行政院國家科學委員會專題研究計畫成果報告

當藥苦素對熱原性發燒大鼠解熱作用之研究

The Antipyretic Effects of Swertiamarin on Pyrogenic Fever in Rats

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一、中、英文摘要

Swertiamarin 為龍膽科植物龍膽(*Gentiana scabra*)的主要成分之一，屬 secoiridoide glycosides；目前研究發現 Swertiamarin (SWE) 除具鎮靜、鎮痛、抗發炎和抗癲癇等作用外，亦可明顯降低正常大鼠之直腸溫度；本實驗室於八十六年度計畫中即針對 SWE 之降溫作用與 serotonergic system 間之關係進行研究，實驗結果顯示：龍膽甲醇萃取物(0.5-3.0g/kg, p.o.)與其主要活性成分 SWE (10-30 mg/kg, i.p.) 均可明顯降低室溫下正常清醒大鼠之體溫，且具劑量依存性；其降溫作用之機轉可能藉由抑制突觸後 serotonin (5-HT) 受體而達成。因此，本計畫繼續針對 SWE 對室溫下熱原性發燒大鼠解熱作用及其作用機轉進行探討；研究結果顯示：SWE (10, 20 mg/kg, i.p.)對致熱原 lipopolysaccharide (LPS) 及 interluckin-1β (IL-1β) 誘發高溫之大鼠具明顯之降溫作用，且可降低 LPS 與 IL-1β 誘發高溫時下視丘 5-HT 濃度增加之現象。又 SWE 對 prostaglandin E_2 (PGE_2)、cAMP 之類似物 (8-Bromo-cAMP)、nitric oxide (NO) donor (S-Nitroso-N-acetylpenicillamine)、NO 釋放劑 (sodium nitroprusside) 和 cGMP 之類似物 (8-Bromo-cGMP) 誘發之高溫均具抑制作用。綜合以上結果，可推知 SWE 具解熱降溫作用，其作用可能與抑制中樞 serotoninergic system 及 PGE_2 與 NO 之活性有關。

關鍵詞：當藥苦素、熱原性發燒、五羥色胺酸、一氧化氮

Abstract

Swertiamarin is a major bitter constitu-ent of *Gentiana scabra*. Swertiamarin (SWE) was reported to have sedative, analgesic, anticonvulsive, antiinflammatory and hypo-thermic effects. In our precedent study, we found SWE caused a dose-related fall in colonic temperature at room temperature, and it may acts through serotoninergic system to induce the hypothermia. The purpose of the present study was intended to investigate the mechanism of SWE on antipyretic effects.

The fever and hypothalamic serotonin (5-HT) release induced by either lipopolysa-ccharide (LPS, 100μg/kg, intraperitoneal injection (i.p.)) or interluckin-1β (IL-1β, 10ng/rat, intracerebroventricular injection (i.c.v.)) were attenuated by treatment with SWE (10, 20 mg/kg, i.p.). SWE inhibited hyperthermia induced by either prostaglandin E_2 (PGE_2, 200 ng/rat, i.c.v.), 8-Bromo-cAMP (cAMP analogue, 40μg/rat, hypothalamic injection (i.h.)), S-Nitroso-N-acetylpenicilla-mine (nitric oxide donor, 10μg/rat, i.c.v.), sodium nitroprusside (NO releaser, 20μg/rat, i.c.v.) or 8-Bromo-cGMP (cGMP anologue, 100μg/rat, i.c.v.). These results indicate that SWE exerts its antipyretic effects mainly through the central nervous serotoninergic, PGE_2 and NO mechanisms.

Keywords: Swertiamarin, Pyrogenic Fever, Serotonin, Nitric oxide

二、緣由與目的

龍膽為龍膽科多年生草本植物龍膽(*Gentiana scabra*)、堅龍膽(*Gentiana rigescens*)、三花龍膽(*Gentiana triflora*)或條葉龍膽(*Gentiana manshurica*)等的乾燥根，始載於神農本草經列為上品，「味苦澀...主骨間寒熱，驚癇邪氣」[1]，名醫別錄曰：「除胃中伏熱、時氣溫熱、熱瀉下痢、去腸中小蟲，益肝膽之氣、止驚悸」[2]。中醫臨床上常用來治療肝炎、腦炎與結膜炎等急性炎症[1]；現代研究顯示龍膽具明顯之保肝、利膽、健胃、抗發炎、抗原蟲及骨骼肌鬆弛等作用[3]。

Swertiamarin (當藥苦素, SWE) 為龍膽的主要成分之一，屬 secoiridoide glycosides [4,5]。實驗証明 SWE 可明顯降低大鼠自發運動量，延長 pentobarbital 誘發之睡眠時間，加強 morphine 鎮痛之時間，抑制 carrageenan 誘發之發炎，以及抗 pentylenetetrazol 誘發之痙攣，具有鎮靜、鎮痛、抗發炎和抗癲癇等作用[6]；1976 年，Bhattacharya SK 等教授研究發現 SWE 除具有上述之中樞抑制作用外，亦可明顯降低正常大鼠之直腸溫度[7]；本實驗室於八十六年度計畫中即針對 SWE 之降溫作用與 serotonergic system 間之關係進行研究，實驗結果顯示：龍膽甲醇萃取物(0.5-3.0g/kg, p.o.)與其主要活性成分 SWE (10-30 mg/kg, i.p.) 均可明顯降低室溫下正常清醒大鼠之體溫，且具劑量依存性；其降溫作用之機轉可能藉由抑制突觸後 serotonin (5-HT) 受體而達成。因此，本計畫繼續針對對室溫下正常大鼠或致熱原(lipopolysaccharide, LPS 或 interleukin-1β, IL-1β)誘發高溫大鼠直腸溫度及清醒大鼠下視丘(體溫調節中樞) 5-HT 變化之影響進行評估，並以臨床上常用之止痛解熱劑 aspirin 作為正對照組。再併用 prostaglandin E_2 (PGE_2)、cAMP 之類似物 (8-Bromo-cAMP)、nitric oxide (NO) donor (S-Nitroso-N-acetylpenicillamine; SNAP)、NO 釋放劑 (sodium nitroprusside; SNP) 和 cGMP 之類似物(8-Bromo-cGMP)等藥物，探討 SWE 之解熱降溫作用與 PGE_2 和 NO 活性之關係，期能深入了解 SWE 解熱降溫作用之機轉，俾有助於中醫藥之現代化。

三、結果與討論

由於傳統上把能引起人體或動物發熱的物質，通稱為致熱原(pyrogen)，一般又可分為外生性致熱原及內生性致熱原[8]。而外生性致熱原(如：endotoxin)可促使白血球與巨噬細胞等釋放內生性致熱原（如：interleukin-1, tumor necrosis factor, interferon等），後者再作用於體溫調節中樞而產熱[9,10,11]。1923年，Seibert 指出引起動物體發燒的物質主要是來自葛蘭氏陰性菌細胞壁受破壞所游離出的毒素，稱為細菌內毒素，其會造成動物畏寒、發燒等症狀，甚至導致敗血性休克(septic shock)而死亡；1943年Shear根據細菌內毒素結構上特性，另命名為 lipopolysaccharide 簡稱為LPS [12]。Interleukin-1 (IL-1) 源自於巨噬細胞 、免疫性T、B淋巴細胞和血管內皮細胞等，隨著血液循環分布全身[13]，亦可由中樞星狀細胞(astrocyte)和微小神經膠細胞 (microglia)合成[14]。IL-1 直接注射入下視丘或腦室給予低劑量 IL-1 均可誘發燒反應[15,16]；而給予對抗 IL-1 抗體或 interleukin-1 receptor antagonist，可抑制內生性 IL-1 致熱作用[17]；IL-1β由側腦室給予大鼠可誘發一具劑量依存性、明顯持久之高溫 (大於6小時)[18]。Aspirin可抑制 cyclooxygenase，進而減少prostaglandins 之合成，是臨床上常用之解熱劑[19]。故本研究利用腹腔給予LPS (100μg/kg)或側腦室給予IL-1β(10ng/rat)誘發一較符合實際引發疾病發熱之動物模式，並以aspirin作為正對照組借以評估SWE之解熱效果。實驗結果顯示SWE (5.0-20 mg/kg, i.p.) 對LPS及內生性致熱原IL-1β誘發高溫之大鼠具明顯之解熱效果（Table 1）。

有關單胺類神經傳遞物質對體溫調節

影響之研究，早在1961年Von Euler即指出下視丘單胺可能在體溫調節中扮演某種角色 [20] ， 1978 年 Myers 及 Waller 指出 serotoninergic system在體溫調節系統中扮演一重要角色[21]，1980年Myerse提出下視丘5-HT之含量與熱的產生有關[22]，降低腦中5-HT之含量會引起尾巴皮膚溫度上升以增加散熱作用[23,24]；而側腦室給予大鼠 IL-1β誘發高溫時，亦可明顯升高大鼠下視丘 5-HT 之濃度[25,26]。因此本計畫利用微透析-高效液相層析法（Microdilalysis- HPLC-ECD）測定SWE對於清醒正常大鼠下視丘 5-HT濃度變化之影響，實驗結果如Table 2所示，LPS或IL-1β均可明顯誘發大鼠下視丘5-HT濃度之增加，室溫下 SWE 對於正常大鼠下視丘 5-HT之濃度並無影響，但在較大劑量 (20mg/kg, i.p.) 對LPS或IL-1β誘發之大鼠下視丘5-HT濃度增加現象具抑制作用，可知SWE對LPS或IL-1β誘發之熱原性發燒大鼠之解熱作用可能與降低下視丘 serotoninergic system 之活性有關。

由於內生性致熱原(IL-1) 之致熱作用可能是經由促進下視丘內 PGE_2 之合成所致[27]，將 PGE_2 直接注入大鼠之側腦室可引起一快速之升溫之現象[28]。其次，1982 年，陽明大學 林茂村教授研究指出在室溫 8-30℃ 時，dibutyryl cyclic AMP（db cyclicAMP）直接注入大鼠下視丘可引起一升溫作用，且此作用並不會被 α- 或 β- adrenergic antagonist (下視丘給予) 前處理所拮抗，但同條件下 PGE_2 或 NE (下視丘給予) 所誘發之升溫作用，則會被 α- 或 β- adrenergic antagonist (下視丘給予) 前處理所拮抗，故可知 PGE_2 誘發之升溫作用可能是經由下視丘之 NE-cyclicAMP 徑路而達成[29]。因此本研究併用 PGE_2 (200ng/rat, i.c.v.)或 cAMP 之類似物(8-Bromo-cAMP, 40μg/rat, i.h.) 以了解 SWE 解熱作用之作用機轉，實驗結果發現 SWE (10, 20mg/kg, i.p.) 可明顯降低 PGE_2 或 8-Bromo-cAMP 所誘發之升溫作用 (Table 3)。

此外，近年來報告指出 cytokines 或 LPS 能刺激腦 astroglia，經由精胺酸路徑合成大量之 cyclic GMP [30]；而最近之研究更進一步發現 IL-1β或 LPS 可藉由 tyrosine kinases 路徑將訊息傳入細胞內，並增加 inducible nitric oxide synthase (iNOS) 及 cyclooxygenase II (COX-2) 之量，促使 NO 及 PGE 等致炎介質合成增加[31,32]；NO 能活化腦細胞製造 cGMP 作為次級神經傳遞物質，產生各種生理或病理反應[(47)]； Kandasamy 等發現腦室給予 cGMP 之類似物可誘發發燒之反應[33]。所以本研究最後乃併用 NO donor SNAP (10μg/rat, i.c.v.) 、NO 釋放劑 SNP (20μg/rat, i.c.v.) 和 cGMP 之類似物 8-Bromo-cGMP (100μg/rat, i.c.v.)等藥物，對 SWE 解熱作用之與 NO 活性之關係進行探討，實驗結果顯示 SWE (10, 20mg/kg, i.p.) 可明顯降低上述藥物所誘發之升溫作用 (Table 4)。

綜合以上結果，顯示 SWE 具明顯之解熱降溫作用，其解熱作用之機轉可能與抑制中樞 serotoninergic system 及 PGE_2 與 NO 之活性有關。

四、計畫成果自評

本研究計畫案計畫書中擬進行之各項實驗除部分藥物給藥方式與劑量，依實際實驗狀況略有修正更動外，均已完成。

有關SWE給藥途徑原擬分就周邊(腹腔)及中樞(側腦室)二不同給藥途徑探討其作用機轉，但為更切合實際與臨床應用之價值，故先針對腹腔投予方式探討之。

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Table 1. The effect of swertiamarin (SWE) on the colonic temperature and hypothalamic serotonin (5-HT) release in the hyperthermia rats induced by lipopolysaccharide (LPS) and interleukin-1β (IL-1β).

Treatment	Change in colonic temperature (Δ°C)		
	Normal	LPS	IL-1β
Saline 0.9% (i.p.)	0.15±0.05	1.51±0.10	2.36±0.32
SWE			
5.0 mg/kg (i.p.)	-0.42±0.09	0.68±0.10*	1.59±0.14*
10.0 mg/kg (i.p.)	-0.71±0.14*	0.50±0.21*	1.37±0.23*
20.0 mg/kg (i.p.)	-1.13±0.24*	0.33±0.17**	1.01±0.21**
Aspirin			
75 mg/kg (i.p.)	-0.12±0.13	0.42±0.17*	1.02±0.12*
150 mg/kg (i.p.)	-0.21±0.21	0.14±0.09**	0.72±0.21**

SWE and aspirin was injected 30 min after LPS (100μg/kg) intraperitoneal injected (i.p.) or 180 min after IL-1β (10ng/kg) intracerebroventricular injected (i.c.v.). The value are mean±SEM of 8 rats per group. Δ, different between the control values before injected and maxium exchange after injected. *P<0.05, **P<0.01, significantly different from the corresponding control values (saline group), ANOVA.

Table 2. The effect of swertiamarin (SWE) on the hypothalamic serotonin (5-HT) release in the hyperthermia rats induced by lipopolysaccharide (LPS) and interleukin-1β (IL-1β).

Treatment	Change in hypothalamic 5-HT release (% basline)		
	Normal	LPS	IL-1β
Saline 0.9% (i.p.)	100.24±8.52	196.27±18.95	258.66±64.25
Swertiamarin			
10.0 mg/kg (i.p.)	95.28±17.27	175.82±50.23	201.12±44.25
20.0 mg/kg (i.p.)	90.78±22.11	128.63±38.14*	169.92±53.18*

SWE and aspirin was injected 30 min after LPS (100μg/kg) intraperitoneal injected (i.p.) or 180 min after IL-1β (10ng/kg) intracerebroventricular injected (i.c.v.). The value are mean±SEM of 5 rats per group. Δ, different between the control values before injected and maxium exchange after injected. The basal 5-HT was 1.2±0.6 pg/20μl. *P<0.05, significantly different from the corresponding control values (saline group), ANOVA.

Table 3. Effects of swertiamarin (SWE) on the hyperthermia induced by intracere-broventricular injection (i.c.v.) of Prostaglandin E_2 (PGE_2) or hypothalamic injection (i.h.) of 8-Bromo-cAMP.

Treatment	Change in colonic temperature (Δ°C)		
	Saline 0.9% (i.p.)	Swertiamarin	
		10 mg/kg (i.p.)	20 mg/kg (i.p.)
Vehicle	0.11±0.15	-0.77±0.17	-1.13±0.24
PGE_2 200ng/rat (i.c.v..)	1.33±0.29	0.64±0.11*	0.37±0.28**
8-Bromo-cAMP 40μg/rat (i.h.)	0.75±0.17	-0.24±0.29*	-0.78±0.32**

SWE was injected 40 min before PGE_2 injected and 20 min after 8-Bromo-cAMP injected. The value are mean±SEM. of 8 rats per group. Δ, different between the control values before injected and maxium exchange after injected. *P<0.05, **P<0.01, significantly different from the corresponding control values (saline group), ANOVA.

Table 4. Effects of swertiamarin (SWE) on the hyperthermia induced by intracere-broventricular injection (i.c.v.) of S-nitroso-N-acetylpenicillamine (SNAP), sodium nitroprusside (SNP) or 8-Bromo-cGMP .

Treatment	Change in colonic temperature (Δ°C)		
	Saline 0.9% (i.p.)	Swertiamarin	
		10 mg/kg (i.p.)	20 mg/kg (i.p.)
Vehicle	0.11±0.15	-0.77±0.17*	-1.13±0.24*
SNAP 10μg/rat (i.c.v..)	1.81±0.20	1.25±0.20*	0.33±0.27**
SNP 20μg/rat (i.c.v..)	1.32±0.21	0.56±0.24*	0.18±0.32*
8-Bromo-cGMP 100μg/rat (i.c.v..)	0.75±0.13	0.19±0.11*	-0.55±0.19*

SWE was injected 120 min after SNAP, 120 min after SNP or 90 min after 8-Bromo-cGMP injected. The value are mean±SEM. of 8 rats per group. Δ, different between the control values before injected and maxium exchange after injected. *P<0.05, **P<0.01, significantly different from the corresponding control values (saline group), ANOVA.