行政院國家科學委員會專題研究計畫 成果報告

白藜蘆醇對大鼠子宮平滑肌收縮及卵巢細胞分泌性類固醇激素的影響(第 2 年)
研究成果報告(完整版)

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行政院國家科學委員會補助專題研究計畫 ■成果報告 □期中進度報告

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中文摘要

現代婦女有一些常見的婦科疾病，而在女性月經期間所引發的眾多不適症狀中，仍然以生理痛的現象為婦科疾病中最常發生的現象。生理痛（dysmenorrhea）又叫經痛或月經困擾症，是指女性在月經的期間，會感覺到強烈的下腹部陣痛，且發生率極高，約佔女性七成以上。主要為前列腺素過度分泌刺激子宮收縮所造成。目前治療生理痛的方式很多，其中利用藥物來降低因子宮平滑肌過度收縮所造成之疼痛較為普遍。而目前一般用來治療經痛的藥物如：即非固醇類抗發炎藥（nonsteroidal anti-inflammatory drugs,NSAID）、前列腺素合成抑制劑（prostaglandin-synthesis inhibitors, PGSI）、口服避孕藥（oral contraceptive pill, OCP）、鈣離子管道阻斷劑（calcium channel blockers）、止痛劑（analgesics）、綜合維他命（vitamins）等。白藜蘆醇(Resveratrol)為自然界存在的化學物質，已被廣泛研究發現具有抗氧化、抗癌等生理活性功能，然而對於是否抑制子宮過度收縮而用來改善經痛的現象，目前尚未有相關研究，本研究計畫擬探討白藜蘆醇，對於大鼠離體子宮平滑肌收縮的影響。由實驗結果發現白藜蘆醇物質確實可以抑制子宮平滑肌過度收縮之效果。此結果將有助於白藜蘆醇對子宮收縮之效應以及部分作用機轉，期望對經痛的改善能有所貢獻。

關鍵字：白藜蘆醇、子宮平滑肌、收縮、經痛

Abstract

Dysmenorrhea is directly related to elevated $PGF_{2\alpha}$ (prostaglandin $F_{2\alpha}$) levels. It is treated with NSAIDs (nonsteroid antiinflammatory drugs) in Western medicine. Since NSAIDs produce many side effects, Chinese medicinal therapy is considered as a feasible alternative medicine. Many special physiological components (ex: Resveratrol) in Chinese medicine have been isolated and identified. Resveratrol have a lot of physiological functions like anti-oxidation and anti-cancer effects. However, the relationship between uterine smooth muscle contraction and resveratrol remains veiled. We studied the *in vitro* effects of resveratrol on uterine smooth muscle contraction. The uterus was separated from female SD rat and uterine smooth muscle contraction activity was measured and recorded. Resveratrol inhibited uterine contractions induced by $PGF_{2\alpha}$, Ca^{2+} channel activator Bay K 8644, and high K^+ in a concentration-dependent manner *in vitro*; furthermore, resveratrol inhibited the Ca^{2+}-dependent uterine contractions. Thus, resveratrol consistently suppressed the increases in intracellular Ca^{2+} concentrations ($[Ca^{2+}]i$) induced by $PGF_{2\alpha}$ and high K^+. Thus, resveratrol probably inhibited uterine contraction by blocking external Ca^{2+} influx, leading to a decrease in $[Ca^{2+}]i$. Thus, resveratrol may be considered as a feasible alternative therapeutic agent for dysmenorrhea.

Key word: Resveratrol, uterine smooth muscle, contraction, dysmenorrhea

前言、研究目的及文獻探討

在眾多女性相關疾病中，經痛是臨床上常見困擾女性的問題(Jones et al., 2004)。根據美國在 1999 年的統計數據中發現約有高達 90%青春期少女有原發性痛經的困擾(Coco et al., 1999)。而從全美國的調查資料中顯示大約有 900 萬到 5500 萬女性有經痛的問題（Bullock et al., 1996）。而 1999 年在澳洲相關的研究統計數據顯示痛經盛行率約為 80%左右（Hillen et al., 1999）。另外 2000 年在西班牙的研究調查資料中發現經痛的盛行率更高達約 85% (Banikarim et al., 2000）。而在 2004 年針對國內約 15-18 歲的女學生調查研究中發現，約有 73%的人有過經痛的症狀。（邱，2004）。由以上的結果顯示經痛確實是一般女性常見的症狀，而經痛不只帶給女性很大的困擾，也會造成國家社會經濟生產力的降低，如影響女性的課業或工作皆會受到嚴重的影響。根據美國的研究統計每年因為經痛造成女性工作時數的損失更高達 14 億小時，而總損失美元約達 20 億（Coco et al., 1999）。經痛會造成女性生理上許多不適的現象產生如生理期疼痛、腸胃不適、頭痛等問題也會造成女性情緒上不穩定的一些問題，有些嚴重的甚至會造成心理上的一些問題障礙如：憂鬱、神經質、焦慮等症狀（周，1994）。故對於女性來說經痛確實是個嚴重困擾女性要的問題。

促使女性生理痛發生的原因有許多，而大部分的原因已知跟子宮肌肉層過度收縮而造成子宮缺血有關。而為何子宮肌肉層過度收縮而造成子宮缺血進而導致疼痛的發生呢？一般研究已知跟子宮釋放前列腺素 E2 (prostaglandin, PGE2)及前列腺素 F2α (prostaglandin, F2α) 造成子宮肌肉的過度收縮有關 (Zondervan et al., 2001)。而隨著經痛的產生會發生一些腸胃道的症狀，如嘔吐、噁心腹瀉或頭痛。另外最常見的是下腹部呈現劇烈的絞痛現象，有些女性有時還會伴隨有疲憊、頭痛、心悸及全身無力等症狀。經痛一般可以分為兩類：原發性經痛和續發性經痛。(一)原發性經痛 (primary dysmenorrhea)：經痛的特性是急劇的疼痛或絞痛。而主要並無明顯的生殖器官實質上發生病變，而通常見於初經後一至二年開始出現經痛的症狀，並此症狀會開始於規則排卵之後 (胡，1997)。疼痛的開始通常在月經來潮前數小時或經血開始來時，在月經期中的第一天最痛，之後疼痛症狀會逐漸減輕，通常持續少於 24 小時左右，很少會疼痛超過 2-3 天，而疼痛的發生處常在在下腹部處，有時疼痛會擴散到大腿或是背部。原發性經痛造成的原因主要是因為子宮肌肉產生過度的收縮或因此而引起子宮的缺血所致，與盆腔疾病無關，而影響子宮平滑肌過度收縮的因子主要為前列腺素 (PGE2)尤其是前列腺素 F2α (PGF2α) (Zondervan et al., 2001)，除了主要因子前列腺素之外研究發現血管收縮素(vasopressin)、血小版活化因子(platelet activating factor)有關(Teng et al., 1990)。(二)續發性經痛 (secondary dysmenorrhea)：一般續發性經痛都可以發現女性骨盆腔中有一些病灶產生，通常引起續發性經痛的原因是子宮內膜異位症、子宮肌瘤、子宮頸狹窄或阻塞、骨盆腔發炎或粘連、子宮內避孕器及子宮內膜息肉等 (Wolf et al., 1999)。而續發性經痛常常發生在初經後多年，通常出現的年紀約在 20 歲之後，續發性經痛通常在經期前幾天就會開始感覺到疼痛，並且會連續疼痛約 2-3 天，有時嚴重的會持續 5-7 天之久，並且嚴重的疼痛甚至會導致休克的情況發生。而上述不管是原發性經痛或是續發性經痛其造成疼痛的結果皆是子宮平滑肌過度收縮所造成的現象。

一般目前用來治療女性經痛的方式有很多，如利用熱敷、指壓按摩等物理性的方式可以用來緩和經痛症狀，但是利用藥物來降低因為子宮平滑肌過度收縮所造成的疼痛症狀則較為普遍。通常使用的藥物為抑制前列腺素合成的相關藥物如前列腺素抑制劑(prostaglandin-synthesis inhibitors, PGSI)、非類固醇類的消炎藥(nonsteroidal anti-inflammatory drugs, NSAID)、前列腺素合成環氧化酶抑制劑(cycloxygenase-2 inhibitor, COX-2 inhibitor)、止痛劑(analgesics)如阿斯匹林(aspirin)、口服避孕藥(oral contraceptive pill, OCP)、鈣離子通道阻斷劑(calcium channel blockers)等藥物(Michelle and Cynthia, 2006)。但藥物有時並未能完

全改善疼痛，有時可能會造成一些副作用如胃腸道潰瘍，或造成肝臟、腎臟的毒性及其它嚴重副作用，故 Coco(1999)建議除了藥物來改善痛經之外，其他療法如另類(傳統)物理療法(alternative treatments)來改善經痛的現象如運動、按摩、休息、熱敷下腹部、熱水浴、熱飲、做瑜珈、耳穴按摩、紅外線貼片及減低鈉的攝取等也可以舒緩子宮肌肉過度緊張收縮並可以用來減輕症狀的產生，然而若日常習慣及物理療法並無法完全改善症狀，另外又不願意服用西藥擔心西藥所造成的副作用時，此時利用中藥或是食療的方式不失為一個可行的方式。而研究指出一些中草藥、食品或營養素確實可以抑制子宮內膜過度收縮而緩和經痛，如維生素 B1(vitamin B1):在研究中發現每天服用約 100 毫克的維生素 B1，連續服用一個月之後約有 87%的女性其症狀會有緩和的現象。而維生素 B6(vitamin B6)或鎂(magnesium):在研究中發現具有減低疼痛的效果，另外魚油(fish oil):在一些研究中發現 n-3 脂肪酸也具有降低疼痛的效果(Michelle and Cynthia, 2006)。而一些中草藥及食品如當歸、厚朴、半夏、白勺、熟地黃、人蔘、甘薑、白朮、薏苡等所組成的方劑具有治療或緩和經痛的效果。

本研究擬研究 Resveratrol 對於大鼠子宮過度收縮的影響，並可以進一步用來改善女性經痛問題;另也進一步研究對於性類醇激素分泌之影響，並可以進一步用來改善多囊性卵巢症狀而希望藉由本計畫可以為提出 Resveratrol 對於抑制子宮過度收縮而改善經痛以及影響卵巢性類固醇激素分泌之科學證據，進一步支持並促進相關保健食品的開發，而用來輔助改善女性疾病的症狀進而提升女性的健康。

材料與方法

一、實驗動物

由陽明大學動物中心購得 Sprague-Dawley（SD）種系，250-300 克之雌性大白鼠飼養於每日 14 小時（0600-2000）人工照明及空調設備（22± 2℃）之動物室，飲水及飼料不限。

二、實驗材料

Resveratrol 採購至 Sigma 公司。

三、子宮平滑肌組織製備

將雌鼠犧牲並由腹腔迅速且小心的分別取出子宮，立刻置入 37℃的 Kreb's solution（118.3 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.2 mM KH2PO4, 2.5 mM CaCl2, 25 mM NaCHO3, 0.026 mM CaEDTA and 11.1 mM glucose），持續以 95% 的 O2 及 5% CO2 充氣。將附著於子宮上的脂肪以及結締組織清除，沿著縱行方向剪開子宮，小心的去除黏膜層，製備成 1 cm 長子宮平滑肌(縱肌)懸掛於組織槽中，內含 5 % Krebs 溶液，溫度保持於 37℃，連續以 95% O2 及 5% CO2 通氣。子宮縱肌肌條一端固定於槽底之固定鉤，另一端連於外側等長傳導器(external isometric force transducer)。子宮平滑肌縱肌肌條之運動則以記錄儀記錄(PowerLab recorder, ML785, Castle Hill, NSW, Australia)，經過約 30-60 分鐘的平衡，將催產素(Sigma, St. Louis, MO, USA)、前列腺素 F2α (Sigma, St. Louis, MO, USA)及測試藥劑以不同濃度加入組織槽中，觀察其對子宮縱肌肌條之作用。另外以催產素受體拮抗劑(atosiban, Ferring, Limhamn, Sweden)、蕈毒鹼受體拮抗劑(muscarinic receptor blocker, atropine, Sigma, St. Louis, MO, USA)、M3 receptor 拮抗劑(4-DAMP, Research Biochemicals International Company, Natick, MA., USA)、鈉離子通道阻斷劑(tetrodotoxin, TTX, Sigma, St. Louis, MO, USA)及 L 型鈣離子通道阻斷劑(nifidipine, Sigma, St. Louis, MO, USA)等不同藥劑來觀察其對子宮縱肌肌條之作用，並紀錄其收縮振幅 (mean contractile amplitude)及收縮頻率(contractile frequency)。

四、子宮平滑肌細胞之分離與培養

將雌鼠犧牲並由腹腔迅速且小心的以無菌鑷子分別取出子宮，上述取得之子宮利用顯小心撕下肌肉束以取得平滑肌縱肌。接著以 0.2% 蛋白質溶解酵素(protease)在 37℃下震盪

20 分鐘，再利用 0.2% trypsin inhibitor 及 0.2% collagenase 混和反應 60 分鐘，經離心沉澱後以分離子宮平滑肌細胞，緊接著細胞加入 DMEM-F12 培養液(含 10% FBS 和 1% antibiotic) 並培養於二氧化碳培養箱中(37℃,5% CO2)。

五、細胞內鈣離子濃度測定

本實驗是根據 Grynkiewicz 等人(1985)之方法，利用 Fura-2/AM 螢光劑(Fura-2 acetoxymethyl ester) (Molecular Probe，Eugene，OR，USA)測定細胞質中鈣離子之濃度(Chien et al., 2000)。Fura-2/AM 為一合成之酯化物具脂溶性，它可輕易穿透細胞膜進入細胞內，被細胞質中之酯解酵素水解成水溶性之 Fura-2，而堆積在細胞質中，因其失去脂溶性故無法再擴散進入胞內其它胞器中。Fura-2/AM 之螢光劑具有二個特性(1)能產生螢光(2)能與鈣離子結合，凡螢光劑皆能自光中吸收適當波長之單色光激發其電子能階上升，當能階下降時則發出螢光，而螢光劑可與鈣離子結合，造成激發時能量需求增加，即激發光波長變短，故研究上所採用波長 380 nm 激發光激發 Fura-2 產生 505 nm 波長之螢光，而 Fura-2 與鈣離子結合後則用波長 340 nm 的激發光以產生 505 nm 波長之螢光，因其以 340nm、380nm 兩波長所激發的螢光比值來代表鈣離子濃度的變化，故採用 Fura-2 測定鈣離子濃度可減少一些不必要校正誤差的煩贅工作，且 Fura-2 對鈣離子結合最靈敏範圍正好與一般細胞內鈣離子濃度變化範圍相符，故本研究選擇 Fura-2/AM 為測定細胞內鈣離子濃度變化之材料。Fura-2/AM 其測定及計算之原理如下：本研究採用 Spex，Model CM1T11I 之細胞內陽離子測定儀為一種特殊設計之螢光儀，在測定時可自光源幾乎同時連續分別發出波長 340 nm，380 nm 之光於測試的標本上，然後分別將測得 505 nm 波長之螢光強度連續記錄，並記錄下標本在處理前後之變化，實驗結束後，可用 Spex 設計之 DM 3000 軟体可自動將 340 nm 激發光產生之螢光強度和來自 380 nm 激發光產生之螢光強度換算成比值以 R 表之(R=F340/F380)而推算出鈣離子濃度。實驗步驟：純化之細胞，加入螢光劑 Fura-2/AM 5 mM，於黑暗中以 37℃ 恆溫水浴槽作用 30 分鐘，再以培養液離心清洗三次，將未進入細胞的螢光劑洗掉，再以含 10% 胎牛血清的培養液將細胞配成 4×106 cells/ml 之懸浮液，分裝至無菌的微量離心管中成 2×106 cells/vial 備用。以上過程皆儘可能避光進行。進行細胞內鈣離子測定之細胞，先以 loading buffer(NaCl, 7.106g；MgCl2・6H2O, 0.1952g；CaCl2・2H2O, 0.2592g；KCl, 0.2976g；Hepes, 1.9072g)清洗二次，再恢復成 2.5 毫升置於比色管內，送入機器開始測定。當測定結束後，利用 DM 3000 軟體將細胞內鈣離子變化轉換成濃度。

結果與討論

目前此國科會計畫之成果已發表於 endocrinology

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Effects of Resveratrol, a Grape Polyphenol, on Uterine Contraction and Ca^{2+} Mobilization in Rats *in Vivo* and *in Vitro*

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Dysmenorrhea is directly related to elevate prostaglandin F $(PGF)_{2\alpha}$ levels. In Western medicine, this condition is treated using nonsteroidal antiinflammatory drugs. Because nonsteroidal antiinflammatory drugs produce many side effects, Chinese medicinal therapy is considered as a feasible alternative for treating dysmenorrhea. Many special physiological components used in Chinese medicine, such as resveratrol, have been isolated and identified. Resveratrol has many physiological functions, such as antioxidation and anticarcinogenic effects. However, the relationship between uterine smooth muscle contraction and resveratrol remains unknown. Here, we studied the *in vitro* and *in vivo* effects of resveratrol on uterine smooth muscle contraction. The uterus was separated from a female Sprague Dawley rat, and uterine smooth muscle contraction activity was measured and recorded. The results demonstrated that 1) resveratrol treatment inhibited $PGF_{2\alpha}$-, oxytocin-, acetylcholine-, and carbachol-induced uterine contractions in rats; 2) resveratrol inhibited uterine contractions stimulated by the Ca^{2+} channel activator (Bay K 8644) and depolarization in response to high K^+ (KCl); 3) resveratrol inhibited $PGF_{2\alpha}$-induced increases in the $[Ca^{2+}]i$ in human uterine smooth muscle cells; 4) resveratrol could mimic Ca^{2+} channel blockers to block Ca^{2+} influx through voltage-operated Ca^{2+} channels in the plasma membrane; and 5) resveratrol inhibited $PGF_{2\alpha}$-induced uterine contractions in rats *in vivo*. Resveratrol inhibited uterine contractions induced by $PGF_{2\alpha}$ and high K^+ in a concentration-dependent manner *in vitro*; furthermore, it inhibited Ca^{2+}-dependent uterine contractions. Thus, resveratrol consistently suppressed the increases in intracellular Ca^{2+} concentrations ($[Ca^{2+}]i$) induced by $PGF_{2\alpha}$ and high K^+ concentrations. It can be assumed that resveratrol probably inhibited uterine contraction by blocking external Ca^{2+} influx, leading to decreased $[Ca^{2+}]i$. Therefore, resveratrol can be considered as a feasible alternative therapeutic agent for dysmenorrhea. **(*Endocrinology* 152: 2090–2099, 2011)**

Dysmenorrhea, or painful menses, is one of the most common gynecological complaints among adolescent and young adult women and is the leading cause of absenteeism (1). It is characterized by cramps and lower abdominal pain and may be associated with nausea, vomiting, diarrhea, headache, dizziness, and/or back pain. Primary dysmenorrhea affects most women throughout the menstrual years. The prevalence of dysmenorrhea varies among women across countries, and women who regularly experience menstrual pain constitute approximately 72–90% of the population of young women in the United States (2, 3), 58% in China (4), 34% in Japan (5), and approximately 73% in Taiwan (6). However, the cause of dysmenorrhea remains unclear. Prostaglandin (PG) release is believed to be a pathogenetic factor in both dysmenorrhea and endometriosis. Previous studies have re-

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Abbreviations: Ach, Acetylcholine; COX-2, cyclooxygenase-2; DMSO, dimethyl sulfoxide; HutSMC, human smooth muscle cell; MLC, myosin light chain; PG, prostaglandin; ROC, receptor-operated channel; VOC, voltage-operated channel.

ported that $PGF_{2\alpha}$ levels are elevated in women with primary dysmenorrhea as compared with their asymptomatic counterparts (7). $PGF_{2\alpha}$ stimulates uterine contraction and vasoconstriction, which may lead to ischemia and the pain symptoms of dysmenorrhea (8, 9). $PGF_{2\alpha}$ could cause the constriction of uterine smooth muscle or small endometrial blood vessels after tissue ischemia and endometrial disintegration, resulting in bleeding and pain (10, 11).

Resveratrol is present in grapes and red wine (12–14). The resveratrol content in red grapes ranges from 1.5 to 7.3 μg/g (13). Recently, many studies have demonstrated that it is a potent antioxidant with antiinflammatory, antiplatelet aggregation, antiproliferation of cancer (*e.g.* ovary and prostate cancer), and vasorelaxation properties (12, 15–20). Although resveratrol has a lot of physical effects, its action on uterine complications and the underlying mechanisms remain unclear. The purpose of the present study was to examine the direct effects of resveratrol on uterine smooth muscle contraction in rats *in vivo* and *in vitro*. This study is the first to report the effects and mechanisms of action of resveratrol in rat uterine smooth muscle tissue and primary human smooth muscle cells (HutSMCs), which is mediated through Ca^{2+} pathway.

Materials and Methods

Drugs and solutions

SMC growth medium-2 and supplement were purchased from PromoCell Co. (Heidelberg, Germany). The Ca^{2+} channel activator Bay K8644 was purchased from Cayman Co. (Ann Arbor, MI). Penicillin G, sodium bicarbonate, oxytocin, streptomycin sulfate, HEPES, $PGF_{2\alpha}$, carbachol, acetylcholine (Ach), mannitol, glucose, potassium chloride, potassium phosphate, magnesium sulfate, calcium chloride, resveratrol, and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO). A stock solution of resveratrol (100 mM), Bay K 8644 (5×10^{-3} M), and $PGF_{2\alpha}$ (5×10^{-3} M) was prepared using DMSO. The final using concentration of DMSO in uterine segments or cells was less than 0.2%. A stock solution of oxytocin (5×10^{-3} M), Ach (5×10^{-3} M), and carbachol (5×10^{-2} M) was prepared using deionized water. Antibodies directed against the following proteins were used in this study: myosin light chain (MLC)20 (sc-15370; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and phospho-Ser19-MLC20 (2249; Cell Signaling, Danvers, MA).

Uterine preparations and measurement of uterine contraction

Female Sprague Dawley rats weighing 200–300 g were housed in a temperature-controlled room (22 ± 1 C) with artificial illumination for 14 h/d (0600–2000 h), with food and water provided *ad libitum*. The investigations were conducted in accordance with the Guide for Care and Use of Laboratory Animals (National Yang-Ming University).

Uterine preparations and measurement of uterine contraction were carried out as previously described (21). In the experiment, the six rats that were confirmed to be in the estrous stage by microscopic examination of a vaginal smear were decapitated, and both uterine horns were surgically removed and placed in a Petri dish containing Krebs's solution [113 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 18 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 5.5 mM glucose, and 30 mM mannitol (pH 7.4)]. After removing the adherent fat and mesenteric attachments, each uterine horn was cut into three equal-length (~10 mm) segments; these were used for the measurement of the uterine oscillatory contraction. The preparations were placed in isolated organ baths, incubated in a physical solution at 37 C, and bubbled with 95% O_2 and 5% CO_2. The preload was 1 g, and the equilibration period was not less than 60 min. The Krebs's solution was changed every 20 min. After equilibration, uterine segments were treated with different drugs (10^{-6} M $PGF_{2\alpha}$, 10^{-6} M oxytocin, 10^{-5} M carbachol, 10^{-6} M Ach, 10^{-6} M Bay K 8644, or 50 mM KCl) to stimulate uterine contraction. Resveratrol was then added to the tissue bath in a cumulative manner at bath concentrations of 10, 25, 50, 75, and 100 μM at 10-min intervals. As solvent control, respective cumulative concentrations of DMSO were applied. The contractions were recorded using force displacement transducers (PowerLab recorder ML785; PowerLab, Castle Hill, New South Wales, Australia) by using Chart 5.1 software (PowerLab). To adjust for variations between individual uterine strips, the mean amplitude and frequency values for the pre- and postexposure intervals were expressed as a percentage of control values. Contractile activity (mean amplitude and frequency) during the control period was taken as 100%.

HutSMCs culture

HutSMCs were purchased from PromoCell Co. HutSMCs were cultured in SMC growth medium-2 containing 5% (vol/vol) fetal calf serum, 0.5 ng/ml epidermal growth factor, 2 ng/ml basic fibroblast growth factor, and 5 μg/ml insulin (PromoCell Co.); seeded in a 24-well plate; and incubated at 37 C in SMC growth medium-2 containing 100 U/ml penicillin and 100 μg/ml streptomycin. After incubation for approximately 24 h, the uterine SMCs were collected and intracellular calcium mobilization was measured.

Measurement of $[Ca^{2+}]i$

HutSMCs were treated with 10, 25, 50, 75, or 100 μM resveratrol and 200 nM $PGF_{2\alpha}$ for 24 h. They were then harvested using the culture medium and washed twice with the same medium. A cell suspension (1×10^6 cells/ml) was loaded with 5 mg of fura 2-acetoxymethyl ester (Fluka Chemical Corp., Milwaukee, WI) dissolved in 5 ml of DMSO. A fluorescent probe was used for monitoring the intracellular calcium concentrations ($[Ca^{2+}]i$). The cells were incubated in the dark for 30 min at 37 C. After extensive washing, 1×10^6 cells were resuspended in 2.5 ml loading buffer (152 mM NaCl, 1.2 mM $MgCl_2$, 2.2 mM $CaCl_2$, 4.98 mM KCl, and 10 mM HEPES). Fluorescence emission at 505 nm was monitored at 37 C by a dual-wavelength spectrometer system, with excitation at 340 and 380 nm. Free $[Ca^{2+}]i$ was calculated using the method developed by Grynkiewicz *et al.* (22) by using the ratio of fluorescence intensities obtained every second with a dissociation constant of 135 nM. The dye was considered saturated after lysis with digitonin at the final concentration of 0.16 mM. Minimum fluorescence was determined by

adding 0.5 ml EGTA (Sigma Chemical Co.) to obtain a final concentration of 8 mM.

Western blotting for MLC20 and phospho-Ser19-MLC20

After uterine muscle strips treatment with vehicle (DMSO) and resveratrol for 20 min at 37 C, the treatment uterine strips were collected and frozen immediately in liquid nitrogen. The strips were homogenized in homogenization buffer (pH 8.0) containing 1.5% Na-lauroylsarcosine, 1×10^{-3} M EDTA, 2.5×10^{-3} M Tris-base, 0.68% phenylmethylsulfonylfluoride, and 2% proteinase inhibitor cocktail and then disrupted by ground-glass homogenizer in ice-cold buffer. Tissue extracts were centrifuged at 13,500 × *g* for 10 min. The supernatant fluid was collected, and the protein concentration was determined. Extracted proteins were denatured by boiling for 5 min in sodium dodecyl sulfate buffer (0.125 M Tris-base, 4% sodium dodecyl sulfate, 0.001% bromophenol blue, 12% sucrose, and 0.15 M dithiothreitol). The proteins (20 μg) in the samples were separated on 12.5% SDS-PAGE at 50 V for 30 min and then at 90 V for 90 min using a running buffer. The proteins were transferred to polyvinylidene difluoride membranes (NEN Life Science Products, Inc., Boston, MA) using a Trans-Blot semidry transfer cell (170–3940; Bio-Rad, Hercules, CA) at 64 mA (for 8 × 10 mm membrane) for 45 min in a blotting solution. Enhanced chemiluminescence detection reagent (PerkinElmer Life Science, Waltham, MA) was used to visualize the immunoreactive proteins on polyvinylidene difluoride membranes after transfer. The quantification software was Multi-Gauge version 3.0.

Measurement of uterine contraction *in vivo*

In the experiment, the rats that were confirmed to be in the estrous stage by microscopic examination of a vaginal smear were used. They were anesthetized with pentobarbitone (18 mg in 0.3 ml, ip). A small midportion of a uterine horn with associated mesometrium was obtained through a ventral incision made in the skin and body wall. A 1- to 2-mm-long incision was made at the distal end of the exposed uterus, and a thin, finger-shaped latex balloon was attached to a polyethylene catheter (Becton Dickinson, Franklin Lakes, NJ), which was a modification of a method described in a previous study (21, 23, 24). The catheter was connected to a transducer (PowerLab recorder ML785). Then, the rats were catheterized via the right jugular vein and injected with $PGF_{2\alpha}$ ($PGF_{2\alpha}$ 0.2 mg/kg) or $PGF_{2\alpha}$ plus resveratrol (0.5, 1, or 2 mg/kg) via the jugular catheter, and the contractions were recorded by this transducer with Chart 5.1 software (PowerLab).

Statistical analysis

Data are presented as the mean ± SEM of several preparations from different animals. The statistical significance of differences between the groups was analyzed by one-way ANOVA by using SPSS software, version 11.0 (SPSS, Inc., Chicago, IL). Comparisons between the mean values of groups were performed using one-way ANOVA and Duncan's multiple-range test. For comparison between two groups, Student's *t* tests were used. Differences between two mean values were considered statistically significant when $P < 0.05$ and highly significant when $P < 0.01$.

Results

Effects of resveratrol on $PGF_{2\alpha}$-induced uterine contractions in the rats

To investigate the potential inhibition of $PGF_{2\alpha}$-induced uterine contraction by resveratrol in rats, we first examined the effect of resveratrol on $PGF_{2\alpha}$-induced uterine contraction. $PGF_{2\alpha}$ is a major factor inducing uterine contractions during dysmenorrhea. As shown in Fig. 1, $PGF_{2\alpha}$ (10^{-6} M) increased contractile force (amplitude) and frequency, and exposure of rat uterine smooth muscles to resveratrol (10, 25, 50, 75, and 100 μM) inhibited the $PGF_{2\alpha}$-induced contraction in a dose-dependent manner (n = 6 rats; n = uterine segments, n = 6; *, $P < 0.05$ or **, $P < 0.01$; Duncan's multiple-range test) (Fig. 1) as compared with the contraction observed in the control group. We also assessed cell viability using a recovery study. We determined that by removing the inhibitory substance and washing the tissue with Krebs's solution for 20 min, then reanalyzing the tissue in the presence of a stimulus (10^{-6} M $PGF_{2\alpha}$). From our results, we found that $PGF_{2\alpha}$ could reverse the inhibition after removing the resveratrol (Fig. 1).

Effects of resveratrol on oxytocin-induced uterine contractions in the rats

Oxytocin is a nonapeptide hormone that produces uterine and mammary gland contractions. Both tissues contain oxytocin receptors. To determine whether resveratrol exerted an inhibitory effect on oxytocin-induced uterine contractions in rats, we observed the effects of resveratrol on uterine contractions induced by oxytocin (10^{-6} M) *in vitro*. Different doses of resveratrol (10, 25, 50, 75, and 100 μM) were administered along with oxytocin (10^{-6} M), and at concentrations of 10–100 μM, resveratrol was found to exert an inhibitory effect on uterine contractile force (amplitude) and frequency (n = 6 rats; n = uterine segments, n = 6; *, $P < 0.05$ or **, $P < 0.01$; Duncan's multiple-range test) (Fig. 2) as compared with the control group.

Effects of resveratrol on Ach-induced uterine contractions in the rats

We observed the effects of resveratrol on uterine contractions induced by 10^{-6} M Ach *in vitro*. The administration of different doses of resveratrol (10, 25, 50, 75, and 100 μM) along with Ach (10^{-6} M) revealed that 10–100 μM resveratrol exerted an inhibitory effect on uterine contractile force (amplitude) and frequency in rats (n = 6 rats; n = uterine segments, n = 6; *, $P < 0.05$ or **, $P < 0.01$; Duncan's multiple-range test) (Fig. 3) as compared with the control group.

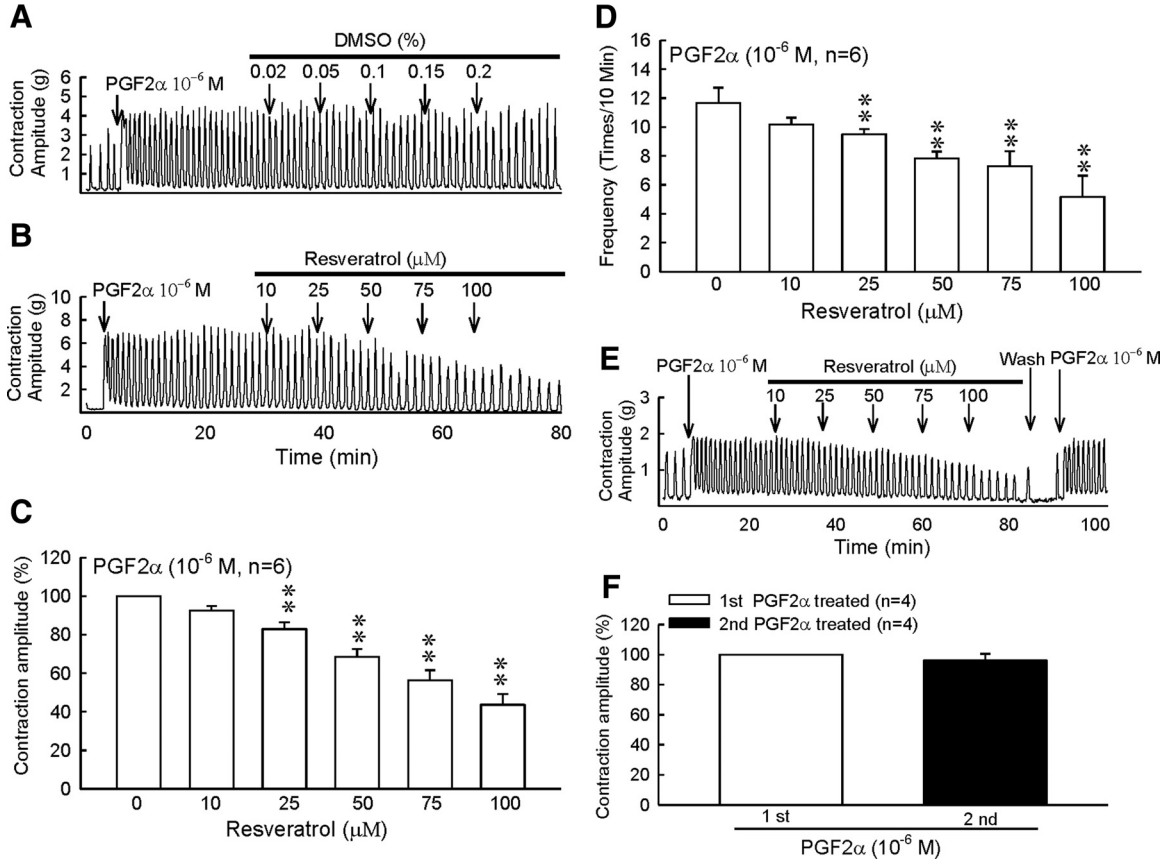

FIG. 1. Effect of resveratrol on $PGF_{2\alpha}$-induced uterine contractions in the rats. Rat uterine segments were treated with $PGF_{2\alpha}$ (10^{-6} M) and exposure of rat uterine smooth muscles to vehicle (DMSO) or resveratrol (10, 25, 50, 75, and 100 μM). Representative recordings of $PGF_{2\alpha}$-induced contractions treated with vehicle (DMSO) only (A) and the effects of cumulative additions of resveratrol (10–100 μM) (B) are shown. C, Dose-dependent effects of resveratrol on the mean peak amplitude. D, Dose-dependent effects of resveratrol on the frequency. E and F, Recovery test about resveratrol on $PGF_{2\alpha}$-induced uterine smooth muscle contractions in the rats. 1st, First $PGF_{2\alpha}$ treated; 2nd, Second $PGF_{2\alpha}$ treated after wash. $PGF_{2\alpha}$-induced contractions before the addition of resveratrol were considered as the control (100%; resveratrol, 0 μM group). n = 6 rats; n = uterine segments, n = 6; *, $P < 0.05$ or **, $P < 0.01$ *vs.* control group, assessed by Duncan's multiple-range test. Each *column* represents the mean ± SEM. Three independent experiments were done and had similar results. Six different uterine segments prepared from different six rats were used in each experiment (n = 6). The results presented are from one of experiment, representative of the group.

Effects of resveratrol on carbachol-induced uterine contractions in rats

Next, we observed the effects of resveratrol on uterine contractions induced by carbachol (10^{-5} M) *in vitro*. We administered 10, 25, 50, 75, and 100 μM resveratrol along with carbachol (10^{-6} M), and we found that 10–100 μM resveratrol inhibited uterine contractile force (amplitude) and frequency in rats (n = 6 rats; n = uterine segments, n = 6; *, $P < 0.05$ or **, $P < 0.01$; Duncan's multiple-range test) (Fig. 4) as compared with the control group.

Effect of resveratrol on $PGF_{2\alpha}$-induced uterine contractions *in vivo*

To confirm the inhibitory effect of resveratrol on uterine contractions *in vivo*, we measured the uterine pressure of the rats. The rats were administered 0.5, 1, and 2 mg resveratrol along with 0.2 mg/kg $PGF_{2\alpha}$ in the rats. Resveratrol treatment (0.5, 1, or 2 mg/kg) significantly reduced the $PGF_{2\alpha}$-induced uterine contractions *in vivo* (n = 6 rats; **, $P < 0.01$; Duncan's multiple-range test) (Fig. 5).

Effect of resveratrol on Ca^{2+}-dependent contractions

Previous studies have reported that Bay K 8644, a Ca^{2+} channel activator, and high K^+ concentrations induce uterine contractions. Administration of resveratrol (50, 75, and 100 μM) along with KCl or Bay K 8644 resulted in a dose-dependent inhibition of uterine contraction. (n = 6 rats; n = uterine segments, n = 6; *, $P < 0.05$ or **, $P < 0.01$; Duncan's multiple-range test) (Fig. 6). To investigate whether the inhibition of uterine contractions by resveratrol occurs due to the blockade of external Ca^{2+} influx, we performed the following experiments in a Ca^{2+}-free medium. In the absence of external Ca^{2+}, the spontaneous contractions were abolished. Further, when the medium was supplied with increasing concentrations of Ca^{2+} from

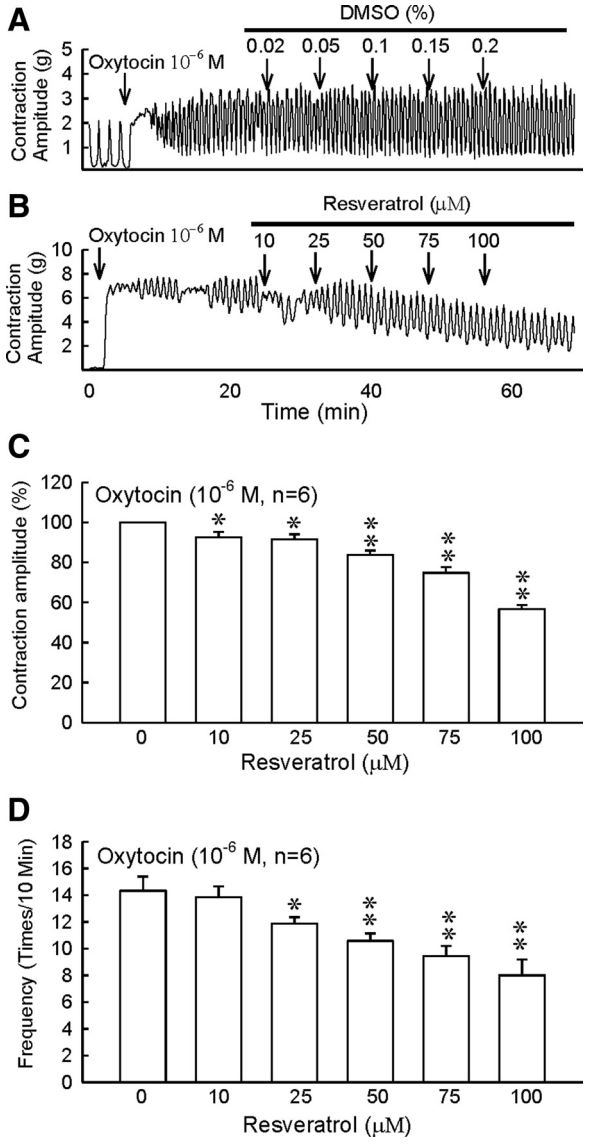

FIG. 2. Effect of resveratrol on oxytocin-induced uterine contractions in the rats. Oxytocin (10^{-6} M) increased contractile force, and exposure of rat uterine smooth muscles to vehicle (DMSO) or resveratrol (10, 25, 50, 75, and 100 μM) inhibited the oxytocin-induced contraction in a dose-dependent manner. Representative recordings of oxytocin-induced contractions treated with vehicle (DMSO) only (A) and the effects of cumulative additions of resveratrol (10–100 μM) (B) are shown. C, Dose-dependent effects of resveratrol on the mean peak amplitude. D, Dose-dependent effects of resveratrol on the frequency. Oxytocin-induced contractions before the addition of resveratrol were considered as the control (100%; resveratrol, 0 μM group). n = 6 rats; n = uterine segments, n = 6; *, $P < 0.05$ or **, $P < 0.01$ *vs.* control group, assessed by Duncan's multiple-range test. Each *column* represents the mean ± SEM. Three independent experiments were done and had similar results. Six different uterine segments prepared from different six rats were used in each experiment (n = 6). The results presented are from one of experiment, representative of the group.

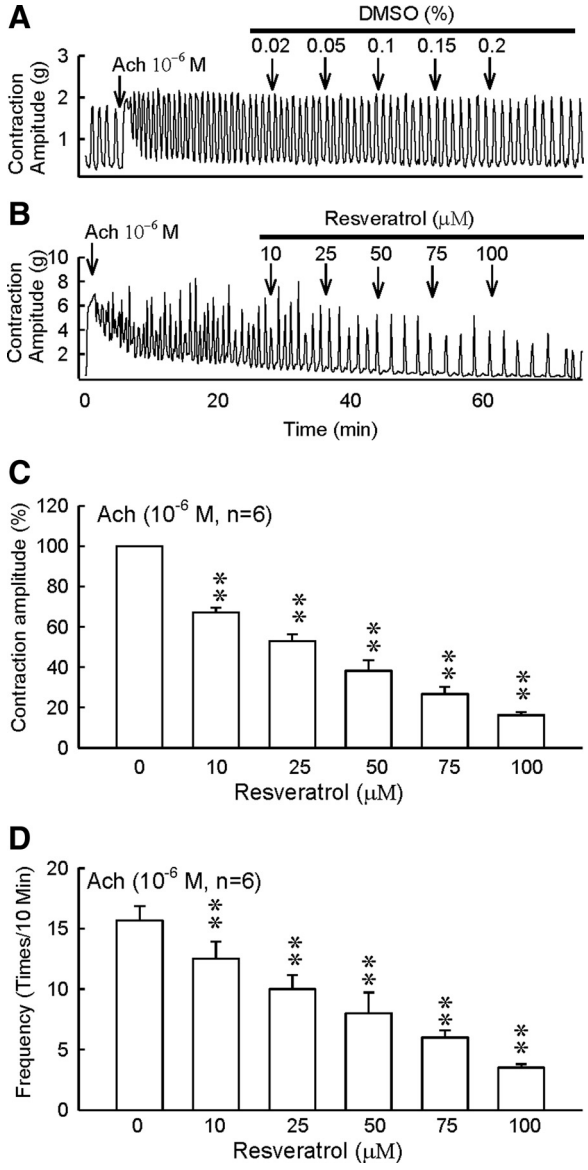

FIG. 3. Effect of resveratrol on Ach-induced uterine contractions in the rats. Rat uterine segments were treated with Ach(10^{-6} M) and exposure of rat uterine smooth muscles to vehicle (DMSO) or resveratrol (10, 25, 50, 75, and 100 μM). Representative recordings of Ach-induced contractions treated with vehicle (DMSO) only (A) and the effects of cumulative additions of resveratrol (10–100 μM) (B) are shown. C, Dose-dependent effects of resveratrol on the mean peak amplitude. D, Dose-dependent effects of resveratrol on the frequency. Ach-induced contractions before the addition of resveratrol were considered as the control (100%; resveratrol, 0 μM group). n = 6 rats; n = uterine segments, n = 6; *, $P < 0.05$ or **, $P < 0.01$ *vs.* control group, assessed by Duncan's multiple-range test. Each *column* represents the mean ± SEM. Three independent experiments were done and had similar results. Six different uterine segments prepared from different six rats were used in each experiment (n = 6). The results presented are from one of experiment, representative of the group.

0.05 to 5 mM, the spontaneous contractions were restored. However, when this Ca^{2+}-containing buffer solution was supplemented with 100 μg/ml resveratrol, the Ca^{2+}-induced uterine contractions were not observed (Fig. 7). We further determined the resveratrol cytotoxicity by removing the resveratrol and washing the tissue with Krebs's solution for 20 min, then reanalyzing the tissue in the presence of a stimulus (10^{-6} M $PGF_{2\alpha}$). From our results, we found that $PGF_{2\alpha}$ could reverse the inhibitory effect after removing the resveratrol (Fig. 7).

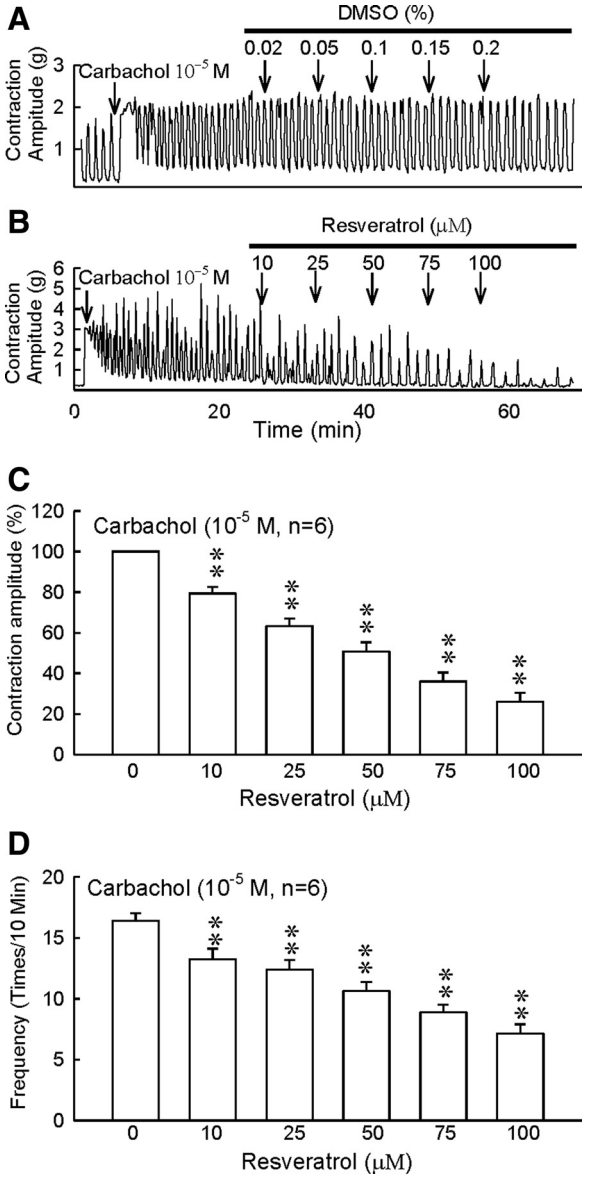

FIG. 4. Effect of resveratrol on carbachol-induced uterine contractions in the rats. Rat uterine segments were treated with carbachol (10^{-5} M) and exposure of rat uterine smooth muscles to vehicle (DMSO) or resveratrol (10, 25, 50, 75, and 100 μM). Representative recordings of carbachol-induced contractions treated with vehicle (DMSO) only (A) and the effects of cumulative additions of resveratrol (10–100 μM) (B) are shown. C, Dose-dependent effects of resveratrol on the mean peak amplitude. D, Dose-dependent effects of resveratrol on the frequency. Carbachol-induced contractions before the addition of resveratrol were considered as the control (100%; resveratrol, 0 μM group). n = 6 rats; n = uterine segments, n = 6; *, $P < 0.05$ or **, $P < 0.01$ *vs.* control group, assessed by Duncan's multiple-range test. Each *column* represents the mean ± SEM. Three independent experiments were done and had similar results. Six different uterine segments prepared from different six rats were used in each experiment (n = 6). The results presented are from one of experiment, representative of the group.

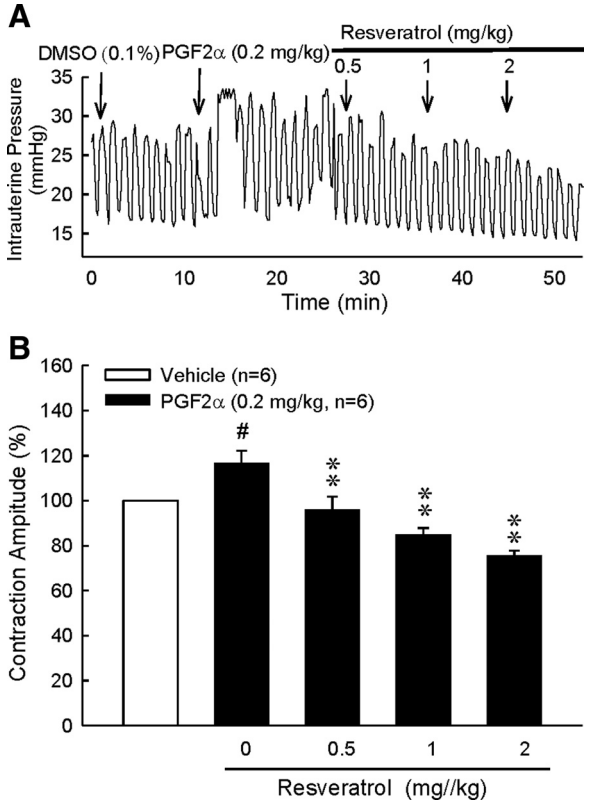

FIG. 5. Effects of resveratrol (0.5, 1, or 2 mg/kg) on $PGF_{2\alpha}$-induced (0.2 mg/kg) uterine contractions *in vivo*. The rats were catheterized via the right jugular vein and injected with $PGF_{2\alpha}$ (0.2 mg/kg) or $PGF_{2\alpha}$ plus resveratrol (0.5, 1, or 2 mg/kg) via the jugular catheter, and the contractions were recorded. A, Representative recordings of $PGF_{2\alpha}$-induced contractions and the effects of cumulative additions of resveratrol (10–100 μM) are shown. B, Dose-dependent effects of resveratrol on the mean peak amplitude. These results are representative of the records of six rats (n = 6). **, $P < 0.01$ *vs.* $PGF_{2\alpha}$-treated group, assessed by Duncan's multiple-range test. #, $P < 0.05$ *vs.* vehicle-treated group, assessed by Student's *t* test. Each *column* represents the mean ± SEM.

Effects of resveratrol on $[Ca^{2+}]i$ and MLC20 phosphorylation

To study whether resveratrol inhibits the increases in $[Ca^{2+}]i$ as a result of which it exhibits an inhibitory effect on muscular contraction, the HutSMCs were treated with resveratrol (10, 25, 50, 75, and 100 μM) along with $PGF_{2\alpha}$ (200 nM). Resveratrol treatment (25–100 μM) significantly reduced the $PGF_{2\alpha}$-induced $[Ca^{2+}]i$ (n = 3; **, $P < 0.01$; Duncan's multiple-range test) (Fig. 8A). In contrast, administration of resveratrol (10–100 μM) had no effect on HutSMCs number. This result imply that the decrease in $[Ca^{2+}]i$ was not attributed to the cytotoxicity of resveratrol on HutSMCs. (Supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). In addition, to study whether resveratrol inhibits the MLC20 phosphorylation, leading to smooth muscle relaxation, the rat uterine smooth muscle was treated with resveratrol (10, 50, and 100 μM) along with $PGF_{2\alpha}$ (10^{-6} M). Resveratrol treatment (50–100 μM) significantly reduced the $PGF_{2\alpha}$-induced MLC20 phosphorylation (n = 3; **, $P < 0.01$; Duncan's multiple-range test) (Fig. 8B).

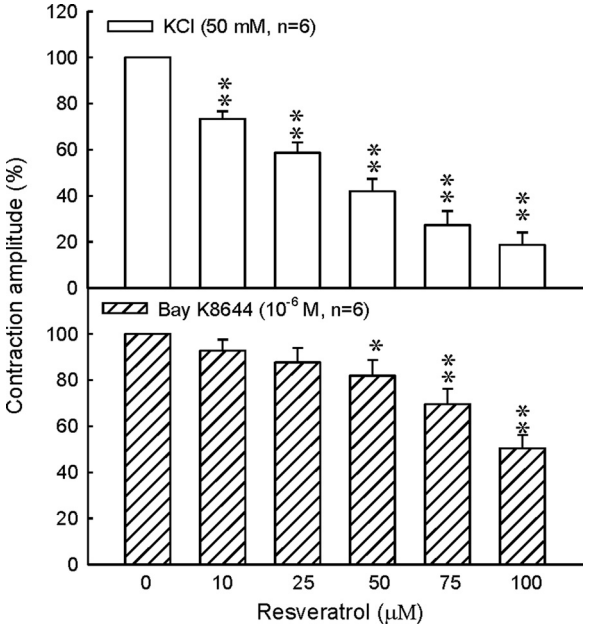

FIG. 6. Effects of resveratrol on high K^+ (KCl) or Bay K 8644-induced uterine contractions in the rats. Rat uterine segments were treated high K^+ (KCl 50 mM) or Bay K 8644 (10^{-6} M) and exposure of rat uterine smooth muscles to vehicle (DMSO) or resveratrol (10, 25, 50, 75, and 100 μM). High K^+ (KCl) or Bay K 8644-induced contractions before the addition of resveratrol were considered as the control (100%; resveratrol, 0 μM group). n = 6 rats; n = uterine segments, n = 6; *, $P < 0.05$ or **, $P < 0.01$ *vs.* control group, assessed by Duncan's multiple-range test. Each *column* represents the mean ± SEM. Three independent experiments were done and had similar results. Six different uterine segments prepared from different six rats were used in each experiment (n = 6). The results presented are from one of experiment, representative of the group.

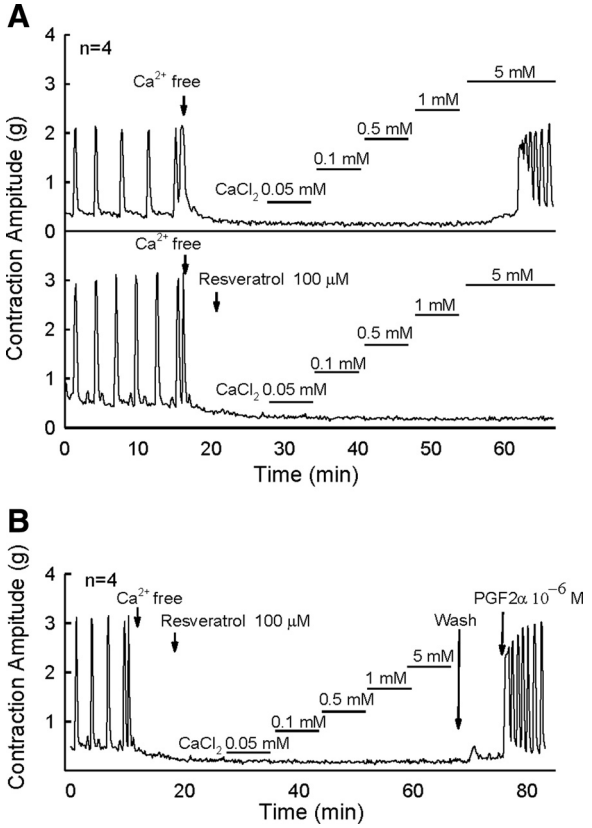

FIG. 7. Inhibitory actions of resveratrol on Ca^{2+}-dependent contractile responses. A, Muscle segments were initially pretreated in a Ca^{2+}-free medium containing the vehicle (0.2% DMSO) (*upper panel*) or resveratrol (100 μg/ml) for 60 min, and calcium (0.05–5 mM) was then cumulatively applied to trigger muscle contraction. B, We further determined the resveratrol cytotoxicity by removing the resveratrol and washing the tissue with Krebs's solution for 20 min, then reanalyzing the tissue in the presence of a stimulus (10^{-6} M $PGF_{2\alpha}$). The results are representative of the records of four independent experiments (n = 4).

Discussion

The present study is the first to demonstrate that resveratrol suppresses $PGF_{2\alpha}$-induced uterine contractions. The results demonstrated that 1) resveratrol treatment inhibited $PGF_{2\alpha}$-, oxytocin-, Ach-, and carbachol-induced uterine contractions in rats; 2) resveratrol inhibited uterine contractions stimulated by the Ca^{2+} channel activator (Bay K 8644) and depolarization in response to high K^+ (KCl); 3) resveratrol inhibited $PGF_{2\alpha}$-induced increases in the $[Ca^{2+}]i$ in HutSMCs; 4) resveratrol could mimic Ca^{2+} channel blockers to block Ca^{2+} influx through voltage-operated Ca^{2+} channels (VOCs) in the plasma membrane; and 5) resveratrol inhibited $PGF_{2\alpha}$-induced uterine contractions in rats *in vivo*. Thus, our study demonstrated that resveratrol may be of potential use in the treatment or improvement of primary dysmenorrhea. To our knowledge, this is the first study demonstrating the effects of resveratrol on uterine contraction *in vivo* and *in vitro*, thus partially explaining the modulatory effects of resveratrol on female reproductive functions.

It has been previously reported that dysmenorrhea leads to increased PG (PGE_2 and $PGF_{2\alpha}$) production, which may result in contraction of the blood vessels and myometrium and insufficient blood flow to the endometrium, which in turn causes ischemia and the pain symptoms associated with dysmenorrhea (8–11). Some studies have reported elevated $PGF_{2\alpha}$ levels in women with primary dysmenorrhea (10, 11). Therefore, the role of PGs is implicated in dysmenorrhea. It is well established that $PGF_{2\alpha}$ increases the $[Ca^{2+}]i$ and then stimulates uterine contraction (7, 25, 26). In the present study, we found that resveratrol inhibited the $PGF_{2\alpha}$-induced uterine contractions both *in vivo* and *in vitro*. Inflammation is also involved in the pathogenesis of dysmenorrhea. A previous study reported that nonsteroidal antiinflammatory drugs could be used for the treatment of dysmenorrhea (27). Several studies have evaluated the effect of a cyclooxygenase-2 (COX-2) inhibitor in treating primary dysmenorrhea (28, 29). A previous study has shown that resveratrol could inhibit lipopolysaccharide-induced COX-2 expression in RAW 264.7 macrophage cells (15); this result indicates the antiinflammatory activity of resveratrol

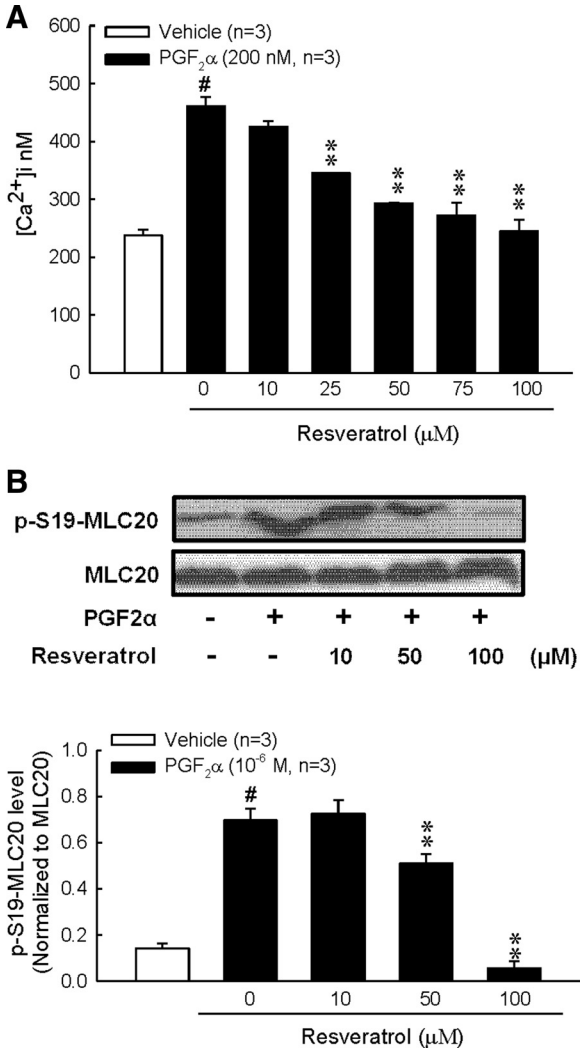

FIG. 8. Inhibition of $PGF_{2\alpha}$-induced increases in $[Ca^{2+}]i$ and MLC20 phosphation by resveratrol in HutSMCs and rat uterine smooth muscle. A, HutSMCs were treated with vehicle (0.2% DMSO) or resveratrol (10, 25, 50, 75, and 100 μg/ml) along with $PGF_{2\alpha}$ (200 nM). B, Rat uterine segments were treated with vehicle (DMSO) or resveratrol (10, 50, and 100 μg/ml) along with $PGF_{2\alpha}$ (10^{-6} M). **, $P < 0.01$ *vs.* $PGF_{2\alpha}$-treated group, assessed by Duncan's multiple-range test. #, $P < 0.05$ *vs.* vehicle-treated group, assessed by Student's *t* test. Each *column* represents the mean ± SEM. The results are representative of the records of three independent experiments (n = 3). p-S19-MLC20, Phospho-Ser19-MLC20.

and further suggests its use for the treatment of dysmenorrhea. In the present study, we demonstrated that resveratrol inhibits $PGF_{2\alpha}$-induced uterine contractions both *in vitro* and *in vivo*. This result suggested that the administration of resveratrol might be beneficial for treating or improving dysmenorrhea.

An increase in the $[Ca^{2+}]i$ in the uterine smooth muscles induces uterine contractions. Previous studies have demonstrated that $[Ca^{2+}]i$ is regulated by two different Ca^{2+} channels: receptor-operated channels (ROCs) and VOCs in the uterine smooth muscles (23–25, 30). In the ROCs, when uterotonic hormones ($PGF_{2\alpha}$, oxytocin, Ach, or carbachol) bind to membrane G protein-coupled receptors to induce uterine contractions, the $[Ca^{2+}]i$ increases via both the influx of extracellular Ca^{2+} through Ca^{2+} channels and by release of intracellular stored Ca^{2+}. Our present study found that the contraction stimulated by different agonists, including $PGF_{2\alpha}$, oxytocin, Ach, and carbachol, was abolished by resveratrol administration. These results demonstrate that the relaxation effects induced by resveratrol are at downstream of receptors. In the VOCs, both Ca^{2+} channel activators (*e.g.* Bay K 8644) and membrane depolarization caused by high K^+ (*e.g.* high concentrations of KCl) can increase the Ca^{2+} influx through the VOCs, resulting in uterine smooth muscle contraction. Both Bay K 8644- and high K^+-induced contractions were also abolished by resveratrol administration. Moreover, the spontaneous contractions stimulated by elevated extracellular Ca^{2+} were also abolished by resveratrol treatment. In addition, $PGF_{2\alpha}$-increased $[Ca^{2+}]i$ concentration was also reduced by resveratrol treatment in HutSMCs. This result demonstrated resveratrol inhibited Ca^{2+} influx, directly. Therefore, the effect of resveratrol on uterine contractions could be due to its interference of ROCs or/and VOCs. Our present study demonstrates that resveratrol could inhibit the $[Ca^{2+}]i$ increases induced by $PGF_{2\alpha}$, oxytocin, Ach, carbachol, KCl, and Bay K 8644 and block the Ca^{2+} influx through ROCs and VOCs.

In the present study, we demonstrated that resveratrol inhibits $PGF_{2\alpha}$-, oxytocin-, Ach-, and carbachol-induced uterine contractions. Resveratrol has previously been shown to inhibit COX-2 expression *in vitro* (15); it could also inhibit cancer proliferation (16, 18). In the rat mesenteric artery, the vasorelaxant effect of resveratrol on the mesenteric artery was due to its interaction with VOCs (31). Resveratrol induced vasorelaxation of mesenteric and uterine arteries from guinea pigs (32). Resveratrol also could inhibit the contractile activity of isolated gallbladder muscle; inhibition of Ca^{2+} influx through VOCs and release of Ca^{2+} from the sarcoplasmic reticulum are suggested to be the mechanisms responsible for the inhibitory effects (33). In uterine smooth muscle contraction, one of the proteins involved is MLC (MLC20), which is phosphorylated at Ser19 and Thr18 by MLC kinase in a Ca^{2+}/camodulin-dependent manner (34). The phosphorylated MLC20 could interact with α-actin filaments, resulting in uterine smooth muscle contraction. Conversely, when MLC20 is dephosphorylated by MLC phosphatase, leading to relaxation (35). In current result, we demonstrate that resveratrol inhibited smooth muscle contraction by affecting MLC20 phosphorylated. The pleiotropic effects of resveratrol on COX-2-related pathway and cell proliferation were also investigated in uterine smooth muscles. Our results suggested that resveratrol also inhibited uter-

ine smooth muscle contraction by affecting COX-2-related pathway in rats (Supplemental Fig. 2). However, the molecular mechanism needs further study in the future. Furthermore, resveratrol did not affect HutSMCs viability (Supplemental Fig. 1). Taken together, these results suggest that resveratrol could inhibit $PGF_{2\alpha}$-induced uterine smooth muscle contraction in rats. Our results provided evidences to explain why this substance can be used for the treatment of dysmenorrhea.

The present study provides some important new insights into the mechanisms of action of resveratrol on uterine contractions. Therefore, whether the dose of resveratrol used in present study corresponds to a reasonable dose for human use is the important issue. At the present study, we can offer a speculative answer only. Red wine with resveratrol content (2–6.5 mg/liter) was administered (36, 37). A recent study suggested that dose translation from animal to human studies should use body surface area as a factor (38). Using this approach, a dose of 2 mg per rat translates to approximately an equivalent of 0.324 mg for a 60-kg human. In the present study, the resveratrol (25–100 μM) is effective in inhibiting $PGF_{2\alpha}$-induced uterine contraction. With regard to the concentrations of resveratrol used in the present study, they correspond to the concentrations used in other *in vitro* studies (39–41). For example, resveratrol inhibited the binding of vascular endothelial growth factor to human umbilical vein endothelial cell at concentrations of 10–100 μM (40). Several animal studies have been done on bioavailability of resveratrol *in vivo*. In the rat model, after oral administration of red wine containing 6.5 mg/liter of total resveratrol (daily dose, 40 μg/kg) for 15 d, the peak serum concentrations of unchanged resveratrol (~33 nM) was observed (42, 43). In humans, the maximum plasma concentration of resveratrol (16.2–71.86 nM) was observed after 0.2 mg/kg, iv (44). In our study, the resveratrol (0.5–2 mg/kg, iv) is effective in inhibiting $PGF_{2\alpha}$-induced uterine contraction *in vivo*. We suggested that the maximum plasma concentration of resveratrol (40.5–179.6 nM) was observed after 0.5–2 mg/kg, iv. At the present study, we can offer a speculative answer that a daily drink of two glasses of wine could attain the effective level. This result indicates that resveratrol at pharmacological concentrations may be effective in inhibiting $PGF_{2\alpha}$-induced uterine contraction.

In summary, the present data demonstrate that the obtained fractions of resveratrol could inhibit the $PGF_{2\alpha}$-induced uterine smooth muscle contraction both *in vitro* and *in vivo*. The inhibition of uterine smooth muscle contraction is, in part, due to the blockade of the ROCs and VOCs in the rat. Thus, resveratrol seems to be of potential use in the treatment or improvement of dysmenorrhea. However, we need additional clinical experiments to further support this finding in the future.

Acknowledgments

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國科會補助計畫衍生研發成果推廣資料表

日期:2011/10/29

國科會補助計畫	計畫名稱: 白藜蘆醇對大鼠子宮平滑肌收縮及卵巢細胞分泌性類固醇激素的影響
	計畫主持人: 夏詩閔
	計畫編號: 98-2629-B-424-001-MY2　　學門領域: 食品及農化

無研發成果推廣資料

98 年度專題研究計畫研究成果彙整表

計畫主持人：夏詩閔	計畫編號：98-2629-B-424-001-MY2
計畫名稱：白藜蘆醇對大鼠子宮平滑肌收縮及卵巢細胞分泌性類固醇激素的影響	

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%		
	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	1	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	1	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	1	1	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	

國科會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）

本成果首次發現白藜蘆醇具有改善經痛之效果已發表於endocrinology並可以進一步開發改善經痛之保健食品