行政院國家科學委員會專題研究計畫成果報告

葛根素對於熱源性發燒大鼠解熱作用之研究

The Antipyretic Effects of Puerarin on Pyrogenic Fever in Rats

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一、中、英文摘要

葛根素 (puerarin) 為葛根之主要成分之一，屬 isoflavonoids；近年來藥理研究發現其為一 beta-adrenoceptor 之阻斷劑，對腦內之 benzodiazepine receptor 亦具致效作用；此外 puerarin 還具有減低酒精吸收，平滑肌舒張，促進腦及心臟血流，保護心肌，降血壓，抗心率不整，降低 2,4-dinitrophenol 誘發之高溫等作用。然有關 puerarin 解熱作用之機轉至今仍並未見有研究報告提出；因此，本研究乃先針對 puerarin 對致熱原 lipopolysaccharide (LPS) 或 interluckin-1β (IL-1β)誘發高溫大鼠之解熱作用進行測定，再利用 prostaglandin E2 (PGE2) 、cAMP 之類似物 (8-Bromo-cAMP) 、NO donor (S-Nitroso-N-acetyl-penicillamine, SNAP)、NO 釋放劑(sodium nitroprusside, SNP) 或 cGMP 之類似物(8-Br-cGMP)等，進行更深入之作用機轉探討。研究結果顯示：puerarin (5, 10, 30mg/kg, i.p.)對 LPS 或 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 dopamine 與 serotonin 濃度增加之現象。又 puerarin 對 PGE2、cAMP、SNAP、SNP 或 cGMP 誘發之體溫升高現像亦具抑制作用。綜合以上結果，可推知 puerarin 具解熱降溫作用，其作用可能與抑制中樞 dopamine、serotonin、PGE2 及 NO 等與體溫調節相關系統之活性有關。

關鍵詞：葛根素、熱原性發燒、單胺、一氧化氮

Abstract

Puerarin is an isoflavone compound isolated from *Puerariae lobata*.. The radix of Puerariae's root has been used for the treatment fever in Chinese. Puerarin, a beta-receptor blocker, was reported to have anti-convulsive, antiarrhythmic and antihyperten-sion effects. It also can reduce the ethanol intake and 2,4-dinitrophenol-induced hyper-thermia. However, the effects and mechanism of puerarin on the antipyretic effect are not investigated even till now. On this account, the purpose of the present study was intended to investigate the mechanism of puerarin on thermoregulatory responses and hypothalamic dopaminergic, serotoninergic, prostaglandin E2 (PGE2) or NO activity.

In unanesthetized rats, puerarin (5, 10, 30mg/kg, intraperitoneal injection (i.p.)) caused a dose-related fall in colonic temper-ature at room temperature. The fever induced by either lipopolysaccharide (LPS, 100μg/kg, i.p.) or interluckin-1β (IL-1β, 10ng/10μl, la-teral cerebral ventricle injection (i.c.v.)) was attenuated by treatment with puerarin (5, 10, 30mg/kg, i.p.). In microdialysis data revealed that puerarin (10,

30mg/kg, i.p.) produced a decrease in hypothalamic dopamine and serotonin concentration of normal or IL-1β-induced fever rat brain. The hyperthermia induced by either PGE2 (200ng/10μl, i.c.v.), 8-Bromo-cAMP (cAMP analogue, 40μg/10μl, hypothalamic injection), S-Nitroso-N-acetylpenicillamine (nitric oxide donor, 10μg/10μl, i.c.v.), sodium nitroprusside (NO releaser, 20μg/10μl, i.c.v.) or 8-Bromo-cGMP (cGMP anologue, 100μg/10μl, i.c.v.) was attenuated by treatment with puerarin (10, 30mg/kg, i.p.) in rats. These results indicate that puerarin exerts its antipyretic effects mainly through the central nervous dopaminergic, serotoninergic, PGE2 and NO mechanisms.

Keywords: Puerarin, Pyrogenic Fever, Monoamines, Nitric oxide

二、緣由與目的

葛根為豆科植物葛 (*Pueraria pseudo-hirsuta* Tang et Wang)、野葛 (*Pueraria lobata* (Willd.) Ohwi)或乾葛藤 (*Pueraria homsonii* Bentn)之乾燥根部;首載於神農本草經列為中品，味甘、平，「主消渴，身大熱，嘔吐，諸痺，起陰氣，解諸毒」[1]，為中醫常用之怯風解表藥，具有解肌退熱、生津、解酒、透疹、升陽止瀉之功。現代研究亦證實葛根對平滑肌之收縮與血壓之調節具雙相調節作用[2,3]，此外亦具抗心率不整[4]，改善腦循環及外週循環[2]等作用。

葛根素(puerarin)為葛根之主要成分之一，屬isoflavonoids；近年來藥理研究發現其為一β-adrenoceptor之阻斷劑[5]，對腦內之 benzodiazepine receptor 亦具致效作用[6]；此外puerarin還具有解酒(減低酒精吸收)[7]，解痙(平滑肌舒張)[2,8]，促進腦及心臟血流，保護心肌[9-12]，降血壓[13,14]，抗心率不整[15]，降低2,4-dini-trophenol誘發之高溫[16]等作用。然有關 puerarin 解熱降溫作用之機轉至今仍並未見有研究報告提出，因此，本研究乃先針對 puerarin 對致熱原 lipopolysaccharide (LPS)或 interluckin-1β (IL-1β)誘發高溫大鼠之解熱作用進行測定，再利用 prostaglandin E2 (PGE2)、cAMP之類似物(8-Bromo-cAMP)、NO donor (S-Nitroso-N-acetylpenicillamine, SNAP)、NO 釋放劑(sodium nitroprusside, SNP)或cGMP之類似物(8-Br-cGMP)等，進行更深入之作用機轉探討，俾有助於中醫藥之現代化。

三、結果與討論

1995年Zhou Y.等人報告指出puerarin能降低2,4-dinitrophenol誘發之高溫[16]。本研究實驗結果亦顯示腹腔注射puerarin(5-30mg/kg) 可明顯降低室溫下正常清醒大鼠之體溫，並具劑量依存性(Fig.1)；其降溫作用為腹腔注射後約120分鐘時可降至最低，持續時間則會隨劑量增加而延長。

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[17]。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[18-20]。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[21]。Inte-rleukin-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[22]，亦可由中樞星狀細胞(astrocyte)和微小神經膠細胞(microglia)

合成[23]。IL-1直接注射入下視丘或腦室給予低劑量時均可誘發燒反應[24,25]；而給予對抗IL-1抗體或IL-1 receptor antagonist，可抑制內生性IL-1之致熱作用[26]；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)[27]。Aspirin可抑制 cyclooxygenase，進而減少prosta-glandins之合成，為臨床上常用之解熱劑[28]。本研究利用腹腔給予LPS (100μg/kg)或側腦室給予IL-1β(10ng/10μl)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puerarin之解熱效果。實驗結果顯示，腹腔給予puerarin (10, 30mg/kg)對LPS或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(dopamine, serotonin)可能在體溫調節中扮演某種角色[29]，1978年Myers及Waller指出serotoninergic system在體溫調節系統中扮演一重要角色[30]，1980年Myerse提出下視丘serotonin之含量與熱的產生有關[31]，降低腦中serotonin之含量會引起尾巴皮膚溫度上升以增加散熱作用[32,33]；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dopamine與serotonin之濃度[34,35]。本計畫利用微透析-高效液相層析法(Microdilalysis-HPLC-ECD)測定puerarin對於清醒正常大鼠下視丘dopamine、serotonin及其代謝物3,4-dihydroxyphenylacetic acid (DOPAC)和5-hydroxyindoleacetic acid (5-HIAA)濃度變化之影響；結果如Table 2所示，IL-1β可明顯誘發大鼠下視丘dopamine、serotonin及其代謝物濃度之增加；室溫下puerarin(30mg/kg, i.p.)會降低正常大鼠下視丘 dopamine 及 serotonin 之濃度，提高5-HIAA之濃度，且可明顯降低IL-1β誘發高溫大鼠下視丘單　濃度增之加現象。可知puerarin對IL-1β誘發熱原性發燒大鼠之解熱作用可能與降低下視丘dopaminergic及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[36]，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[37]。其次，1982年林茂村教授研究指出 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[38]。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 puerarin 解熱作用之作用機轉，實驗結果發現 puerarin (10, 30mg/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 能刺激腦內 astroglia，經由精胺酸路徑合成大量之 cyclic GMP [39]；而最近之研究更進一步發現 LPS 或 IL-1β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[40,41]；Kandasamy 等發現腦室給予 cGMP 之類似物可誘發發燒之反應[42]。所以本研究最後乃併用 NO donor SNAP (10μg/10μl, i.c.v.)、NO 釋放劑 SNP (20μg/10μl, i.c.v.) 和 cGMP 之類似物 8-Bromo-cGMP (100μg/10μl, i.c.v.) 等藥物，對 puerarine 解熱作用之與 NO 活性之關係進行探討，實驗結果顯示 puerarine(10, 30mg/kg,) 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 puerarine 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 dopaminergic system、seroto-ninergic system、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。

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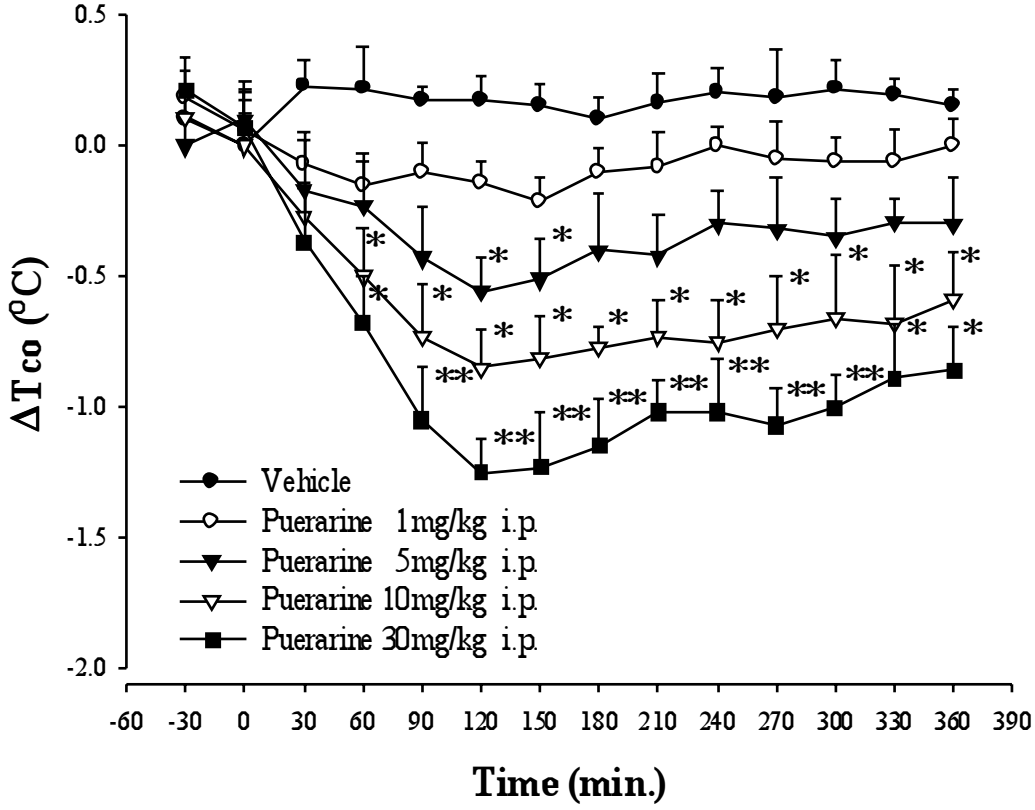

Fig 1. Time course of the effects of puerarine on colonic temperature in rats. Puerarin (1, 5, 10, 30 mg/kg, i.p.) was injected at 0 min. The colonic temperature of vehicle-injected rats was 37.82±0.20°C at time 0 min. Δ, denote the difference between the control values before injected and exchange after injected. The values are mean±SEM of 8-12 rats per group. *P<0.05, **P<0.01, significantly different from corresponding control values (vehicle group), ANOVA.

Table 1. The effect of puerarin on the colonic temperature in the hyperthermia rats induced by lipopolysaccharide (LPS) and interleukin-1β (IL-1β).

Treatment	Change in colonic temperature (Δ°C)		
	Normal	LPS	IL-1β
Vehicle (i.p.)	0.14±0.07	1.28±0.22	1.98±0.24
Puerarin			
5 mg/kg (i.p.)	-0.56±0.13*	1.11±0.19	1.56±0.16
10 mg/kg (i.p.)	-0.85±0.21*	0.65±0.15*	1.10±0.20*
30 mg/kg (i.p.)	-1.25±0.20**	0.30±0.17**	0.88±0.21**
Aspirin			
75 mg/kg (i.p.)	-0.14±0.12	0.45±0.18*	1.06±0.18*
150 mg/kg (i.p.)	-0.25±0.191	0.13±0.19**	0.77±0.21**

Puerarin was injected 10 min after LPS (100μg/kg) intraperitoneal injected (i.p.) or 120 min after IL-1β (10ng/10μl) intracerebroventricular injected (i.c.v.), and aspirin was injected 30 min after LPS injected or 180 min after IL-1β injected. The values are mean±SEM of 8 rats per group. Δ, denote the difference between the control values before injected and maximum exchange after injected. *P<0.05, **P<0.01, significantly different from the corresponding control values (vehicle group), ANOVA.

Table 2. The effect of puerarin on the hypothalamic dopamine (DA), serotonin (5-HT), 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) release in the hyperthermia rats induced by interleukin-1β (IL-1β).

Treatment	DA	DOPAC	5-HT	5-HIAA
	percent baseline			
Vehicle (i.p.)	100.24±20.52	102.48±21.27	99.12±34.25	106.33±38.14
IL-1β 10ng/10μl (i.c.v.)	192.18±30.55**	136.29±25.18*	384.52±70.82***	194.63±42.03**
Puerarin 30mg/kg (i.p.)	28.71±18.26***	96.23±34.64	21.61±13.36***	204.92±60.58***
IL-1β 10ng/10μl + Puerarin 30mg/kg	132.68±36.14#	114.63±19.96	259.74±40.84###	122.46±27.88##

Puerarin was injected 120 min after IL-1β intracerebroventricular injected (i.c.v.). The values are mean±SEM of 4 rats per group. The vehicle-treated control values for extracellular DA, 5-HT, DOPAC and 5-HIAA release in the hypothalamus are 6.16±2.21, 1.78±0.65, 2.94±1.02

and 217.26±18.48 pg/18μl/30min. *P<0.05, **P<0.01, ***P<0.001 significantly different from the corresponding control values (vehicle group), ANOVA. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 significantly different from the corresponding control values (IL-1β group), ANOVA.

Table 3. Effects of puerarin on the hyperthermia induced by intracerebroventricular 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)		
	Vehicle	Puerarin	
		10 mg/kg (i.p.)	30 mg/kg (i.p.)
Saline 0.9% (i.c.v.)	0.11±0.15	-0.85±0.21*	-1.25±0.20**
PGE_2 200ng/10μl (i.c.v..)	1.33±0.29	0.50±0.16*	-0.30±0.28**
8-Bromo-cAMP 40μg/10μl (i.h.)	0.65±0.17	-0.13±0.29*	-0.74±0.15**

Puerarin was injected 100 min before PGE_2 injected and 10 min after 8-Bromo-cAMP injected. The values are mean±SEM. of 8 rats per group. Δ, denote the difference between the control values before injected and maximum exchange after injected. *P<0.05, **P<0.01, significantly different from the corresponding control values (vehicle group), ANOVA.

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

Treatment	Change in colonic temperature (Δ°C)		
	Vehicle	Puerarin	
		10 mg/kg (i.p.)	30 mg/kg (i.p.)
Saline 0.9% (i.c.v.)	0.12±0.15	-0.85±0.21*	-1.25±0.20**
SNAP 10μg/10μl (i.c.v..)	1.78±0.20	0.97±0.25*	0.70±0.27**
SNP 20μg/10μl (i.c.v..)	1.40±0.23	0.91±0.21*	0.20±0.22**
8-Bromo-cGMP 100μg/10μl (i.c.v..)	0.75±0.16	0.20±0.15*	-0.83±0.30**

Puerarin was injected 120 min after SNAP, 10 min after SNP or 120 min after 8-Bromo-cGMP injected. The values are mean±SEM. of 8 rats per group. Δ, denote the difference between the control values before injected and maximum exchange after injected. *P<0.05, **P<0.01, significantly different from the corresponding control values (vehicle group), ANOVA.