit IgG-HRP, or donkey anti goat IgG-HRP (Santa Cruz) for 1 hour and diluted 500-fold in TBS buffer. The immunoblots were then washed in TBS buffer for 10 min three times. The immunoblotted proteins were visualized using an enhanced chemiluminescence ECL western Blotting luminal Reagent (Santa Cruz, CA, USA) and quantified using a Fujifilm LAS-3000 chemiluminescence detection system (Tokyo, Japan).

H&E staining, Masson Trichrome Staining, and TUNEL

The brains were excised, saturated in formalin, dehydrated using graded alcohols and then embedded in paraffin wax. The 2 μm thick paraffin sections of brain tissue were taken from the paraffin wax blocks. The sample blocks were immersed in Xylene and then rehydrated to allow removal of the tissue sections. The tissue samples were then dyed with hematoxylin and eosin for Hematoxylin-eosin staining (H&E staining). For Masson Trichrome Staining, the slices were dyed by Masson Trichrome. After rinsing with water, samples were dehydrated using graded alcohols then soaked in Xylene two times. Zeiss Axiophot microscopes were used to obtain photomicrographs.

The sections were incubated by proteinase K for Terminal Deoxynucleotide Transferase-mediated dUTP Nick End Labeling (TUNEL) assay, washed in Phosphate-Buffered Saline (PBS), incubated with permeabilisation solution, blocking buffer, and cleaned twice again with PBS. For detection, we used a Rosche apoptosis detection kit of terminal deoxynucleotidyl transferase and fluorescein isothiocyanate-dUTP for 60 minutes at 37 °C (Roche Applied Science, Indianapolis, IN, USA). TUNEL-positive nuclei (fragmented DNA) fluoresced bright green were observed at 450-500 nm. The mean number of TUNEL-positive cells were calculated for at least 5-6 separate fields x 2 slices x 3 regions of the left ventricle (upper, middle, lower) excised from 6 mice brains in each group. All results were collected by at least two independent individuals in a blind manner.

Statistical Analysis

The protein levels and cortex TUNEL-positive apoptotic cells were compared among the Control, the Hypoxia, the Hypoxia+S10 and the Hypoxia+S30 groups using analysis of variance of test with pre-planned contrast comparison to negative control group and positive control group "Hypoxia". In all cases $P<0.05$ was considered significant.

Results

Upstream components of cortex Fas receptor dependent apoptotic pathways

To investigate whether Salidroside prevent the upstream components of cortex Fas-dependent apoptotic signaling pathways after chronic intermittent hypoxia, the protein levels expression of Fas ligand, Fas receptor and FADD in four groups were examined by Western blotting. Compared with the Control group, Fas ligand, Fas receptor, and FADD were significantly increased in the Hypoxia group (Fig 1). Fas ligand, Fas receptor, and FADD in the the Hypoxia+S10, and the Hypoxia+S30 groups were significantly lower than those in the Hypoxia group (Fig 1).

Upstream components of cortex mitochondria-dependent apoptotic pathways

To further understand whether salidroside prevent upstream components of mitochondria-dependent apoptotic pathways after chronic intermittent hypoxia, we examined the protein levels of the Bcl-2 family (Bcl-xL, Bcl-2, Bax) in four groups by Western Blotting. Mitochondrial related pro-apoptotic proteins of Bax, and Bax-to-Bcl2 ratio were significantly higher in the Hypoxia group than the Control group as well as those in the Hypoxia+S10, and the Hypoxia+S30 groups were lower than those in the Hypoxia group. Anti-apoptotic Bcl-xL and Bcl-2 proteins were increased in the Hypoxia+S10 and Hypoxia+S30 groups when compared to the Hypoxia group (Fig 2).

Main intracellular molecule signaling mediator from Fas to mitochondrial pathway

To investigate the therapeutic effect of salidroside on cortex main intracellular molecule signaling mediator from Fas to mitochondrial pathway after chronic intermittent hypoxia, we examined the protein levels of t-Bid from the Control, the Hypoxia and the Hypoxia+S10 and the Hypoxia+S30 groups. The protein level of t-Bid were increased in the Hypoxia groups, compared with the Control group (Fig 3). The protein level of t-Bid in the Hypoxia+S10, and the Hypoxia+S30 groups was

significantly lower than the Hypoxia group (Fig 3).

Downstream components of cortex Fas and mitochondria dependent apoptosis

In order to identify whether salidroside prevent downstream components of cortex Fas receptor (caspase 8 and 3) and mitochondrial (caspase 9 and 3) dependent apoptotic pathways, the caspase 8, 9 and 3 was measured by Western blotting in the brains excised from the Control group, the Hypoxia group and the Hypoxia+S10 group and the Hypoxia+S30 group. Western blot analysis revealed that, compared to the Control group, the protein products of activated caspase 8, 9, and 3 were increased in the Hypoxia groups but not changed in the salidroside group (Fig 4).The protein level of activated caspase 8, 9, and 3 in the treatment of the Salidroside group was significantly lower than those in the Hypoxia group (Fig 4).

Discussion

Our study identified the protective effects of salidroside treatment on brain disease associated with chronic intermittent hypoxia. The summarized findings are: (1) The activity of cortex Fas receptor-dependent apoptotic pathway in mice with chronic intermittent hypoxia was decreased after salidroside treatment, which was based on reductions in Fas ligand, Fas receptor and FADD, activated caspase-8 and activated caspase-3. (2) The activity of cortex mitochondrial-dependent apoptotic pathway in mice with chronic intermittent hypoxia was decreased with treatment of salidroside, which was based on increases in anti-apoptotic Bcl-xL, Bcl-2, and Bid levels as well as decreases in pro-apoptotic Bax, t-Bid, activated caspase-9 and activated caspase 3. Subsequent to merging our results with prior apoptotic theories, we offer a hypothesized illustration (Fig 6).

This study showed that salidroside treatment potentially protects from cortex damage and apoptosis after chronic intermittent hypoxia which is an animal model of

severe sleep apnea. Since cortex tissues are not easily extracted from human brains, the current animal model under chronic intermittent hypoxia-induced cardiac apoptosis and pre-treatment of salidroside might provide some information as to how salidroside treatment prevents neurodegeneration or apoptosis-related brain diseases in humans with severe sleep apnea. Our study proved that salidroside treatment may prevent activated Fas-mediated and mitochondria-mediated cortex apoptosis after chronic intermittent hypoxia. Further studies are required to clarify the exact mechanisms responsible for this therapeutic effect in chronic intermittent hypoxia and the potential therapeutic application in humans with severe sleep apnea.

Acknowledgement:

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Figure Legend

Fig 1. (A) The representative protein products of Tumor Necrosis Factor alpha (TNF-α), TNF receptor one (TNF-R1), Fas ligand, Fas receptor and Fas-associated death domain (FADD) extracted from the left ventricles of excised hearts in the Control, the Hypoxia, the Hypoxia+S10, and the Hypoxia+S30 groups were measured by Western Blot analysis. (B) The bars represent the relative protein quantification of Fas receptor, Fas ligand and Fas-associated death domain (FADD) to α-tubulin, respectively, and indicates Mean values ±SD (n=6 in each group). *P<0.05,**P<0.01, are the significant differencess from the Control. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, are the significant differencess from the Hypoxia group.

Fig 2. (A) The representative protein products of Bax, Bcl-2, Bax, and Bcl-xL extracted from the left ventricles of excised hearts in the Control, the Hypoxia, the Hypoxia+S10 and the Hypoxia+S30 groups were measured by Western Blotting analysis. (B) The bars represent the ratio of Bax to Bcl2 and Bak to Bcl-xL indicates Mean values ±SD (n=6 in each group). **P<0.01, are the significant differences from the Control. $^{\#}$P<0.05, $^{\#\#}$P<0.01, are the significant differences from the Hypoxia group.

Fig 3. (A) The representative protein products of t-Bid and Bid extracted from the left ventricles of excised hearts in the Control, the Hypoxia, the Hypoxia+S10 and the Hypoxia+S30 groups were measured by Western Blotting analysis. (B) The bars represent the relative protein quantification of t-Bid and Bid to α-tubulin and indicates Mean values ±SD (n=6 in each group). *P<0.05, **P<0.01 are the significant differences from the Control. $^{\#\#\#}$P<0.001 are the significant differences from the Hypoxia group.

Fig 4. (A) The representative protein products of activated caspase 8, activated caspase 9, and activated caspase 3 extracted from the left ventricles of excised hearts in the Control, the Hypoxia, the Hypoxia+S10, and the Hypoxia+S30 groups were measured by Western Blotting analysis. (B) The bars represent the relative protein quantification of activated caspase 8, activated caspase 9, and activated caspase 3 to α-tubulin, respectively, and indicates Mean values ±SD (n=6 in each group). *P<0.05, **P<0.01 are the significant differencess from the Control. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, are the significant differencess from the Hypoxia group.

Fig 1

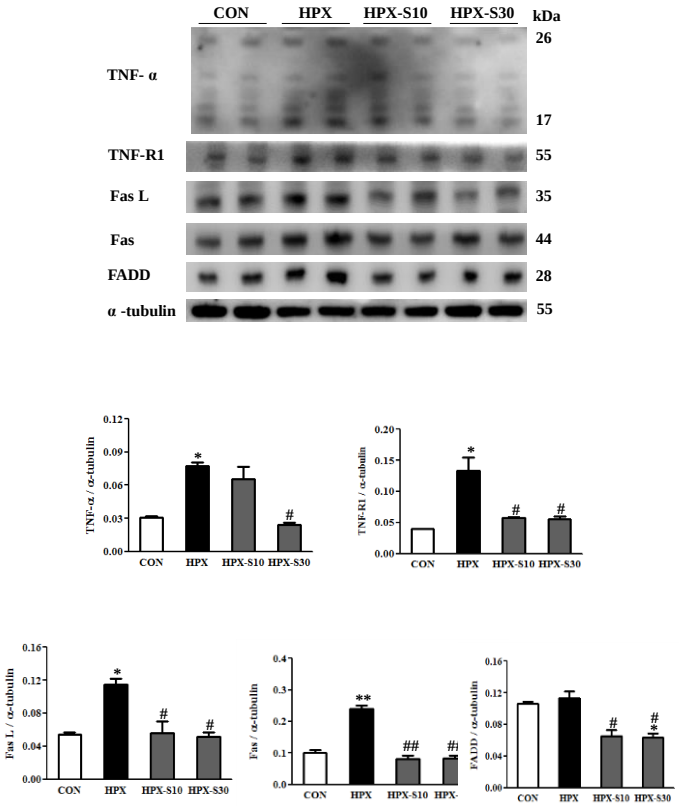
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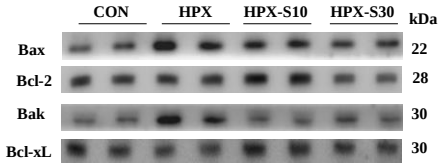
Fig 2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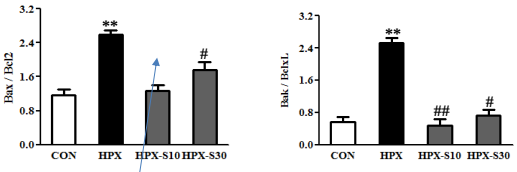
kDa
Bax
22
Bcl-2
28
Bak
30
Bcl-xL
30


Bax / Bcl2
3.2
2.4
1.6
0.8
0.0
**
#
CON
HPX
HPX-S10
HPX-S30
Bak / BclxL
3.2
2.4
1.6
0.8
0.0
**
##
#
CON
HPX
HPX-S10
HPX-S30

#

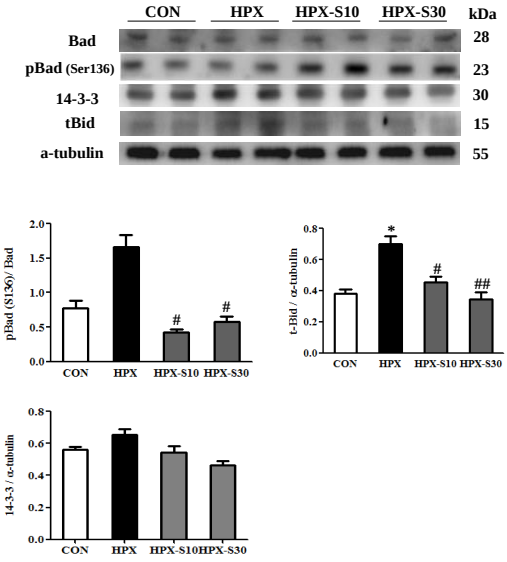
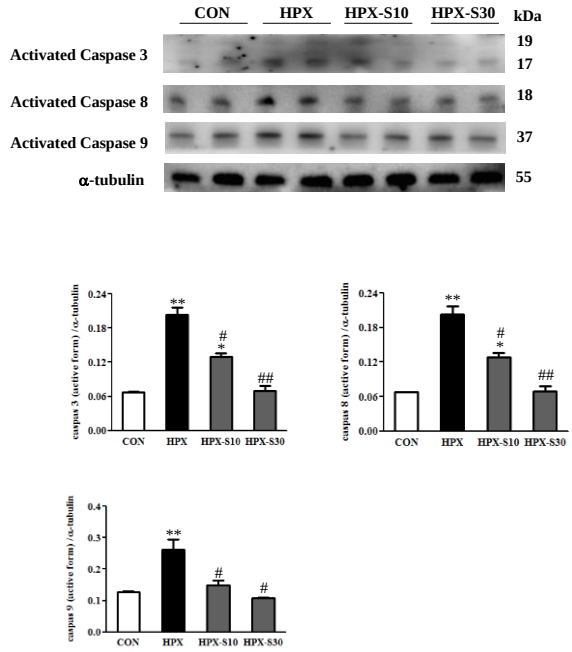

CON
HPX
HPX-S10
HPX-S30
kDa
Bad
28
pBad (Ser136)
23
14-3-3
30
tBid
15
a-tubulin
55
pBad (S136)/ Bad
2.0
1.5
1.0
0.5
0.0
#
#
CON
HPX
HPX-S10
HPX-S30
t-Bid / α-tubulin
0.8
0.6
0.4
0.2
0.0
*
#
##
CON
HPX
HPX-S10
HPX-S30
14-3-3 / α-tubulin
0.8
0.6
0.4
0.2
0.0
CON
HPX
HPX-S10
HPX-S30

Fig 4


CON
HPX
HPX-S10
HPX-S30
kDa
Activated Caspase 3
19
17
Activated Caspase 8
18
Activated Caspase 9
37
α-tubulin
55
caspas 3 (active form) /α-tubulin
caspas 8 (active form) /α-tubulin
caspas 9 (active form) /α-tubulin
CON
HPX
HPX-S10
HPX-S30

科技部補助計畫衍生研發成果推廣資料表

日期:2015/11/30

科技部補助計畫	計畫名稱: Salidroside配合運動治療對睡眠呼吸終止症誘發大腦細胞凋亡的療效探討
	計畫主持人: 丁化
	計畫編號: 103-2314-B-040-001- 學門領域: 復健科

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：丁化	**計畫編號：**103-2314-B-040-001-
計畫名稱：Salidroside配合運動治療對睡眠呼吸終止症誘發大腦細胞凋亡的療效探討	

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	0	0	100%		
		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	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%		
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	0	0	100%		
		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（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	1	1	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。）		無					

	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）

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）
   Salidroside對睡眠呼吸終止症誘發大腦細胞凋亡有保護作用應具臨床應用價值